

# Investigation of the Wzy O antigen polymerase and the Wzz co-polymerase interface

---

**Vincenzo Leo**

Bachelor of Science (Hons)



THE UNIVERSITY  
*of* ADELAIDE

Submitted for the Degree of Doctor of Philosophy

Research Centre for Infectious Diseases

School of Biological Sciences

The University of Adelaide

Adelaide, South Australia

August 2021

## **Declaration**

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Program Scholarship.

Vincenzo Leo

April 2021

# Contents

Abstract .....	ix
Acknowledgements .....	x
Abbreviations .....	xiii
Thesis Style and Layout .....	xv
Chapter 1: Introduction .....	2
1.1 <i>Shigella flexneri</i> .....	2
1.2 <i>Shigella flexneri</i> pathogenesis .....	4
1.3 Bacterial Polysaccharides .....	5
1.4 Lipopolysaccharide .....	6
1.4.1 The O-Antigen .....	7
1.5 LPS Biosynthesis .....	9
1.5.1 Lipid A biosynthesis .....	9
1.5.2 Core biosynthesis .....	11
1.5.3 O antigen biosynthesis in <i>S. flexneri</i> .....	12
1.6 Proteins of the WzyB dependent pathway .....	13
1.6.1 WzyB Oag polymerase .....	13
1.6.2 <i>S. flexneri wzyB</i> mutants .....	15
1.6.3 WzzB Oag co-polymerase .....	16
1.6.4 Wzz <sub>pHS2</sub> Oag co-polymerase .....	19
1.6.5 The Wzy/Wzz complex .....	20
1.6.6 Wzx flippase .....	21
1.6.7 LPS export .....	21
1.7 Enterobacterial common antigen .....	23
1.8 <i>Shigella</i> vaccines .....	24
1.9 Research Hypothesis and Aims .....	25
Chapter 2: Materials and Methods .....	28
2.1 Bacterial strains and growth conditions .....	28

2.1.1	Strains and plasmids .....	28
2.1.2	Growth media and conditions .....	28
2.1.3	Antibiotics and additives .....	28
2.1.4	Growth and maintenance of bacterial strains.....	28
2.2	Antibodies.....	28
2.3	DNA techniques.....	29
2.3.1	Plasmid isolation.....	29
2.3.2	Whole chromosome DNA purification.....	29
2.3.3	DNA quantification .....	29
2.3.4	Restriction digests.....	29
2.3.5	Oligonucleotides .....	29
2.3.6	PCR.....	29
2.3.7	Preparation of “boiled lysate” for PCR .....	30
2.3.8	PCR DNA purification .....	30
2.3.9	DNA gel extraction.....	30
2.3.10	<i>In vitro</i> oligonucleotide annealing .....	30
2.3.11	Inverse PCR for site-directed mutagenesis.....	30
2.3.12	DNA phosphorylation.....	30
2.3.13	DNA ligation .....	31
2.3.14	Lambda Red mutagenesis .....	31
2.3.15	Random mutagenesis .....	31
2.3.16	Agarose gel electrophoresis.....	32
2.3.17	DNA sequencing.....	32
2.3.18	Whole genome DNA sequencing .....	32
2.4	Bacterial transformation .....	32
2.4.1	Preparation of electro-competent cells .....	32
2.4.2	Electro-pulse transformation .....	32
2.4.3	Preparation of chemically competent cells .....	32



2.4.4	Ultra-competent bacterial cells .....	33
2.4.5	Heat-shock transformation.....	33
2.5	LPS techniques .....	33
2.5.1	Preparation of LPS samples.....	33
2.5.2	Analysis of LPS by silver-stained SDS-PAGE .....	33
2.6	Protein techniques.....	34
2.6.1	Whole cell lysate protein samples .....	34
2.6.2	Purification of WzyB.....	34
2.6.3	<i>In vivo</i> protein crosslinking .....	34
2.6.4	SDS-PAGE for Western transfer.....	35
2.6.5	Western transfer.....	35
2.6.6	Immunoblotting .....	35
2.6.7	Analysis of ECA by SDS-PAGE.....	35
2.6.8	Coomassie Blue staining.....	35
2.6.9	Colloidal Blue staining .....	36
2.7	Mass spectrometry .....	36
2.8	Anti-bacterial sensitivity assays .....	36
2.8.1	Colicin E2 spot assay.....	36
2.8.2	Colicin E2 swab assay .....	36
2.9	Bioinformatical techniques.....	37
2.9.1	Alignment of whole genome sequencing to reference sequence.....	37

Chapter 3:	Research Article 1: Polysaccharide co-polymerase WzzB/WzzE chimeras reveal transmembrane 2 region of WzzB is important for interaction with WzyB.....	39
3.1	Statement of Authorship.....	39
3.2	Article Abstract.....	41
3.3	Article Introduction .....	42
3.4	Article Methods .....	45

3.4.1	Ethics statement. ....	45
3.4.2	Bacterial strains, growth media and growth conditions.....	45
3.4.3	Chimera construction by inverse PCR.....	45
3.4.4	DNA methods. ....	45
3.4.5	LPS SDS-PAGE and silver staining. ....	46
3.4.6	Protein SDS-PAGE.....	46
3.4.7	Western immunoblotting. ....	46
3.4.8	Colicin spot assay. ....	46
3.4.9	Purification of WzyB from <i>S. flexneri</i> strains. ....	47
3.4.10	<i>In vivo</i> protein crosslinking. ....	47
3.5	Article Results .....	49
3.5.1	WzzE is able to partially complement a <i>wzzB</i> mutant.....	49
3.5.2	Functional analysis of the transmembrane regions within the co-polymerases. .....	50
3.5.3	The effect of the chimeric proteins on resistance to colicin E2.....	53
3.5.4	The effect of the chimeric proteins on ECA production and modal length control. ....	54
3.5.5	Interaction of the Wzz chimera proteins with WzyB. ....	55
3.6	Article Discussion.....	60
3.7	Article References .....	63
3.8	Article Supporting Information .....	66
3.8.1	Supporting Figures .....	66
3.8.2	Supporting Table .....	71
3.9	Article Acknowledgements .....	72
Chapter 4: Research Article 2: Identification of a region in <i>Shigella flexneri</i> WzyB disrupting the interaction with WZZ <sub>pHS2</sub> . ....		74
4.1	Statement of Authorship .....	74
4.2	Article Abstract.....	76
4.3	Article Introduction .....	77

4.4	Article Methods .....	79
4.4.1	Ethics statement.....	79
4.4.2	Bacterial strains, growth media and growth conditions.....	79
4.4.3	DNA methods.....	79
4.4.4	Construction of WzyB-FLAG-LacZ $\alpha$ vector.....	79
4.4.5	Random mutagenesis.....	79
4.4.6	Colicin swab assay.....	80
4.4.7	LPS SDS-PAGE and silver staining.....	80
4.4.8	Protein SDS-PAGE.....	80
4.4.9	Western immunoblotting.....	80
4.4.10	Purification of WzyB from <i>S. flexneri</i> strains.....	80
4.4.11	<i>In vivo</i> protein crosslinking.....	81
4.5	Article Results .....	82
4.5.1	Construction and complementation analysis of a WzyB-FLAG-LacZ expression construct.....	82
4.5.2	Random mutagenesis of the <i>wzyB</i> coding region.....	84
4.5.3	Alanine scanning mutagenesis of WzyB aa 351 to 357 .....	85
4.5.4	Further site-directed mutagenesis of WzyB aa 352-354.....	89
4.5.5	Interaction of the WzyB mutant proteins with WzzB and Wzz <sub>pHS2</sub> .....	92
4.6	Article Discussion.....	95
4.7	Article References .....	99
4.8	Article Supporting Information .....	102
4.8.1	Supporting Figures .....	102
4.8.2	Supporting Tables.....	108
4.9	Article Acknowledgements .....	110
Chapter 5: Identification of WzyB binding partners .....		112
5.1	Introduction.....	112
5.2	WzyB purification .....	112

5.3	Upscaled WzyB purification and band excision.....	114
5.4	Mass spectrometry analysis .....	115
5.5	Discussion.....	127
Chapter 6: Identification of novel LPS genes.....		131
6.1	Statement of Authorship.....	131
6.2	Introduction.....	131
6.3	Mutagenesis of select mutants in <i>E.coli</i> W3110.....	132
6.4	Mutagenesis of select mutants in <i>E.coli</i> BW25113 .....	134
6.5	Whole genome sequencing of the identified mutants.....	135
6.6	Discussion.....	142
Chapter 7: Conclusions .....		146
7.1	WzzB interaction with WzyB via TM2 .....	146
7.2	Identification of a region in WzyB that disrupts the novel interaction with Wzz <sub>pHS2</sub> .....	146
7.3	Identification of WzyB binding partners .....	147
7.4	Identification of novel LPS genes.....	148
7.5	Summary.....	149
Appendix A - Bacterial strains generated .....		152
Appendix B – Constructs generated.....		161
Appendix C – Oligonucleotides generated .....		163
Appendix D – Mass spectrometry raw data.....		166
Appendix E – Whole genome sequencing full data set .....		239
Appendix F – Thesis bibliography.....		292

## Abstract

Complex polysaccharide chains such as the O antigen (Oag) component of lipopolysaccharide (LPS) and the enterobacterial common antigen (ECA) are located on the bacterial cell surface. Oag and ECA are synthesised by the most common polysaccharide synthesis pathway in bacteria known as the Wzy-dependent pathway. The Oag and ECA are polymerized into chains via the inner membrane proteins WzyB and WzyE respectively, while the co-polymerases WzzB and WzzE respectively, modulate the number of chains or “the modal length” of the polysaccharide. The objective of this thesis is to understand how the Wzy and Wzz proteins interact to control Oag and ECA modal length, using *Shigella flexneri* as a model. Particular focus was placed on WzyB and its binding partners; throughout this thesis each of the co-polymerases, WzzB, WzzE and Wzz<sub>pHS2</sub>, were all shown to physically interact with WzyB. For the first time “cross-talk” between Oag and ECA synthesis was shown and identified novel physical protein-protein interactions between proteins in these systems. These findings further the understanding of how these systems function to control polysaccharide chain length. The data suggests that the TM 2 region of the co-polymerases is likely the binding site to WzyB. Additionally, the data suggests that the aa 352-354 region of WzyB appears to be the binding site of Wzz<sub>pHS2</sub> but is also likely an active site involved with Oag modal length control. Using co-purification experiments, novel WzyB binding partners with no previous link were also discovered via mass spectrometry. The physical interaction between the WzyB and Wzz members of the Wzy-dependent pathway appear to be the only members of the pathway that interact. This interaction is likely required for function and as such furthering the understanding of this interaction is of critical importance, which has great implications for novel biotechnologies and/or the combat of bacterial diseases. Finally, whole genome sequencing of previously identified colicin E2 sensitive mutants reveals novel genes involved with unknown aspects of LPS synthesis, transport or length regulation. Future mutagenic investigation may shed light on these genes which may be critical to LPS.

## Acknowledgements

First and foremost, I would like to thank my supervisor Renato Morona. Your constant support, enthusiasm and knowledge throughout my PhD has helped me immensely. Always excited to see new results and ideas, always ready to help with any question that I had. Conversations that went on for hours in your office each time a new result was produced. This inspired and motivated me to continue to work hard and continue to think and of new and exciting ways to test our theories. This support has made my PhD the most enjoyable and worthwhile achievement in my life. I will never forget the morning coffees and lab lunches shared with you, fantastic memories that I will cherish forever. I will forever be in your debt and I cannot thank you enough Ren, I appreciate everything.

Next, I would like to thank Dr. Elizabeth Tran, my PhD would not have been possible without your constant help. You literally have endless patience, and are always willing to help me and others in the lab, Alex and Brian are extremely lucky to have a mum like you! Your constant support in the lab made my PhD easy, because I knew I could always rely on your help should anything go wrong or I had a problem. Thank you for taking to time to help me draft any of my documents and put up with all the dodgy grammar and spelling. Because of your kindness and determination to our lab, it was not a group of people from the lab it was a family. Thanks Liz!

Next I would like to thank Dr. Min Teh, your help though out my PhD will never be forgotten. You were always there to answer any silly question I had and always there to offer advice inside and outside the lab. You are fantastic member of the Morona family and I'm glad that our paths crossed during my PhD, Thank you very much Min! Please don't bring anymore durian to the lab I still have nightmares.

Thank you to all the past and present members of the Morona lab, the friendships made throughout my PhD will last forever! Brad, lucky dragon, Geelong, Sindorei, Qin, I can't count the number of times we had each other in tears! Thank you for all the help and advice you gave me throughout my PhD, you were seriously a massive help and I could not have done it without you! You are a great friend and I will never forget the moments we shared during my PhD! Xbox is rat, Petrol prices are on a cycle that I can predict, *The tip of the dragons tail smells in winter*. I love you mate! Zuleeza, thank you for all the great memories and laughs we shared during our PhDs. You were a fantastic member of the Morona family and I hope we can visit leaf hut for some mie goreng soon! Sir Nicholas Polazn prince of Poland Maczuga! Thank you for being a great friend throughout my PhD! You have helped me more than you know, our constant meaningful conversations about science, politics and forest architecture will never be forgotten.

Lewis Hamilton is a flog, FORZA THE ORANGES AND RED NUMBER THREE, has it crashed? Iron forever baby! Alice “hot dogs” Ascari, thank you for all the memories and laughs we shared during my PhD. It was nice to have a true choc in the lab to correct my useless Italian. Keep munging your hands, PG10, Gene HAAS, RATAQ, L'Chaim, the guy in the card shop, Gel tank with a cigarette! You are a true friend thank you for everything! Thank you both for all the memories of F1s and League I will never forget our friendship!

The morning coffee crew! Thank you to Ali, Tony and Kate! I have always enjoyed morning coffee and it wouldn't have been the same without you all! You all have helped me during my PhD and I will never forget the laughs and chats we all shared during my PhD!

I would like to thank all my friends! A PhD or (Ph Dude) is a hard and long process and you all have helped me throughout my studies by being a great bunch of friends!

Next, I would like to thank my parents Anti Anti and Pino!!! I love you both very, much thank you for the endless support and love throughout my whole studies during and before my PhD. You have made this process very easy and enjoyable! Thank you for always asking how long until you finish, this made me take my time and slow down and ensure my PhD was perfect.

Thanks must be also given to my grandparents and my dear Zia Lina! I love you all so much thank you for everything you have done for me not only during my PhD but my whole life! You were always there for me and help raised me to be the man I am today thank you all very much!

I would also like to thank my brother Gianni, for his love and support, I am appreciative for all the good times we had together throughout my PhD, and you have helped me more than you know! Thank you for all the movies, Minecraft sessions, table tennis and Mr olympica updates, move your rat car! I love you bello!

Marissa! I am very grateful for all the love and support you have given me! You have been there for me since day one of my university career and you have made it so easy for me to study! This PhD would have been impossible without you, have been my best friend and always there to help and comfort me when I was stressed about exams or scared about giving talks, and always there after a long stressful day to cheer me up with your beautiful smile, because of this and with your help I was able to get the opportunity to do my PhD without you I wouldn't have even had the confidence to even try. I appreciate everything you have done for me over my PhD your confidence in me is truly amazing and will never forget it! I Love you so much bestie!

A genius scholar by the name as Chenzleo11 once said said “*at the end of the day, It’s night time*”  
and “*The fart bends corners when winds are high*”

Mn=Mg

Coke zero

Signing out,

Chenzleo11 (The LPS Master)



## Abbreviations

AGRF	Australian Genome Research Facility
Amp	Ampicillin
CDS	Coding sequence
Cml	Chloramphenicol
ECA	Enterobacterial common antigen
g	Grams
GlcNAc	<i>N</i> -acetylglucosamine
hrs	Hours
IL	Interleukin
Kan	Kanamycin
Kdo	3-deoxy-D-manno-oct-2-ulosonic acid
LB	Lysogeny broth medium
LBA	Lysogeny broth agar
LPS	Lipopolysaccharide
mA	Milliamps
mg	Micro grams
min	Minutes
MOP	Multidrug/Oligosaccharidyl-lipid/Polysaccharide
MQ	Milli-Q
ng	Nana Grams
nm	Nanometres
Oag	O Antigen
OD600	Optical density at wavelength of 600 nm
OM	Outer membrane
OMV	Outer membrane vesicle
ON	Overnight
PAGE	Polyacrylamide gel electrophoresis
PEtN	Phosphoethanolamine
PMNL	Polymorphonuclear neutrophil leukocytes
Rha	L-rhamnose
R-LPS	Rough lipopolysaccharide
Ro	Reverse osmosis

RT	Room temperature
SDS	Sodium dodecyl sulphate
Sec	Seconds
S-LPS	Smooth lipopolysaccharide
S-Oag	Short O antigen
SR-LPS	Semi-rough lipopolysaccharide
STn	Sialyl-Tn
T3SS	Type three secretion system
TBS	Tris-buffered saline
Tet	Tetracycline
TTBS	Tris-buffered saline tween
UTR	Untranslated region
V	Volts
v/v	Volume per volume
VL-Oag	Very long O antigen
w/v	Weight per volume

## **Thesis Style and Layout**

This thesis is submitted in the style of a “Combination of conventional thesis and thesis by publication formats”. As such, the result chapters are two research publications (Chapter 3 & 4) and traditional two results chapter (Chapter 5 & 6). In Chapter 1, the studies and research in the field of this work is reviewed and research gaps identified as Aims for investigation. In Chapter 2, the Materials and Methods used in this thesis are outlined in detail.

As for publication purposes, Chapter 3 and 4 are presented as per the requirement of the journal, which includes all the information that will be submitted for publication. Author contributions for each publication are stated in the Statement of Authorship section. For Chapter 5 and 6, materials and methods used are outlined in Chapter 2 and are cross-referenced. Each result chapter has a discussion section to explore the underlying meaning of the work. Chapter 7 draws conclusions of the outcomes and the significance of this study, and points out the future research directions.

# Chapter One

---

## INTRODUCTION

---

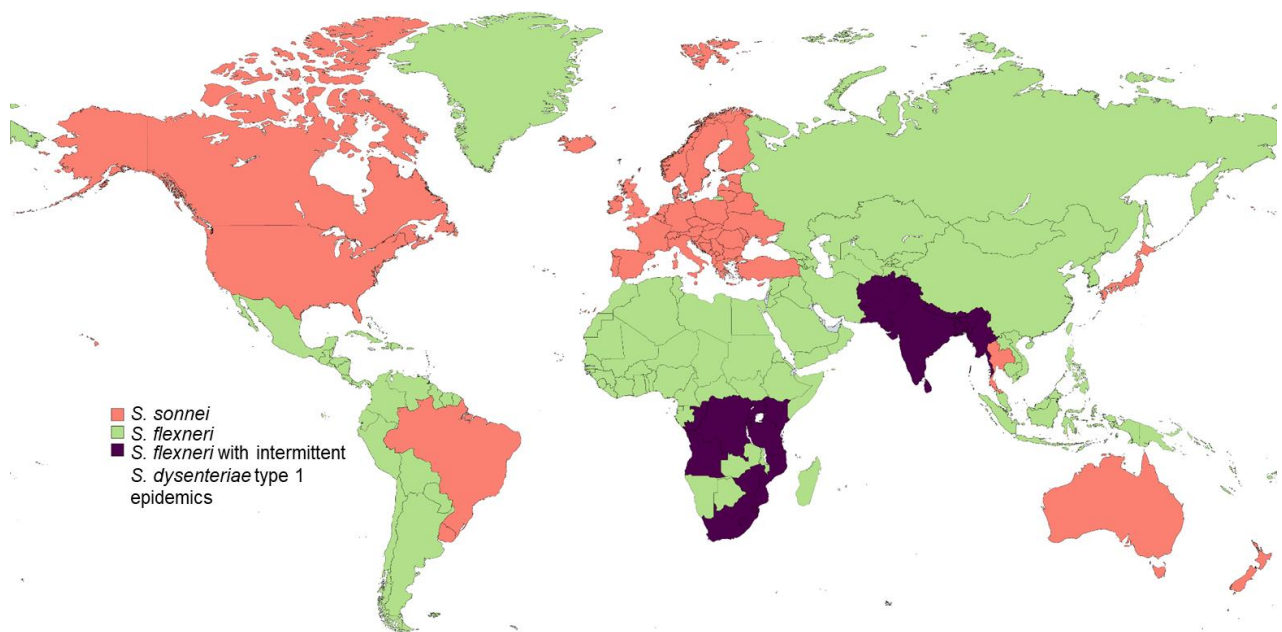
## Chapter 1: Introduction

---

### 1.1 *Shigella flexneri*

---

*Shigella flexneri* is a human specific Gram negative bacterial pathogen that causes the diarrheal disease known as shigellosis. Shigellosis is an acute intestinal infection with symptoms that range from watery diarrhoea to bacterial dysentery, and is characterised by strong abdominal cramps, fever and stools containing blood and mucus. This disease may be life-threatening to patients that are malnourished, immunocompromised or lack adequate medical care. This is a major health problem in several parts of the world (as shown in Figure 1.1). Shigellosis is endemic in most developing countries, with estimations indicating that at least 188 million cases of bloody diarrhoea and 164,000 deaths each year are caused by this disease. Of these deaths, 99% occurred in developing countries where poor hygiene and non-sufficient access to clean drinking water promote the spread of enteric diseases (Kotloff et al. 2018).



---

**Figure 1.1: Species distribution of *Shigella flexneri* throughout the world.**

*Shigella flexneri* is a major problem throughout the world especially in countries lacking clean drinking water which promotes the spread of the enteric pathogen. Figure from Bennish and Khan (2012)

---

*S. flexneri* is very similar to *Escherichia coli* K-12, with only 1.5% sequence divergence between the two bacterial species (Lan & Reeves 2002). The differences between the species is the result of changes to the bacterial chromosomes, and also the virulence plasmid that is only present in *Shigella*. This virulence plasmid expresses genes that encode proteins essential for *Shigella* virulence, including the proteins involved in the machinery for invasion known as the type III secretion system, or T3SS, a molecular syringe that is prevalent in pathogenic Gram

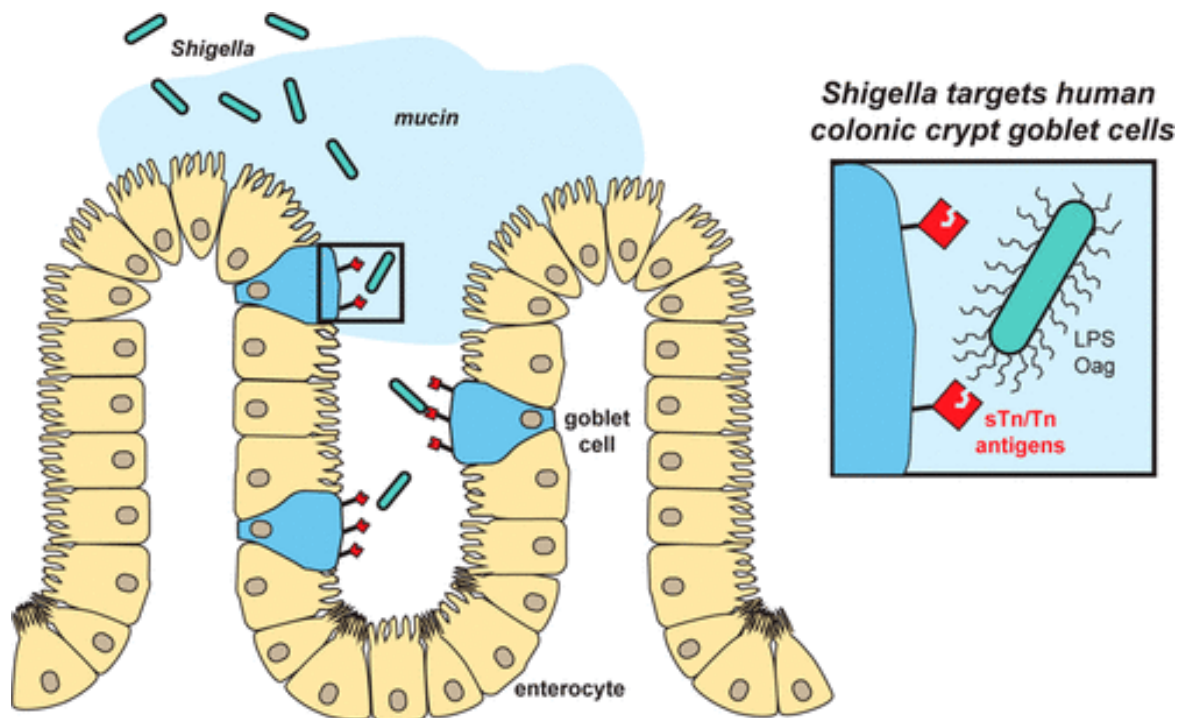
negative bacteria and used to deliver effector proteins into the target eukaryotic cell (Philippe 2004). It also encodes the proteins involved in bacterial adherence to target epithelial cells, as well as the proteins required for viability inside host cells. A summary of the well characterised virulence genes is shown in Table 1.1.

**Table 1.1: Genes on the *Shigella* virulence plasmid associated with virulence and their encoded protein functions.**

Gene	Function	Reference
<i>virF</i>	AraC-linked transcription regulator; activates the transcription of <i>virB</i> and <i>icsA</i>	(Sakai, T et al. 1988)
<i>virA</i>	Facilitation of entry and intracellular motility by degradation of microtubules	(Yoshida et al. 2006; Yoshida et al. 2002)
<i>invA (mxiB)</i>	Required for invasion	(Andrews et al. 1991)
<i>mxiA</i>	Required for invasion	(Andrews et al. 1991)
<i>ipaB</i>	Control of type III secretion, translocon formation, phagosome escape, macrophage apoptosis	(Blocker et al. 1999; Chen et al. 1996)
<i>ipaD</i>	Control of type III secretion, membrane insertion of translocon	(Espina et al. 2006)
<i>ipaC</i>	Translocon formation, filopodium formation, phagosome escape, disruption of EC tight junctions	(Blocker et al. 1999)
<i>ipaA</i>	Efficient invasion, actin cytoskeleton rearrangements, disassembly of cell-matrix adherence	(DeLeo 2004)
<i>icsA (virG)</i>	Recruitment of actin-nucleating complex required for actin-based motility and intercellular spread; adherence	(Bernardini et al. 1989; Zumsteg et al. 2014)
<i>ipaH7.8</i>	Efficient phagosome escape	(Fernandez-Prada et al. 2000)
<i>ipH9.8</i>	Host cell transcriptome modulation, reduction of inflammation	(Okuda et al. 2005)
shET2	Enterotoxin	(Fasano et al. 1995)

## 1.2 *Shigella flexneri* pathogenesis

*Shigella flexneri* is transmitted by the faecal oral route and therefore enters the body following ingestion of either contaminated food or drinking water. *S. flexneri* are enteroinvasive meaning they invade and multiply inside epithelial cells and eventually cause death of the epithelial cells. This ultimately leads to destruction of the gut tissue and a reduction in the surface area needed for absorption of fluids and nutrients. *S. flexneri* invades the epithelial cells of the colon from the basolateral cell surface by initially translocating through the gut epithelium via specialised cells called the colonic crypt goblet cells (Tran et al. 2020). Prior to invasion *S. flexneri* adheres to the goblet cells by interacting with Tn/sialyl-Tn (sTN) antigens via glycan-glycan interactions with their lipopolysaccharide (Figure 1.2). Following translocation *S. flexneri* encounter resident macrophages and survive bacterial cell death by inducing pyroptosis of the macrophage. This subsequently releases proinflammatory cytokines such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-18 (Sansonetti et al. 2000). The bacteria are now free to invade the epithelial cells via the basolateral side and replicate in the cell cytoplasm. *S. flexneri* move into adjacent cells via actin based motility by utilizing the host cell's actin; this process is ideal as *S. flexneri* does not interact with the extracellular components of the host's immune defence (Bernardini et al. 1989).

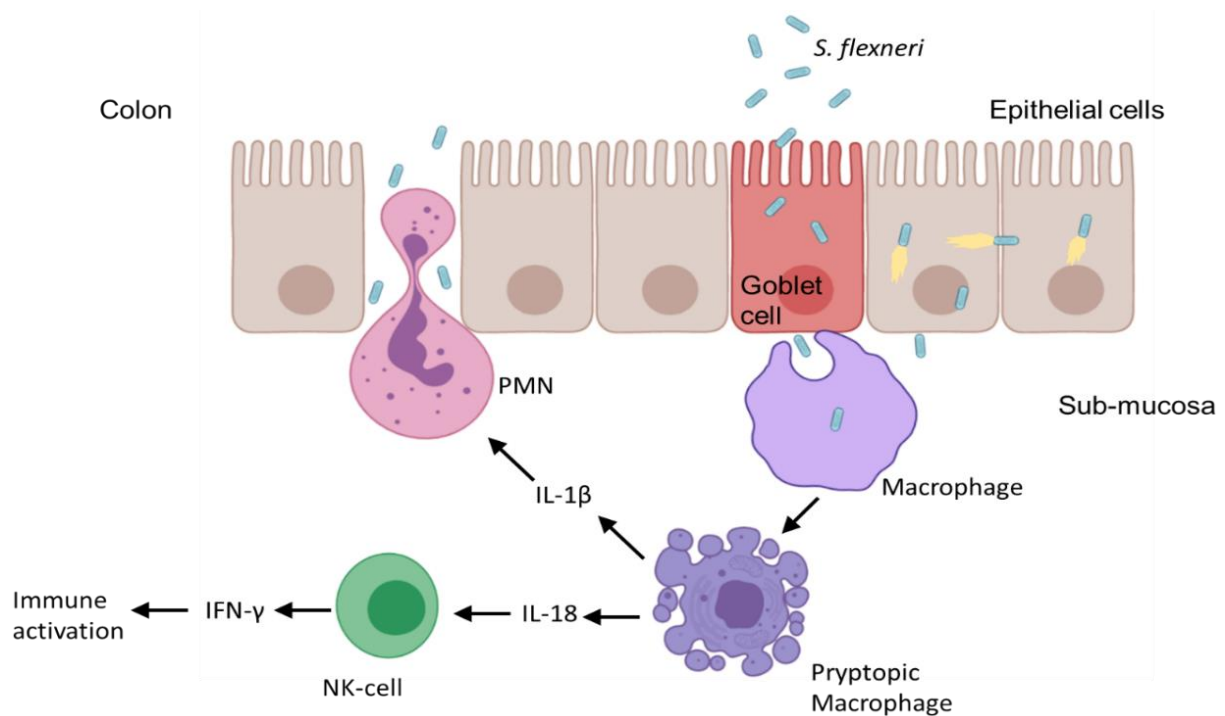


**Figure 1.2:** *S. flexneri* adhering to goblet cells via sTn/Tn antigens.

Graphical representation of *S. flexneri* using LPS to adhere to human colonic crypt goblet cells via the sTn/Tn antigens as the receptor. Figure from Tran et al. (2020)

The invasion of *S. flexneri* also triggers the release of cytokines which results in a massive inflammatory response that includes an influx of polymorphonuclear neutrophil leukocytes

(PMNL) to the gut epithelium. Invading PMNLs disrupt the tight junctions between adjacent epithelial cells thus enabling more *Shigella* to invade the submucosa without the need to invade goblet cells. It has also been shown that *S. flexneri* weaken the tight junction by altering the tight junction protein composition (Sakaguchi et al. 2002). Tissue damage as well as the profound inflammatory response with the increase of PMNLs and mucus results in the characteristic dysenteric stool (Bennish & Khan 2012). The *Shigella* pathogenesis process is summarised in Figure 1.3.



**Figure 1.3: Cellular pathogenesis of *S. flexneri*.**

*Shigella* pass through the epithelial cell barrier by passing through goblet cells where they encounter resident macrophages in the sub-mucosa. To ensure immediate survival, *Shigella* induce pyroptosis of the macrophages which leads to the release of pro-inflammatory cytokines. The released bacteria then invade the epithelial cells on the basolateral side and spread to adjacent cells via actin based motility. Furthermore the proinflammatory cytokines released by the epithelial cells and the macrophages activate the innate immune response leading to natural killer cells attracting polymorphonuclear leukocytes (PMNL) which squeeze between horizontal epithelial cells, thus allowing more bacteria to cross the epithelial cell barrier to the sub-mucosa.

### 1.3 Bacterial Polysaccharides

The ability of bacteria to synthesise complex polysaccharide chains has wide biological functions for the bacteria which includes: symbiosis, biofilm formation and immune system avoidance. They also function to assist in bacterial resistance to antimicrobials such as antibiotics, bacteriocins, bacteriophages, etc. Importantly, Gram negative bacterial pathogenesis is reliant on the production of these polysaccharides. For example the exopolysaccharide capsule, a



polysaccharide loosely attached to the outer surface of bacteria is responsible for aiding the bacteria's survival in harsh conditions, while also acting as adhesion receptors during colonization of tissues (Nwodo et al. 2012). Another example is Oag, a polysaccharide responsible for acting as a physical barrier to antimicrobials and the host immune system. Thus, there is a great importance to further our understanding of these complex polysaccharides and their biosynthetic pathways.

Aside from their principle role in the biological setting, polysaccharides also have a wide range of biotechnological, industrial, and commercial applications. An example of this is the production of the food gelling agent Xanthan gum by *Xanthomonas campestris* (Margaritis & Zajic 1978). Additionally, polysaccharides have been used in bioemulsifiers, which are surface active compounds that decrease surface tension and help solubilise hydrophobic substrates (Satpute et al. 2010). An example of this is the polysaccharide Emulsan from *Acinetobacter calcoaceticus* that acts as a powerful emulsion stabiliser (Pines & Gutnick 1986). Finally, in depth understanding of polysaccharide systems may allow for engineered synthesis of polysaccharides for vaccine production. For example, encapsulated bacteria such as *Haemophilus influenza* serotype B (Hib), *Neisseria meningitidis* and *Streptococcus pneumoniae* cause major disease worldwide. Vaccines against these pathogens targeting their capsular polysaccharide protect against the disease. In particular Pneumococcal vaccines which contain purified polysaccharides have been available for over half a century and have been effective in preventing infection (Leinonen et al. 1986). These vaccines however act in a T-independent immune response, characterised by lack of memory. By conjugating the polysaccharides to carrier proteins it is possible to elicit a strong T-dependent immune response (Lesinski & Westerink 2001). The glycoconjugate vaccine against Hib for example, has been successful in reducing the incidence of the invasive disease (Peltola et al. 1992). Thus for these reasons the study and understanding of bacterial polysaccharide systems are crucial for the fundamental development of novel biotechnologies and/or the combat of bacterial diseases.

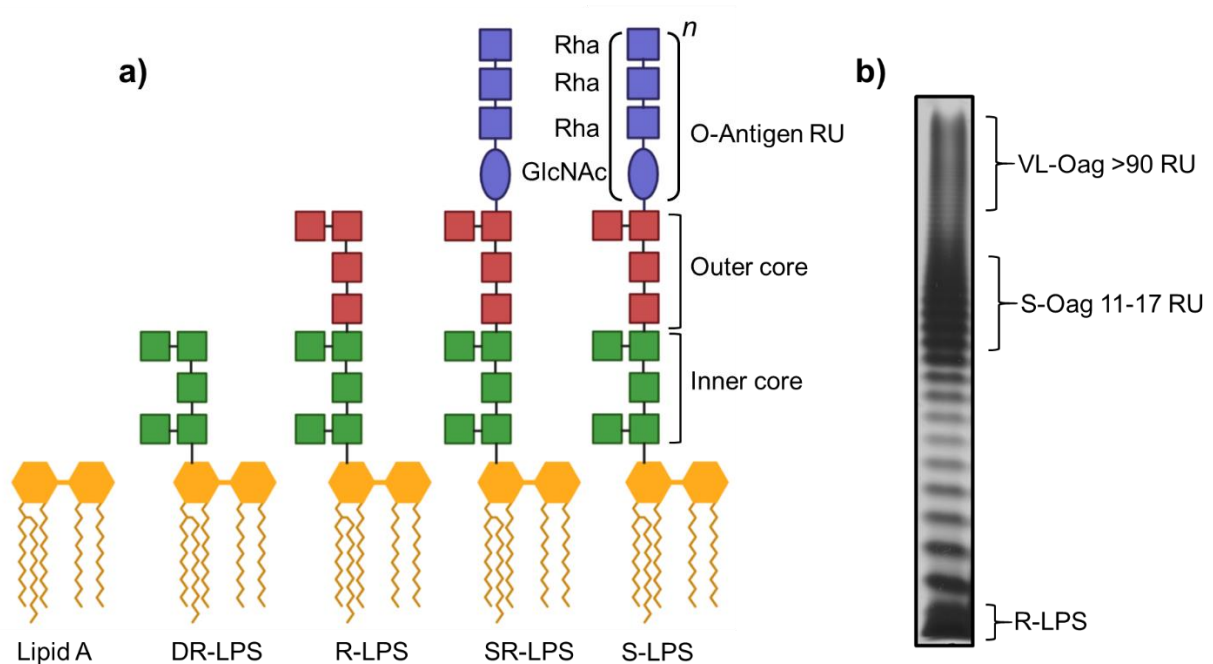
#### **1.4 Lipopolysaccharide**

---

A major virulence determinant of *S. flexneri* is lipopolysaccharide (or LPS), particularly the O antigen (Oag) polysaccharide component. LPS is also crucial for the environmental survival of *Shigella* as it acts as a barrier against a number of environmental pressures, conferring resistance to complement and also colicins (Tran et al. 2014). LPS molecules are major components of the outer membrane (OM) covering approximately 75% of the cell surface (Jacobson et al. 2015). LPS consists of three moieties: 1) lipid A, which is the region that anchors the LPS molecule to the OM, 2) the core oligosaccharide, which can be divided further into the inner core and the outer

core (Polissi & Sperandeo 2014), and 3) the Oag polysaccharide repeat, which can also vary in the number of repeats (Putker et al. 2015) (Figure 1.4).

LPS also affects the virulence of *S. flexneri* as it influences actin based motility and is also needed for adherence (Teh et al. 2012; Tran et al. 2020). LPS is released during bacterial replication and destruction of epithelial cells, and this causes the release of pro-inflammatory cytokines. These cytokines are responsible for the activation of the host innate immune response and also for the clinical symptoms of shigellosis (Ranallo et al. 2010). The lipid component A is also an endotoxin responsible for activation of the innate immune system via Toll-like receptor 4 (Poltorak et al. 1998; Rosenfeld & Shai 2006). Following activation the innate immune response results in the production of cytokines, including, TNF- $\alpha$ , IL-1, and IL-6. Uncontrolled, massive innate immune responses, due to a large quantity of toxic LPS can lead to more severe symptoms of sepsis or septic shock.



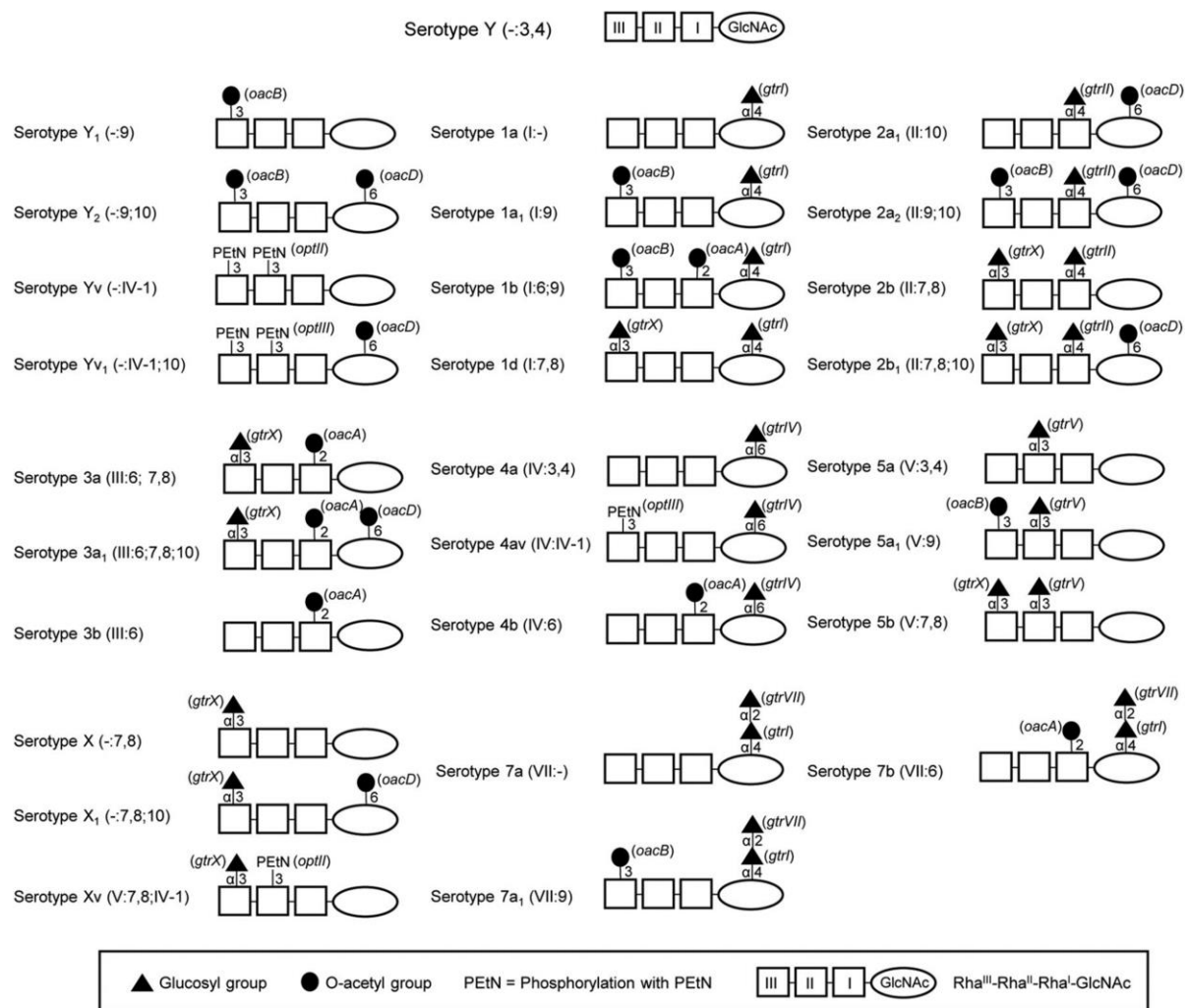
**Figure 1.4: Lipopolysaccharide structure and forms.**

a) LPS consists of three distinct regions; lipid A, core OS and Oag polysaccharide. LPS structures with the Oag repeat unit (RU) are termed smooth LPS (S-LPS), LPS structures with only one Oag RU are termed semi rough LPS (SR-LPS), LPS structures without the Oag are termed rough LPS (R-LPS) and LPS structures without Oag and outer core sugars are termed deep-rough LPS (DR-LPS). (B) Silver-stained-PAGE separation of the LPS from *S. flexneri*. The various types of LPS are indicated on the right.

### 1.4.1 The O-Antigen

The Oag component of the LPS has a remarkable range of components and arrangements in the structure of Oag which defines at least 19 serotypes in *S. flexneri* [1a, 1b, 1c/7a, 1d, 2a, 2b,

3a, 3b, 4a, 4av, 4b, 5a, 5b, 6, 7b, X, Xv, Y and Yv] (Liu, B et al. 2014; Perepelov et al. 2012; Stenutz et al. 2006) (Figure 1.5). All of the named *S. flexneri* serotypes share the same basic polysaccharide backbone consisting of three L-rhamnosides (Rha) molecules, and one N-acetylglucosamine (GlcNAc), with the exception of serotype 6. The basic Oag structure is known as the Y serotype, whereby the Rha residues are linked by  $\alpha$  linkages while the Rha and GlcNAc are linked by  $\beta$  linkages. The differing serotypes are the result of the addition of glucosyl, O-acetyl or phosphoethanolamine (PEtN) groups to the tetrasaccharide repeats by different linkages (Knirel et al. 2015). Glucosylation of the Oag is the result of the *gtr* cluster (*gtrA*, *gtrB* and *gtr* (type specific)), whereby *gtrA* and *gtrB* are conserved, but *gtr* (type specific) is unique and encodes a glucosyltransferase that attaches a glucosyl group to the specific sugar in the tetrasaccharide repeat unit. Additionally the *oacB* gene encodes an O-acetyl transferase responsible for O-acetylation of the Oag (Clark et al. 1991). Glycosylation can occur at any of the residues of the tetrasaccharide repeat unit, while O-acetylation occurs at position two of Rha I (Jakhetia et al. 2014). The PEtN modification can occur at position 3 of Rha II and/or Rha III, uniquely this is encoded by the plasmid borne *opt* gene (Jakhetia et al. 2014). The hyper-variability of Oag observed across these pathogens is thought to be a result of many environmental selective pressures, such as the host adaptive immune system and Oag specific bacteriophage which use the Oag as a site of attachment (Whitfield & Trent 2014).



**Figure 1.5: *S. flexneri* serotypes**

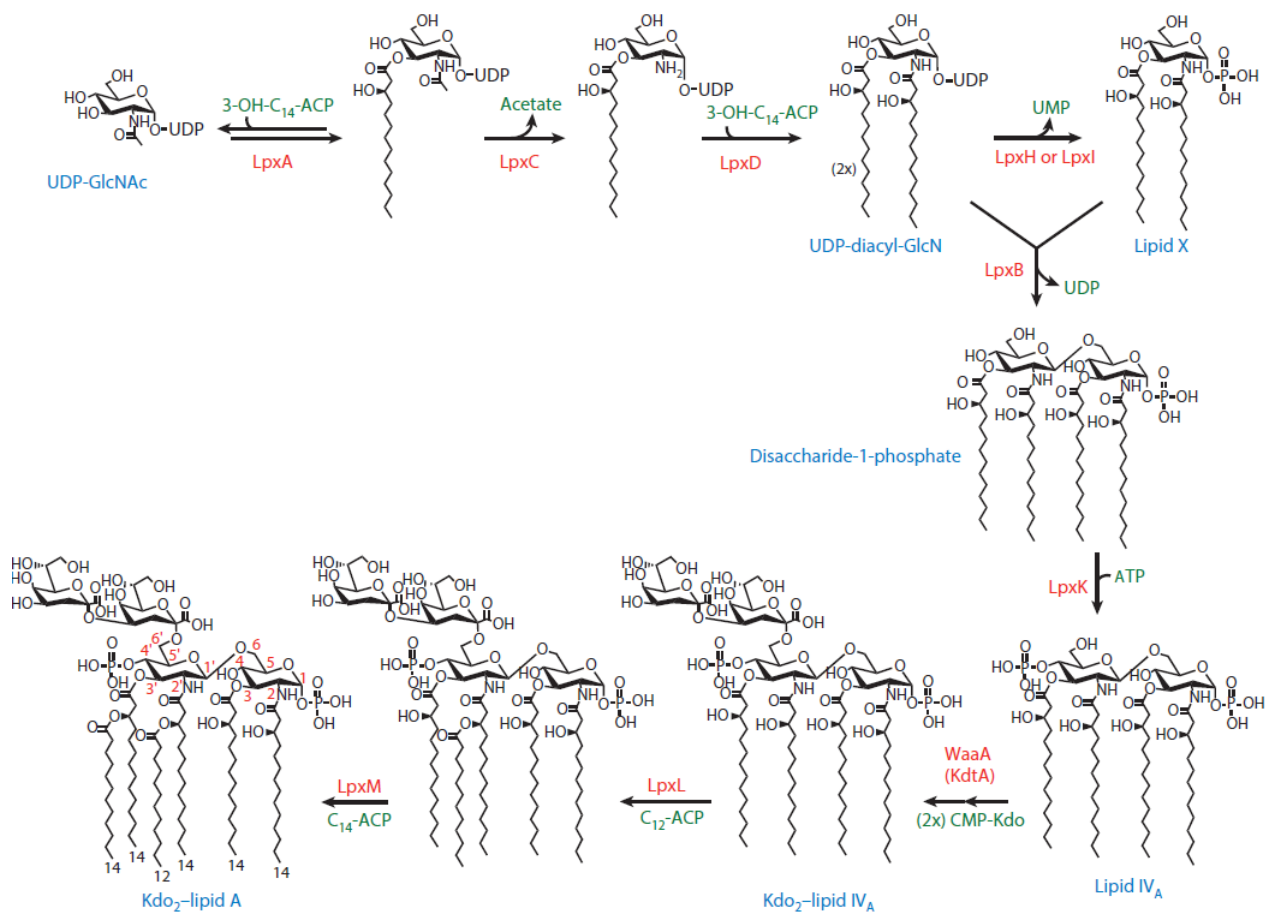
Graphical representation of the chemical structures of *S. flexneri* serotypes. The basic O-polysaccharide backbone or the Y serotype consists of repeat units including three l-rhamnose residues (Rha<sup>III</sup>-Rha<sup>II</sup>-Rha<sup>I</sup>) and one *N*-acetylglucosamine (GlcNAc). Each serotype differs by the addition of a glucosyl group, O-acetyl group, or phosphoethanolamine (PEtN) the addition of these groups can occur on different sugars within the repeat unit via the linkages indicated. Specific Oag modification genes are indicated in brackets. Each serotype has one type-specific (Roman numeral) and one or more group-specific antigenic determinants. Serotypes 2a, 3b, 4a, 5a, and Y possess group O-factor 3, 4, which is associated with the O-polysaccharide backbone but is omitted from the antigenic formula when other group O-factors are present. Figure adapted from Teh, MY et al. (2020)

## 1.5 LPS Biosynthesis

### 1.5.1 Lipid A biosynthesis

The lipid A component of the LPS anchors the polysaccharide to the OM by hydrophobic interactions via the fatty acids, laurate and myristate. Lipid A is comprised of an ester linked diglucosamine with ester and amide-linked pyrophosphates and fatty acids. In *S. flexneri* fatty acetylation occurs by four (R)-3-hydroxy fatty acids at positions O-2, O-3, O-2' and O-3 (Steimle et al. 2016). The biosynthesis of the lipid A component of the LPS is via the nine-step Raetz pathway which is highly conserved in most Gram negative bacteria (Figure 1.6). The pathway is

undertaken by the Lpx enzymes, which do not have mammalian counterparts and could be potential novel drug targets (Raetz & Whitfield 2002). Lipid A synthesis begins with uridine diphosphate GlcNAc acetyltransferase (UDP-GlcNAc) named LpxA. LpxA acetylates the sugar nucleotide UDP-GlcNAc via the carrier R-3-hydroxymyristoyl-acyl protein, to form UDP-3-O-(R-3-hydroxymyristoyl)-GlcNAc. Next, LpxC deacetylates the O-acetylated UDP-GlcNAc and LpxD transfers a second acyl group from R-3-hydroxymyristoyl-ACP to O-acetylated UDP-GlcNAc forming UDP-2, 3-diacylglucosamine. UDP-2, 3-diacylglucosamine is the immediate precursor of lipid A. A proportion of UDP-2,3-diacylglucosamine is cleaved at the phosphate bond by LpxH to create diacylglucosamine-1-phosphate named lipid X. LpxB uses UDP-2,3-diacylglucosamine with lipid X to form the disaccharide and releases UDP. Following phosphorylation of this disaccharide generates lipid IV<sub>A</sub>. Next, two Kdo residues are added to form keto-doxy-octonate 2 lipid A. 12-carbon fatty acid laurate and 14-carbon fatty acid myristate are linked to two of the four 3-OH-myristic acids available on keto-doxy-octonate 2 lipid A. LpxL catalyses the acyl-oxyacyl linkage of laurate to the 3' hydroxymyristate that is itself linked to the 2' position of the glucosamine, while LpxM catalyses the acyl-oxyacyl linkage of myristate on the hydroxy-myristate that is itself linked to the 3' position of the glucosamine disaccharide (Raetz et al. 2007).

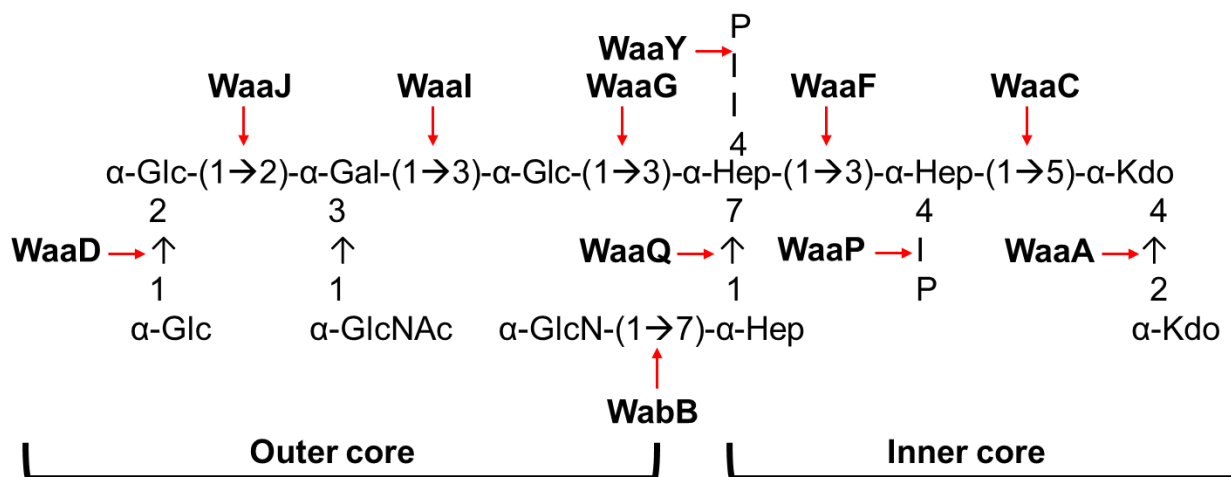


**Figure 1.6: Biosynthesis of the Kdo<sub>2</sub> – the Raetz pathway.**

The Raetz pathway consists of nine enzymes displayed in red. These enzymes catalyse the reactions that lead to the different major structural intermediates labelled in blue. All reactions catalysed by LpxH occur at the cytoplasmic side of the inner membrane. Abbreviations: UDP-GlcNAc, uridine diphosphate N-acetylglucosamine; CMP, cytidine monophosphate; UMP, uridine monophosphate. Figure from Whitfield and Trent (2014)

### 1.5.2 Core biosynthesis

There are 5 core types in *E. coli/Shigella* R1-R4 and K-12 (Knirel et al. 2011). *S. flexneri* 2a has an R3 core type generated by the proteins encoded by the chromosomal *waa* locus (Kaniuk et al. 2004) (Figure 1.7). WaaA transfers the first two KDO residues to the core, WaaC and WaaF are heptosyltransferases. WaaP transfers phosphate to heptose I and WaaY to heptose II. WaaQ transfers heptose III to heptose II. WaaG is a UDP-glucosyl transferase, which attaches a glucose (Glc) to heptose II, WaaI attaches a galactose (Gal) unit to the first Glc and then WaaJ attaches a second Glc to Gal. WaaD is a  $\alpha$ -1, 2-glucosyltransferase which adds terminal glucose molecules to the side branch (Heinrichs et al. 1998; Kaniuk et al. 2004).

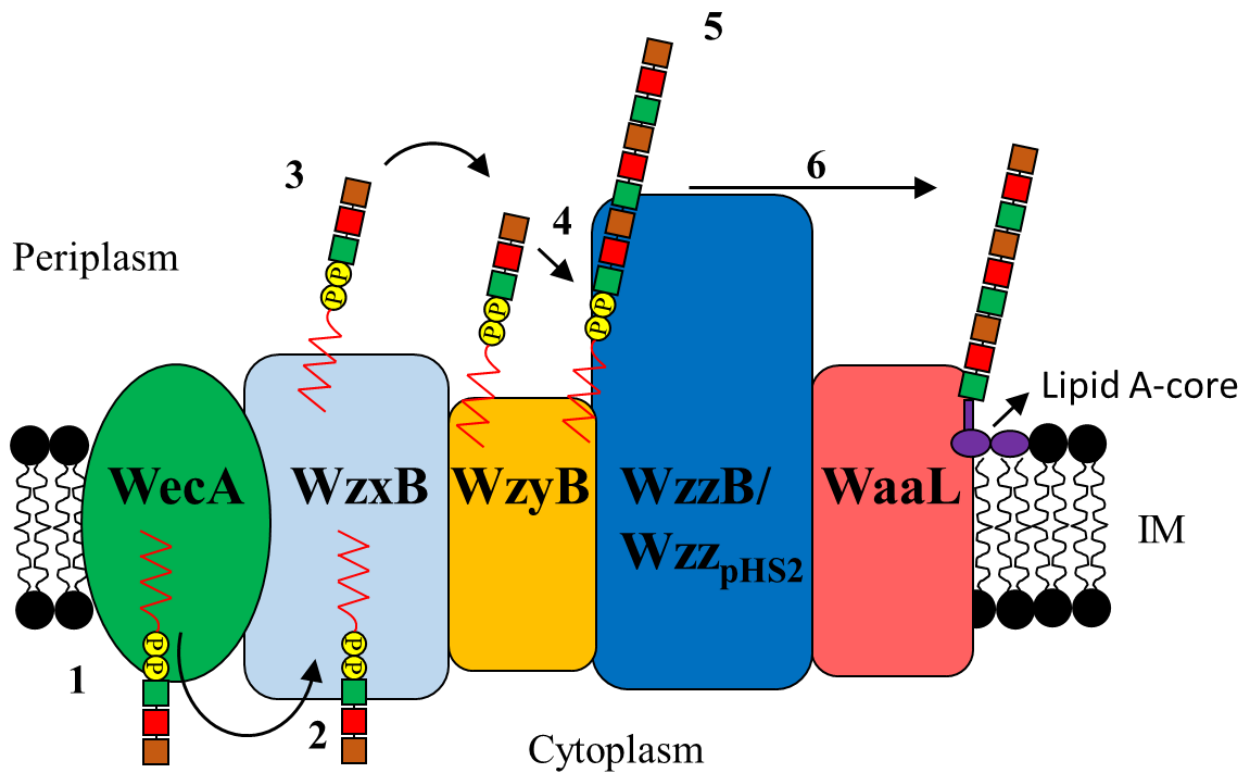


**Figure 1.7: Synthesis of R3 type LPS core**

Summary of the reactions and enzymes responsible for the synthesis of R3 core type LPS in *S. flexneri*. Red arrows indicate which step the named enzymes catalyse the reaction.

### 1.5.3 O antigen biosynthesis in *S. flexneri*

The Wzy-dependent polysaccharide synthesis mechanism is the most commonly used system in bacteria to produce Oag which takes place in the inner membrane, and requires a set of integral membrane proteins (Figure 1.8). In *S. flexneri* O antigen biosynthesis begins with GlcNAc-phosphate (GlcNAc-1-P) being transferred from UDP-GlcNAc to undecaprenol phosphate (Und-P) at the cytoplasmic of the inner membrane by the enzyme WecA. Rhamnosyl transferases RfbG and RfbF add Rha residues from the carrier dTDP-Rha to the GlcNAc forming the O unit (Macpherson et al. 1994; Morona et al. 1994). The Oag chain is synthesised by the Oag polymerase, WzyB. The individual RUs bound to the lipid carrier UndPP are transported across the inner membrane from the cytoplasm into the periplasm by the Wzx flippase. Here, the UndPP bound RU are polymerised in a block wise manner by WzyB, whereby the transfer of the growing chain from its lipid carrier, is to the non-reducing end of an incoming Wzx transported repeat unit. The Oag chain length is regulated by polysaccharide co-polymerase (PCP) proteins which form oligomeric “bell” structures; in *S. flexneri* they are WzzB and Wzz<sub>pHS2</sub>. Finally, the WaaL protein ligates the lipid linked polymerised Oag chain to the previously synthesised lipid A + core sugar molecules (Figure 1.8).



**Figure 1.8: Wzy-dependent polymerisation of O antigen.**

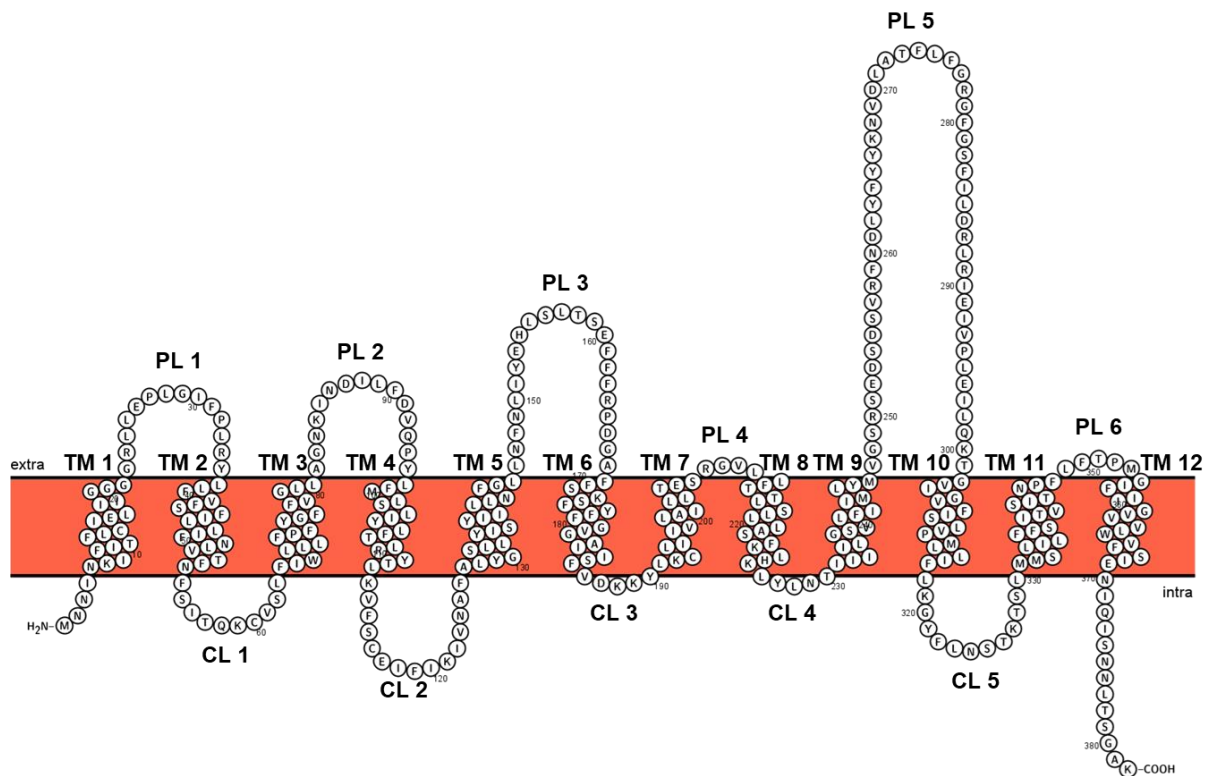
1) The Oag RUs are synthesised on the lipid carrier undecaprenyl pyrophosphate (UndPP) by a series of glycosyltransferases including WecA. 2) The UndPP-linked Oag subunit is flipped by the cation-driven WzxB protein from the cytoplasmic to the periplasmic side of the inner membrane (IM). 3) The UndPP-linked Oag subunits are then transferred to the Oag chain polymerase WzyB. 4) Subunits of the UndPP-linked Oag are polymerised into chains to the reducing end of the growing chain. 5) The growing chain length of the Oag is modulated by the WzzB/Wzz<sub>pHS2</sub> protein. 6) The polymerised Oag chain is ligated by WaaL to the Lipid A-core oligosaccharide molecule (which is produced in an independent pathway) to complete the full LPS molecule.

## 1.6 Proteins of the WzyB dependent pathway

### 1.6.1 WzyB Oag polymerase

The *S. flexneri* WzyB protein is approximately 43.7 kDa with 12 transmembrane domains, 6 periplasmic loops and 5 cytoplasmic loops (Figure 1.9) (Daniels et al. 1998). Wzy proteins are difficult to overexpress and purify and thus there is no X-ray crystal structures available for any Wzy homologs at present, leading to a reliance upon genetically determined topology maps (Figure 1.9).

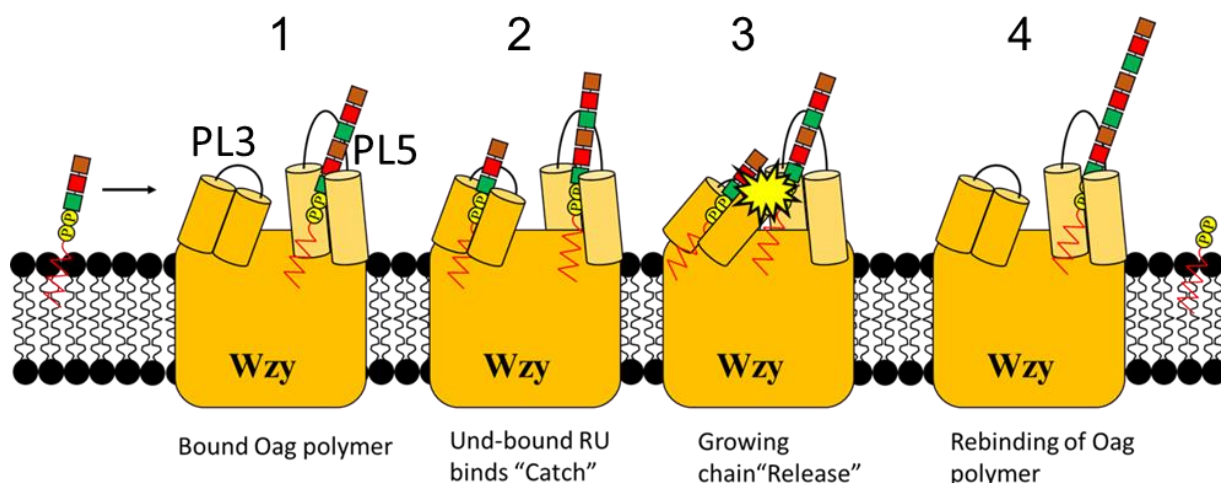




**Figure 1.9: *S. flexneri* WzyB topology map.**

*S. flexneri* WzyB is an integral inner membrane protein responsible for Oag synthesis. The protein is made up of 12 transmembrane domains, and 2 notably large periplasmic loops (PL3 and PL5). Periplasmic loops (PL1-6), transmembrane domains (TM1-12) and cytoplasmic loops (CL1-5) are indicated above. Topology map was drawn using “Protter”. (URL: <https://wlab.ethz.ch/protter/start/>)

WzyB proteins from different Oag producing bacteria have little to no sequence homology, which makes it difficult to identify key regions that are conserved among all WzyB proteins. In *Pseudomonas aeruginosa* serotype O5, the Wzy protein harbours two amino acid motifs (named RX<sub>10</sub>) in the periplasmic loops 2 and 5, where the arginine residues were found to be important for polymerase activity (Islam et al. 2010). The proposed “catch and release” model suggests that the Und-PP-RU binds a site on the third periplasmic loop and is then transferred to a second site on the fifth periplasmic loop, mediated by the loop differences in pI, basic vs acidic, respectively (Figure 1.10). This specific motif is commonly seen in WzyB proteins from non-Enterobacterial species (Islam et al. 2013). Conversely in *S. flexneri*, the WzyB protein contains a slightly different motif (RX<sub>15</sub>) in which the arginine residues were shown to affect activity, however the pI of the loops appeared to play less of a role as both were shown to be similar and basic (Nath et al. 2015).

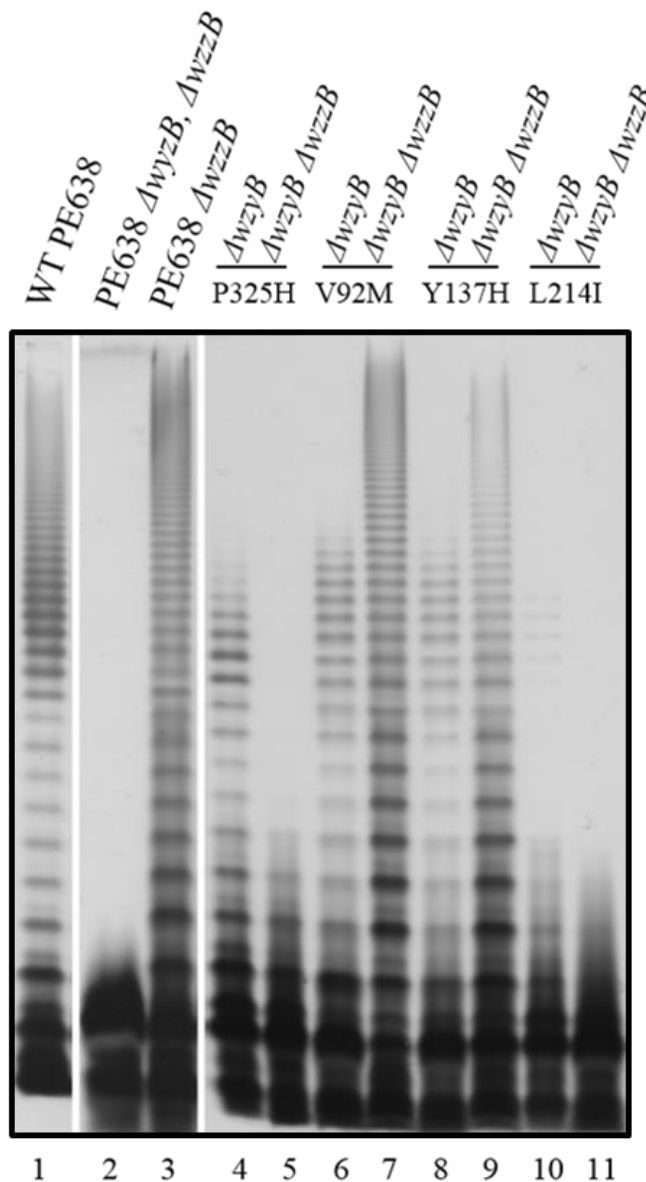


**Figure 1.10: Catch and release model from *P. aeruginosa* Wzy**

1) The Und-linked Oag repeat unit is bound to negatively charged PL5 mediated by its  $RX_{10}G$  motif. 2) “Catching” of a new Und-linked Oag to the positive charged PL3 via the  $RX_{10}G$  motif. 3) Transfer of the Oag repeat to the retention arm of PL 5 and polymerisation at the reducing terminus of the growing Oag chain, this results in the release of the growing chain to accommodate chain elongation. 4) Rebinding of the polymerised chain at the newly add Oag RU via the  $RX_{10}G$  motif.

### 1.6.2 *S. flexneri* *wzyB* mutants

Nath et al. (2015) generated a number of WzyB mutants via random mutagenesis. The mutagenesis experiments produced a pool of plasmids that were screened in a *wzyB/wzzB* double mutant background for phenotypic alterations, and revealed a number of key amino acids that appeared to be critical for WzyB activity and stability. While some mutations were found to inactivate the activity of WzyB, other mutations resulted in WzyB activity that was dependent on the presence of WzzB. These mutants were separated into two distinct categories; 1) mutants that were only active in the presence of WzzB (V92M, Y137H), and 2) mutants that were only active in the absence of WzzB (P352H and L214I) (Figure 1.11). Interestingly, the WzyB production levels of these different mutants were affected by the presence/absence of WzzB, suggesting that WzzB may be impacting the stability of WzyB (Nath et al. 2015). The LPS profiles seen in Figure 1.11 along with protein production data (Nath et al. 2015) suggests that WzzB interacts with WzyB not only to modulate Oag modal length but also WzyB polymerisation activity, WzyB cellular production level and/or its stability.



**Figure 1.11: Analysis of WzzB dependent WzyB mutants by SDS-PAGE and silver staining.**

Silver-stained PAGE separation of the LPS from *S. flexneri* Y serotype (PE638) and mutant strains. Mutant WzyB proteins were encoded on plasmids and tested in both a *wzyB* single mutant and *wzyB/wzzB* double mutant as indicated. The position of the amino acid substitution of each WzyB mutant is indicated. Figure from Nath et al. (2015).

### 1.6.3 WzzB Oag co-polymerase

In *S. flexneri* WzzB confers the short type Oag ~11-17 RU (S-Oag) (Figure 1.4) modal length and is a 39 kDa inner membrane protein that is anchored by two transmembrane domains which are located at the N and C terminus of the protein, however, the majority of the protein resides in the periplasmic space. WzzB is a member of the polysaccharide co-polymerase group 1 (PCP1) family of proteins (Morona et al. 2009). Despite low sequence identity among members the PCP1 family, the monomeric structures of the proteins are remarkably similar (Figure 1.12). Structural studies on PCP1 proteins show that each form a distinct “bell” shaped structure with

different numbers of monomers. The number of monomers that make up each protein is controversial as formation of both hexamers (Larue et al. 2009) and octamers (Kalynych et al. 2015) have been reported. Additionally, *in situ* WzzB has been shown to exist in multiple oligomeric forms at equilibrium with the monomeric form (Papadopoulos & Morona 2010; Tran & Morona 2013)



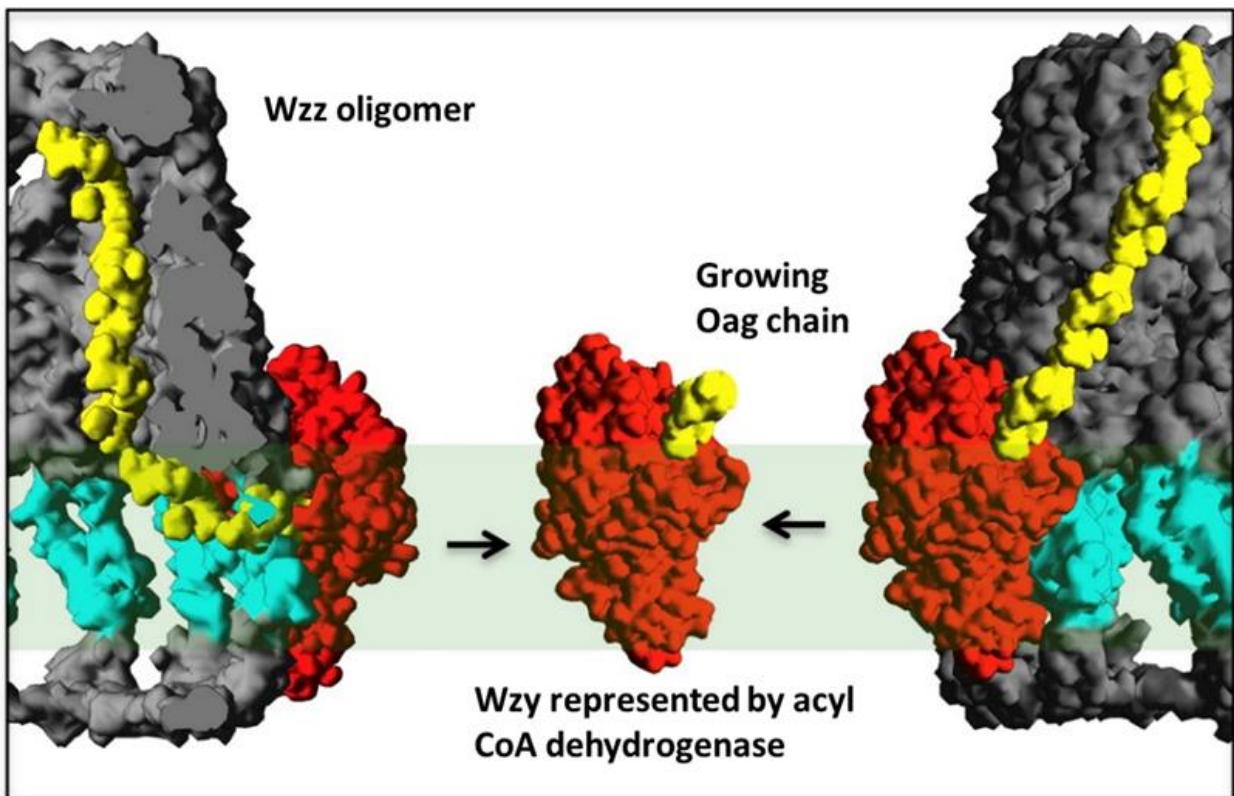
---

**Figure 1.12: comparison of the members of the PCP1 protein family.**

3-D structures of periplasmic WzzB, WzzE, and FepE from *E. coli*. Figure adapted from Wiseman et al. (2021)

---

The full length oligomeric structure of WzzB<sup>ST</sup> from *Salmonella enterica* serovar Typhimurium was resolved using cryoelectron microscopy experiments (Collins et al. 2017). These experiments revealed a potential binding pocket for the Wzy protein, and proposed a new mechanism of action based on Wzy/Wzz synergy. The growing Oag chain (Figure 1.13, in yellow) associates with the external surface of the Wzz oligomer (Figure 1.13, right) or is polymerised in the barrel formed within Wzz oligomer (Figure 1.13, left). Finally the Oag chain extension stops once the polysaccharide binding capacity is reached and Wzy disassociates from the complex (Collins et al. 2017).

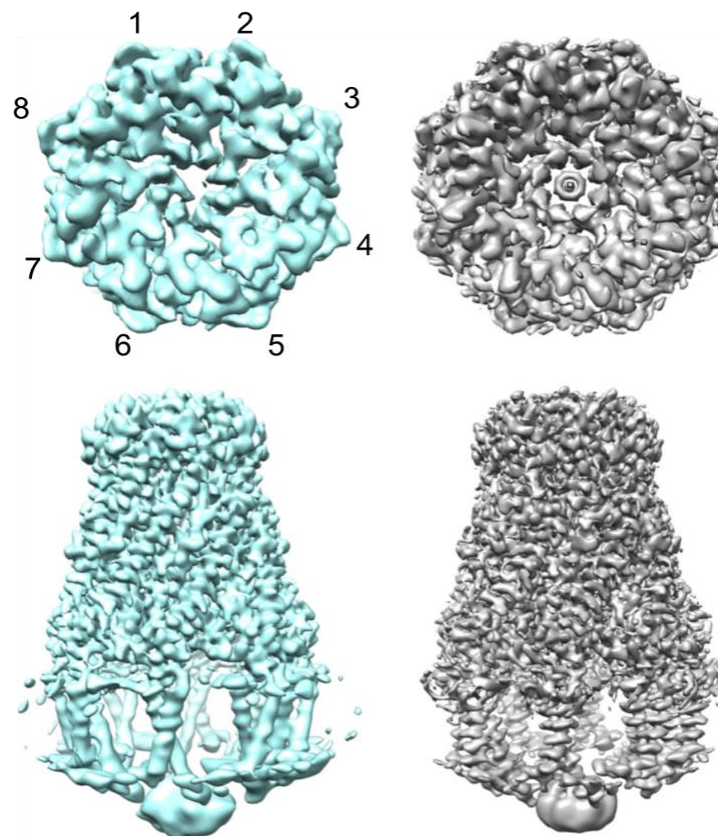


**Figure 1.13: Model of the proposed Oag model length determination mechanism.**

Wzy represented by the structure of acyl-CoA dehydrogenase (red structure) interacting with Wzz (grey and cyan) during the polymerization of the Oag (yellow). The model in which the growing Oag chain is polymerised into the barrel structure produced by Wzz is shown on the left. The model in which the growing O antigen chain is polymerised onto the external surface of the Wzz protein structure is shown on the right. Figure adapted from Collins et al. (2017)

More, recently the full length oligomeric structure of WzzB from *E.coli* was solved using cryoelectrom microscopy experiments (Figure 1.14) (Wiseman et al. 2021). Uniquely, this study provided high resolution of the transmembrane regions of WzzB, which provided insight into the specific roles of each of the transmembrane (TM) regions. The study hypothesised that TM 1 was likely involved with homo-ologomeric interactions, and that TM 2 was involved with an interaction with WzyB.






---

**Figure 1.14: Structure of *E. coli* WzzB**

Full-length WzzB solved via cryo electron microscopy. Individual protomers are numbered. Figure adapted from Wiseman et al. (2021)

---

#### 1.6.4 Wzz<sub>pHS2</sub> Oag co-polymerase

The plasmid encoded *wzz<sub>pHS2</sub>* gene in *S. flexneri* is responsible for conferring the very long Oag modal length (VL-Oag) (>80 RU) (Figure 1.4) (Stevenson et al. 1995). This Wzz<sub>pHS2</sub> is an inner membrane protein of 41 kDa that is another member of the PCP1 family. Wzz<sub>pHS2</sub> is closely related to the *E. coli* protein FepE, and shares approximately 62% amino acid identity and a very similar function of regulating the VL-Oag chains of their respective species. The basic topology of Wzz<sub>pHS2</sub> comprises of two cytoplasmic domains, two transmembrane domains with a large periplasmic domain. *in silico* analysis of the sequence revealed three coiled-coil regions (CCR) and mutagenesis studies showed that CCR 2 and 3 were necessary for wild-type Oag chain regulation, and that the protein forms homo-oligomers like the other members of the PCP-1 family (Purins et al. 2008). More recent studies performed on FepE in *E. coli* revealed that mutation of aa L168 and D268 resulted in a shortened chain lengths of less than 80 Oag RU (Tran & Morona 2013). These mutations appeared to be present in the inner cavity of the bell forming structure, thus providing insight into the Oag regulation.

### 1.6.5 The Wzy/Wzz complex

The Oag biosynthesis pathway described above is believed to function as a large protein complex. Studies in *E. coli* K-12 revealed genetic evidence of protein interactions, via cross-complementing a *wzx* mutation with *wzx* genes from other *E. coli* Oag types; only related proteins in the system successfully complemented the mutation (e.g. *wzy/wzx/wzz*) (Marolda et al. 2006). In 2010, the *E. coli* O86 oligosaccharide biosynthetic pathway was reconstituted *in vitro* (Woodward et al. 2010). This involved the expression and purification of the WzyB<sup>O86</sup> polymerase protein via a chaperone co-expression system which allowed for the demonstration of polymerization of O86 oligosaccharide *in vitro*. Similarly, specific polysaccharide chain length modality was also demonstrated and resulted from the action of the co-polymerase Wzz<sup>O86</sup> (Han et al. 2010). Additionally, studies on the Wzy/Wzz proteins in *Rhizobium leguminosarum* detected physical interactions via a bacterial two-hybrid system (Marczak et al. 2013). Recently Nath and Morona (2015) produced the first direct evidence of a biochemical interaction between WzzB and WzyB through the use of *in vivo* chemical crosslinking using dithiobis-succinimidylpropionate (DSP) (Nath & Morona 2015). This recent breakthrough paves the way for further understanding of the Wzy/Wzz system. Despite these advances in characterising the interaction of Wzz and Wzy there is still no agreed upon model to explain how the Wzz protein controls modal length.

Many proposed models suggest an association between the Oag and the Wzy/Wzz complex may be involved in modal length control (Daniels & Morona 1999; Kalynych et al. 2011; Tocilj et al. 2008). Additionally the “molecular-clock” and “molecular chaperone” models have also been proposed as mechanistic hypotheses (Bastin et al. 1993; Morona et al. 1995). In the molecular-clock model, Wzz acts as a molecular clock and regulates Wzy activity between two states: the extension state (where polymerisation is favoured) and the transfer state (whereby ligation is favoured). The molecular chaperone model describes Wzz as a typical molecular chaperone whereby it controls the ratio of Wzy and WaaL via a complex which controls the enzyme kinetics of the ligation reaction (Morona et al. 1995). More recently the “molecular ruler” model was proposed, whereby, the Wzz periplasmic bell acts as a ruler and the length of the Oag chain is determined by a direct interaction with the outside of the bell. As the size of the bell decreases the number of Oag RU also lowers as the area of interaction between Wzz and Oag is reduced (Kintz & Goldberg 2011). Recent data involving the construction of WzzB chimeras between *S. typhimurium* and *S. flexneri* suggest a model in which the growing Oag chains adapt a higher order structure, thus interfering with the positioning of the Wzy binding site. Additionally the growing chain binds the Wzz, and after achieving a certain length the Oag can no longer bind the Wzz, and dissociates completing the cycle, thus highlighting that the outer surface of the bell structure is

important for chain length control (Kalynych et al. 2012). Conversely, mutagenesis of FepE in *E. coli* suggests that residues inside the oligomeric bell are important for Oag chain length regulation (Tran & Morona 2013). The most recent proposed model is a hybrid model by Islam and Lam (2014) based on the aforementioned Kintz and Goldberg (2011) and Kalynych et al. (2012) models. The proposed hybrid model suggests that Wzy dimers bind to the TMs of the Wzz promoter within the bell shaped structure. Wzy polymerises the Oag until the higher order Oag structure destabilises the interaction of the growing chain to Wzz, thus ending the polymerisation. Furthermore, when the tip of the growing chains reaches the top of the bell structure the mechanical feedback of the interaction transmits through the Oag chain to the basal Oag RU in the Wzy active site, thus disassociating the Oag from Wzy. As there is evidence to suggest the growing chain binds to the outside and inside of the bell there is still no model that has been fully supported; this is due to the technical difficulties in studying the proteins of the Wzy-dependent pathway.

### **1.6.6 Wzx flippase**

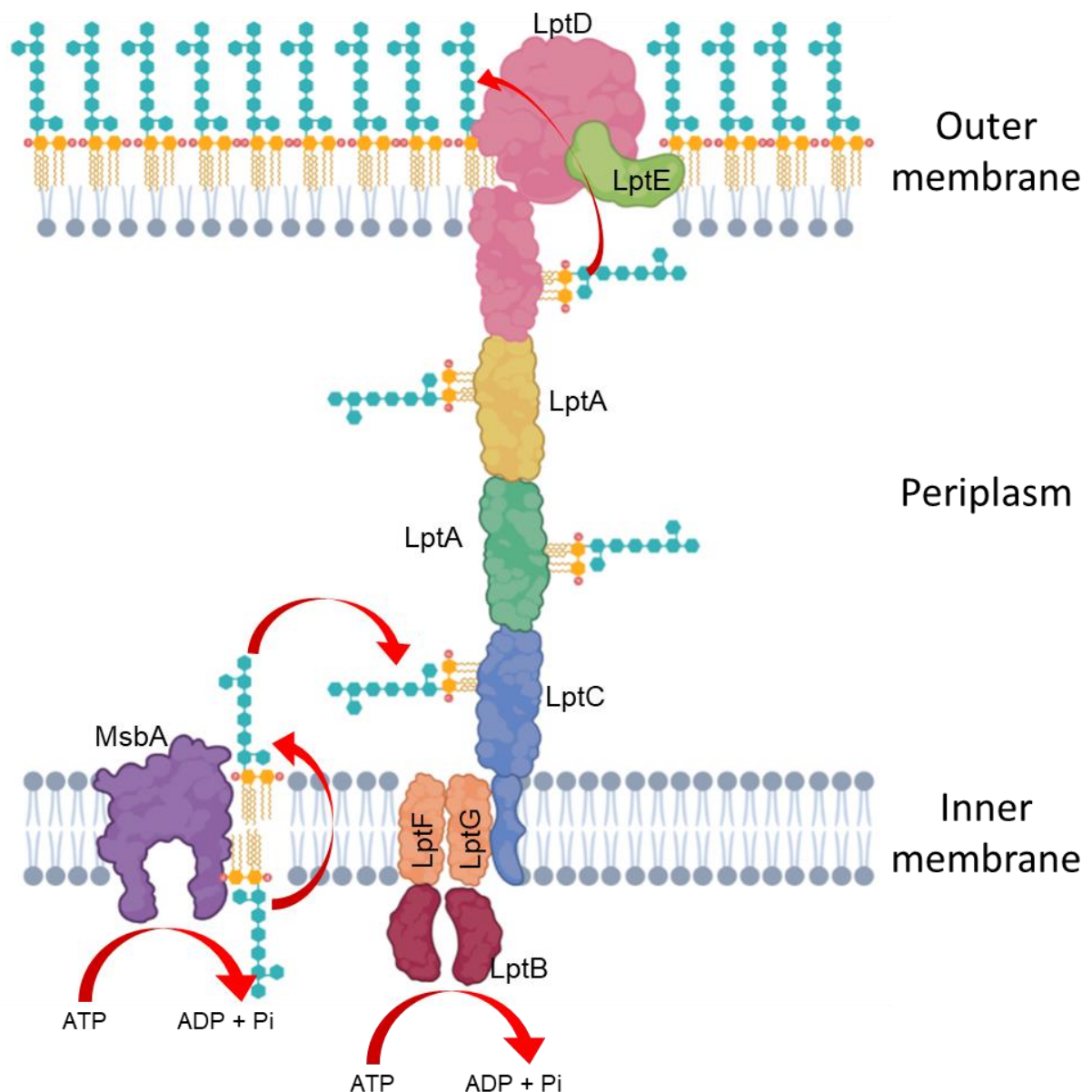
Wzx is a member of the multidrug/oligosaccharidyl-lipid/polysaccharide (MOP) flippase family of transporters. This protein translocates Oag across the inner membrane to the periplasm, however the details of substrate recognition are poorly understood. There is evidence that Wzx can export non-native substrates when overexpressed. This suggests that Wzx may be able to translocate different substrates depending on substrate availability and concentration (Liu et al. 2019). There is no structural data available for Wzx however structural and mechanistic insights are provided by the related MurJ flippase that exports Und-PP-linked peptidoglycan. A Wzx member from the exopolysaccharide system was shown to complement a MurJ mutation when overexpressed, suggesting that there are conserved mechanisms in this class of exporters (Sham et al. 2018). Structures of MurJ reveal a two-lobed structure with a cation lumen that can be accessed by a lateral gate. Structural studies showed that the MurJ can be found in different conformations with the substrate binding to the lateral gate and then captured by the lumen in the inward-facing confirmation. The substrate is then proposed to be translocated by a “rocket switch” mechanism which shrinks the lumen forcing the substrate out (Kuk et al. 2019; Kuk et al. 2017; Kumar et al. 2019). As both are members of the same family, it is predicted that both Wzx and MurJ flip substrates via a similar mechanism.

### **1.6.7 LPS export**

The lipid A/core moiety is transported to the periplasmic side of the inner membrane by a transporter named MsbA. Once in the periplasm, Oag is added if present, and the completed LPS is transported across the OM to the cell surface by the Lpt machinery (Whitfield & Trent 2014). However there is currently no experimental data that supports the transport of mature LPS via the



Lpt machinery; experimental data only supports an essential role in transport of R-LPS. The Lpt machinery is made up of seven Lpt proteins; LptB/LptC/LptF/LptG are located at the inner membrane, LptA is present in the periplasm and LptD/LptE are present at the OM. Each protein functions independently as any deletion of these seven proteins results in failure to transport LPS to the cell surface, and an accumulation occurs at the outer leaflet of the inner membrane (Sperandeo et al. 2011). Figure 1.15 provides a model for the LPS transport pathway which starts in the cytoplasm and involves crossing two membranes to reach the cell surface.



**Figure 1.15: Mechanism of LPS transport model.**

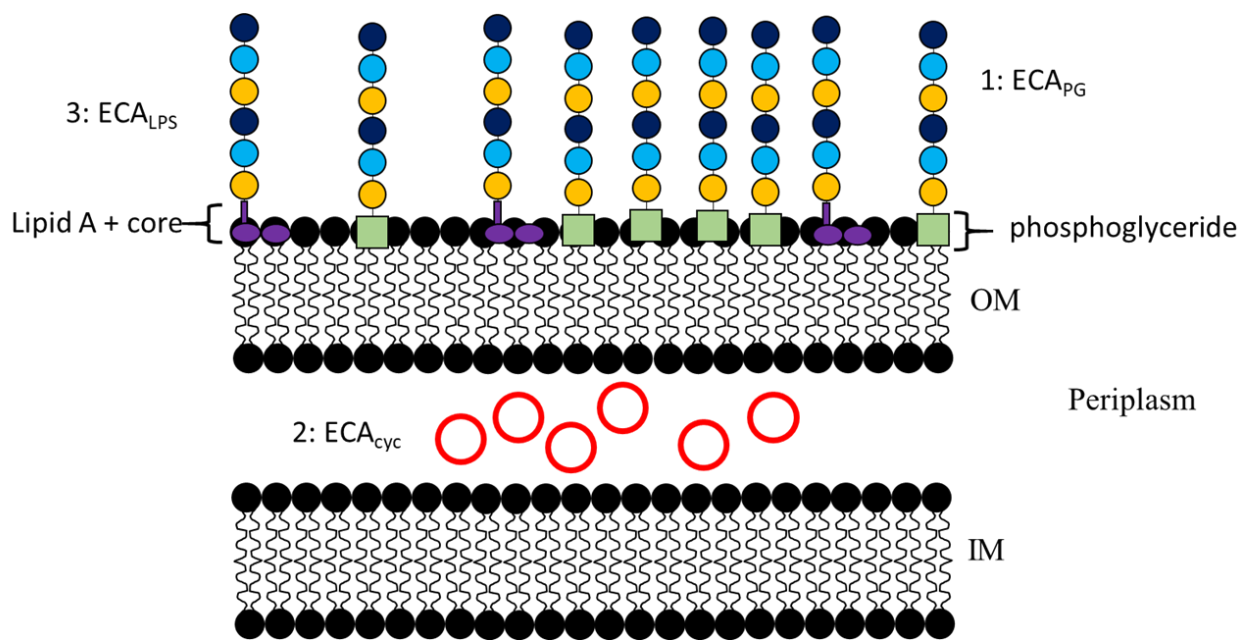
Firstly the Lipid A/core is flipped from the cytoplasmic to the periplasm via an essential ABC transporter named MsbA facilitated via the hydrolysis of an ATP molecule. The LPS is then detached from the inner membrane via another ABC transporter structure comprised of LptB, LptF and LptG that is again mediated by the hydrolysis of ATP. Transport then occurs via a bridge across the periplasm formed by structurally similar LptC, and LptA proteins. The LPS is then inserted into the outer leaflet of the outer membrane via the β-barrel of LptD and LptE. Figure from Putker et al. (2015)

## 1.7 Enterobacterial common antigen

---

Enterobacterial common antigen (ECA) is predominantly a surface polysaccharide antigen that is also polymerised by the Wzy - dependent pathway. It is a polysaccharide expressed by Gram negative bacteria that are from the Enterobacteriaceae family (Gozdziewicz et al. 2014). ECA has been studied extensively in *E. coli* implicating the polysaccharide as being important for cell wall integrity, flagellum expression, and resistance to harmful compounds (bile salts) found in the environment. The ECA chain contains, N-acetyl-D-glucosamine (GlcNAc), N-acetyl-d-mannosaminuronic acid (ManNAcA), and 4-acetamido-4, 6-dideoxy-d-galactose (Fuc4NAc) and these sugars are linked together to form a linear trisaccharide RU (Lugowski et al. 1983; Mannel & Mayer 1978). In *E. coli* K-12 the ECA trisaccharide RUs are comprised of a modal population of polymers ranging in chain length from 1 to 14 RUs, with a modal value of 6 to 7 RUs (Barr et al. 1999a). The ECA polysaccharide has been observed in three distinct forms, 1) phosphoglyceride-linked ECA (ECA<sub>PG</sub>) the most common form of ECA which has been estimated to account for approximately 0.2% of the cellular dry weight of *E. coli* K-12 (Mannel & Mayer 1978), 2) Cyclic ECA (ECA<sub>CYC</sub>), ECA polysaccharides that lack the phosphoglyceride of ECA<sub>PG</sub> and reside in the periplasm and, 3) LPS-linked ECA (ECA<sub>LPS</sub>), the only immunogenic form of ECA where ECA RUs are covalently linked to the core component of LPS molecules (Figure 1.16).

WzyE, the ECA polymerase, is an inner membrane protein involved in the polymerisation of ECA polysaccharide chains, however unlike WzyB involvement in Oag synthesis, little is known about WzyE involvement in ECA synthesis. Like WzzB, the ECA biosynthetic pathway involves a co-polymerase, known as WzzE, to modulate the modal length of the ECA polysaccharide chains, and has been shown to be required for the production of the cyclic form of ECA (ECA<sub>cyc</sub>) (Kajimura et al. 2005), which has been shown to be important to maintain the outer membrane permeability (Mitchell et al. 2018). Recent studies suggest that a *wzzE* knockout mutant results in lowered flagellum expression and the induction of the Rcs (regulator of capsule synthesis) stress pathway (Castelli & Vescovi 2011). Despite low sequence identity between WzzB and WzzE, the 3D structures are near identical, suggesting a similar protein activity. To date, although both the ECA and the Oag polysaccharides rely on Wzy-dependent systems, it has always been assumed that these systems function alongside one another but remain independent to each other. Similarities in these proteins structures however suggest that it may be possible for the systems to act on one another in certain situations.



**Figure 1.16: Three distinct forms of ECA**

Graphical representation of the three forms of ECA. 1) Phosphoglyceride-linked ECA ( $ECA_{PG}$ ). 2) Cyclic ECA ( $ECA_{cyc}$ ). 3) LPS-Linked ECA ( $ECA_{LPS}$ ).

## 1.8 *Shigella* vaccines

The polysaccharide Oag is the major surface antigen of *Shigella* and as a result has been the obvious target of many vaccine studies. Since Oag is a polysaccharide it is a T-independent antigen which results in lower induction of an antibody response, immunological memory and affinity maturation in infants (Gonzalez-Fernandez et al. 2008). In order to overcome this problem Oag can be conjugated to a protein carrier to become more immunogenic, and a number of vaccine candidates have been developed.

One vaccine example used the Oag non-covalently linked to *Shigella* ribosomal particle carriers (Levenson et al. 1988). This candidate showed efficacy in guinea pigs, mice and monkeys against *S. flexneri* 2a (Shim et al. 2007). However standardisation and characterisation issues limited the success of this approach.

Another example of conjugate vaccines against *Shigella* has been prepared using the recombinant *Pseudomonas aeruginosa* exoprotein A and the *Corynebacterium diphtheriae* toxin to successfully deliver the *S. flexneri* and *S. sonnei* Oag, respectively, both of which has reached Phase III clinical trials (Passwell et al. 2010; Passwell et al. 2003; Passwell et al. 2001). However due to the regions (Israel) tested being considered endemic the results obtained could be attributed to boosting the response to previously exposed volunteers. Therefore, it is unclear if the vaccine is effective in naïve volunteers or children under the age of three, whom are the most at risk and

affected age group for *Shigella* infection. Additionally the vaccines faced problems with standardisation issues and the use of parenteral routes requiring multiple doses meant that the vaccines were limited in mass administration.

Invaplex is the name of a conjugate vaccine in which LPS, IpaB and IpaC are complexed using extracts of virulent *Shigella* species. The first generation of the vaccine was able to stimulate a protective response in mice and guinea pigs, however required multiple doses via the intranasal route (Oaks & Turbyfill 2006). The second generation of this particular vaccine resulted in improved immunogenicity and protection, and is in clinical trials.

The outer membrane vesicle (OMV) vaccine strategy has been quite successful against serotype B meningococcal disease (Lewis et al. 2009) as well as being a promising vaccine candidate against other pathogens such as: *Pseudomonas*, *Vibrio*, *Salmonella* and *Acinetobacter baumannii* (Alaniz et al. 2007; Ellis et al. 2010; McConnell et al. 2011; Schild et al. 2009). A vaccine candidate generated from the OMV of *S. flexneri* 2a, which contained LPS and Ipa proteins has been tested to be successful in mice (Camacho et al. 2011).

More recently, a *S. flexneri* 2a  $\Delta wzy$  strain has been used as a whole cell vaccine. The lack of the Oag polymerases results in the *S. flexneri* only being able produce one LPS with Oag RU, thus exposing and broadening the immune response to surface proteins. This vaccine candidate induced a potent antibody response to the outer membrane protein PSSP-1 and the type III secretion system proteins IpaB and IpaC in mice (Kim et al. 2018). Additionally, it showed cross-protective immunity against *S. flexneri* 3a, *S. flexneri* 6, and *Shigella sonnei* in a mouse pneumonia model. Thus this vaccine is a promising candidate for a universal vaccine, however, as a whole cell vaccine, future safety studies will evaluate this vaccine candidate success in the future.

## **1.9 Research Hypothesis and Aims**

---

The ability of bacteria to synthesise complex polysaccharide chains at a controlled number of repeating units has wide implications for a range of biological activities that include: symbiosis, biofilm formation and immune system avoidance. Complex polysaccharide chains such as the Oag component of lipopolysaccharide and ECA are synthesised by the most common polysaccharide synthesis pathway used in bacteria, known as the Wzy-dependent pathway. As such, complete understanding of the Wzy-dependent pathway is critical to the future success of the development of novel antimicrobial strategies. Recent studies show an interaction between Wzy and Wzz and, recent structural data of WzzB has predicted binding pockets for the interaction of WzyB to most likely be present in the transmembrane region of Wzz. These aspects will be investigated in this thesis via mutagenesis. Wzy also most likely interacts with other members of the PCP family such

as WzzE and Wzz<sub>pHS2</sub>, as such these will also be investigated. Due to unpublished data suggesting WzyB may interact with other proteins previously un-linked to the Wzy-dependent pathway, co-purification experiments will be performed to reveal novel WzyB binding partners. Finally, due to the complexity of LPS, genes with no prior link to LPS generated by a previous preliminary screen of the Keio collection will be further investigated.

**Aims:**

1. To investigate WzzB and WzzE via the generation of chimera proteins and investigate the impact on interaction with WzyB
2. To perform random mutagenesis on Wzy and identify regions that affect Oag polymerisation in a *wzzB* negative background
3. To investigate the interaction between WzyB and Wzz<sub>pHS2</sub>.
4. To identify novel WzyB binding partners using mass spectrometry.
5. To identify novel genes that affect LPS synthesis, transport or length regulation.

# Chapter Two

---

## MATERIALS AND METHODS

---

## Chapter 2: Materials and Methods

---

### 2.1 Bacterial strains and growth conditions

---

#### 2.1.1 Strains and plasmids

The bacterial strains used during this study are listed in **Appendix A**. Plasmids are listed in **Appendix B**

#### 2.1.2 Growth media and conditions

Bacterial strains were routinely grown on lysogeny broth (LB) agar (10 g tryptone L<sup>-1</sup>, 5 g yeast extract L<sup>-1</sup>, 5 g NaCl L<sup>-1</sup>, 15 g agar L<sup>-1</sup>) or in LB containing the appropriate antibiotics.

#### 2.1.3 Antibiotics and additives

For selection, the following antibiotics were used at the following concentrations; 100 µg ampicillin (Ap) ml<sup>-1</sup>, 25 µg chloramphenicol (Cml) ml<sup>-1</sup>, 25 µg Rifampicin (Rif) ml<sup>-1</sup>, 50 µg kanamycin (Kan) ml<sup>-1</sup> and 10 µg tetracycline (Tet) ml<sup>-1</sup>. Strains carrying pQE30 or pBCKS<sup>(+)</sup> constructs requiring induction were grown in LB at 37 °C with aeration for 16 h, subcultured 1/20 into fresh broth with 0.01 mM IPTG and grown for another 4 h. Strains carrying pBAD33 constructs requiring induction were grown in LB at 37 °C with aeration for 16 h, subcultured 1/20 into fresh broth with 0.2 % (w/v) L-arabinose and grown for another 4 h. For blue white colony selection, LBA plates were supplemented with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) 20 mg mL<sup>-1</sup>

#### 2.1.4 Growth and maintenance of bacterial strains

All strains were grown at 37°C in liquid cultures using 20 mL McCartney bottles with aeration. Bacterial strains were stored at -80°C in glycerol medium. These bacterial strains were streaked out onto LBA from single colonies before storage. Cultures were prepared from glycerol stocks by streaking out a small quantity onto selective agar and incubating at 37°C overnight. A single colony was then used to inoculate a liquid broth as required.

### 2.2 Antibodies

---

The antibodies used in this study for Western immunoblotting are as follows: polyclonal rabbit anti-WzzB (Daniels & Morona 1999), polyclonal rabbit anti-WzzE (Morona Lab), polyclonal rabbit anti-ECA (Morona Lab), polyclonal rabbit anti-Wzz<sub>PHS2</sub> (Purins et al. 2008), mouse monoclonal anti-FLAG (Sigma), monoclonal anti-His (GeneScript) HRP-conjugated goat anti-mouse IgG (KPL) and HRP-conjugated goat anti-Rabbit IgG (KPL).

## **2.3 DNA techniques**

---

### **2.3.1 Plasmid isolation**

Plasmid DNA was purified from overnight bacteria cultures (LB 10 mL) according to the QIAprep Spin Miniprep kit (Qiagen) protocol. MilliQ (MQ) H<sub>2</sub>O (18Ωcm) was used for elution and final preparations were stored at -20°C.

### **2.3.2 Whole chromosome DNA purification**

Chromosomal DNA was purified from overnight bacterial cultures (LB 10 mL) according to the Wizard Genomic DNA Purification Kit (Promega). MQ H<sub>2</sub>O was used for elution and final preparations were stored at -20°C.

### **2.3.3 DNA quantification**

The NanoDrop 2000c spectrophotometer (Thermo scientific) was used for quantification of all Plasmid DNA samples using 1 μL at an absorbance of 260nm.

### **2.3.4 Restriction digests**

All restriction digests were performed using New England Biolabs (NEB) enzymes following the supplied protocols. Where possible the enzymes were heat inactivated after use.

### **2.3.5 Oligonucleotides**

The oligonucleotides used for this study were purchased from Integrated DNA Technologies (IDT) (Singapore) and are listed in **Appendix C**. Oligonucleotides were resuspended in MQ H<sub>2</sub>O at a concentration of 100 μM for storage at -20°C. Oligonucleotides was diluted to a working stock of 10 μM for use in polymerase chain reaction (PCR).

### **2.3.6 PCR**

PCR reactions were conducted according to the supplied protocols using either Taq DNA polymerase or Q5 DNA polymerase (NEB) using the Eppendorf Mastercycler Gradient PCR thermocycler with 25-35 amplification cycles adjusted according to the DNA being amplified. Each standard cycle involved denaturation of the template at 95°C for 30 sec, annealing of primers to the DNA at a temperature ranging from 55-70°C for 30 sec, and extension at 72°C for 1 min/kb of DNA. Deoxynucleic triphosphates (dNTPs) (Sigma) were used at a final concentration of 200 μM.



### **2.3.7 Preparation of “boiled lysate” for PCR**

Single colonies of bacteria were resuspended in 100  $\mu\text{L}$  of MQ water, followed by incubation at 100°C for 5 min. Lysates were then centrifuged (16,250 $\times$ g, 1 min) before the supernatant was used in PCR reactions as the DNA template.

### **2.3.8 PCR DNA purification**

PCR DNA was purified using PCR DNA and Gel Band Purification Kit (GE Healthcare) according to the supplied protocols. DNA was eluted with 20  $\mu\text{L}$  of MQ H<sub>2</sub>O and stored at -20 °C.

### **2.3.9 DNA gel extraction**

DNA fragments from gel electrophoresis were purified using the PCR DNA and Gel Band Purification Kit (GE Healthcare). DNA was eluted with 20  $\mu\text{L}$  of MQ H<sub>2</sub>O and stored at -20 °C.

### **2.3.10 *In vitro* oligonucleotide annealing**

1 nmol of each of the oligonucleotides to be annealed were added to annealing buffer [100 mM potassium acetate, 30 mM HEPES, 2 mM magnesium acetate, pH 7.5] for a total volume of 50  $\mu\text{L}$ . Samples were then placed in an Eppendorf Mastercycler Gradient thermocycler for temperature cycling (98 °C, 5 minutes; 85 °C, 5 minutes; 70 °C, 10 minutes) following the last temperature the samples were left to slowly cool to RT. Samples were then further cooled with 5 minute cycles at 15 °C, 10 °C, and 4 °C. Annealed fragments were then phosphorylated using T4 PNK (NEB) according to the supplied protocol and ligated to the digested plasmids.

### **2.3.11 Inverse PCR for site-directed mutagenesis**

For targeted in-frame deletion of protein coding regions within plasmids, oligonucleotide primers were designed to anneal immediately adjacent the region to be deleted. The entire plasmid was PCR amplified (using Q5 DNA polymerase) with extension proceeding outwards from the region to be deleted, thus excluding it from the amplicon. Each standard cycle involved annealing of primers to the DNA at a temperature ranging from 55-70°C for 30 sec, and extension at 72°C for 1 min/kb of DNA. The amplicon was simply phosphorylated and blunt end ligated.

### **2.3.12 DNA phosphorylation**

Linear DNA fragments were subjected to 5' phosphorylation using T4 polynucleotide kinase (NEB) according to the supplied protocols. Samples were then heat treated to 70 °C for 20 minutes to deactivate T4 polynucleotide kinase.

### 2.3.13 DNA ligation

Ligation of linear DNA fragments was performed using T4 DNA Ligase (NEB) according to the supplied protocols. Samples were incubated at 25 °C for 2 hours or 16 °C over night.

### 2.3.14 Lambda Red mutagenesis

Bacterial gene deletion was performed using  $\lambda$  Red mutagenesis as described by (Datsenko & Wanner 2000). Oligonucleotides were designed to PCR amplify the *frt-kan-frt* cassette from the pKD4 plasmid with 50 bp nucleotides homologous to the adjacent region of the target gene. Using these primers, ten identical PCR reactions were combined and the PCR product was purified and eluted to a total volume of 50  $\mu$ L (Section 2.3.6), the parental plasmid was digested using DpnI. Strains to be mutated were first transformed with pKD46, then were grown at 30 °C in the presence of 0.2% (v/v) arabinose and made electro-competent. The purified PCR product was then electroporated into 100  $\mu$ L of the electro-competent cells. The transformants that grew at 37 °C were then patched onto LB agar containing ampicillin and kanamycin, to confirm the loss of pKD46 and the incorporation of the resistance cassette. The successful replacement of target gene with the kanamycin resistance gene cassette was then confirmed by qualitative PCR. In order to subsequently remove the kanamycin cassette, a plasmid, pCP20, carrying yeast FLP recombinase recognising *frt* sequences was then transformed in the confirmed mutant. Colonies were picked and used to inoculate a 10 mL LB broth which was grown at 30 °C for 16 hr, then incubated at 42 °C for another 3 hr. Cultures were diluted and spread on an LB agar plate and incubated at 37 °C for 16 hr. The loss of pCP20 and the resistance gene cassette was then confirmed by patching colonies onto LB agar with or without kanamycin antibiotics. Mutants were confirmed by PCR (Section 2.3.6) with the appropriate primers and DNA sequencing (Section 2.3.17).

### 2.3.15 Random mutagenesis

Random mutagenesis of a target coding region was performed using the GeneMorph EZ-Clone Domain Mutagenesis kit (catalogue number: 200552; Agilent) according to the manufacturer's instructions with designed primers. 500 ng of purified plasmid was used in the reaction with 30 cycles to yield a "medium" mutation frequency. For the second PCR reaction 500 ng of mega primer was used as the *wzyB* gene is > 1 kb. The mutagenized plasmids were transformed into competent *E. coli* DH5 $\alpha$  cells (if target plasmid possess Cml<sup>R</sup>) or provided competent XL-10 Gold *E. coli*. Plasmid DNA was isolated from random colonies, isolated and transformed into *S. flexneri* for screening.

### **2.3.16 Agarose gel electrophoresis**

PCR amplicons (10  $\mu$ L) were mixed with loading dye (5  $\mu$ L) and separated on 1% (w/v) agarose gels with 1 $\times$  Tris-borate-EDTA (TBE) buffer at 120V for 40 min. *Bacillus subtilis* SPP-1 bacteriophage DNA digested with *EcoRI* was used as a DNA maker (2.5  $\mu$ L) (Morona laboratory). Gels were then visualised and photographed using the GelDoc XR system (BioRad).

### **2.3.17 DNA sequencing**

Oligonucleotide primer was added at a final concentration of 0.8  $\mu$ M to 1000 – 1500ng of purified DNA and adjusted to a final volume of 12  $\mu$ L using MQ H<sub>2</sub>O. The samples were submitted to and sequenced by Australian Genome Research Facility (AGRF).

### **2.3.18 Whole genome DNA sequencing**

Samples for whole genome sequencing were generated at a concentration of 12.5 ng  $\mu$ L<sup>-1</sup>. Samples were sent to BGI TECH SOLUTIONS (Hong Kong) for Bacterial whole-genome resequencing.

## **2.4 Bacterial transformation**

---

### **2.4.1 Preparation of electro-competent cells**

Mid-exponential phase (OD<sub>600</sub> ~0.5) bacteria were grown in 10mL LB broths and harvested via centrifugation (2,200 x g, 10 min, 4 °C), washed 2x with 10 mL of ice-cold MQ H<sub>2</sub>O, before being resuspended in 200  $\mu$ L of 20% (v/v) glycerol.

### **2.4.2 Electro-pulse transformation**

2  $\mu$ L of purified DNA was added to 100  $\mu$ L of electro-competent cells and left on ice for 20 min. The sample was then transferred into an electroporation cuvette (0.2 cm) (BioRad), the cuvette was then transferred into the BioRad Gene Pulser and electroporation performed using the *E. coli* conditions. Following the pulse the sample was added to 900  $\mu$ L of SOC recovery media [20 g L<sup>-1</sup> tryptone (BD), 5 g L<sup>-1</sup> yeast extract (BD), 8.6 mM NaCl, 2.5 mM KCl, 10 mM MgSO<sub>4</sub>, 0.25 (v/v) glucose], cells were then incubated at 37 °C for 1 hr and then plated and grown at 37 °C O/N.

### **2.4.3 Preparation of chemically competent cells**

Mid-exponential phase (OD<sub>600</sub> ~0.5) bacteria were grown in 10 mL LB broths and harvested via centrifugation (2,200 x g, 10 min, 4 °C), washed with 10 mL of ice-cold MQ H<sub>2</sub>O, washed in 5 mL of ice-cold 0.1 mM MgCl<sub>2</sub> and resuspended in 1 mL of ice-cold 100 mM CaCl<sub>2</sub>.

Cells were then incubated for 20 minutes on ice before being harvested via centrifugation (16,000x g, 1 min, 4 °C) and resuspended in 200 µL CaCl<sub>2</sub> plus 20% (v/v) glycerol, then stored at -80 °C.

#### **2.4.4 Ultra-competent bacterial cells**

Bacteria were grown overnight at 37 °C for ~ 8 h to reach stationary growth phase, and then were diluted in 250 mL of SOB medium [20 g/L tryptone [BD], 5 g/L yeast extract [BD] and 0.5 g/L NaCl, 2.5 mM KCl, 10 mM MgCl<sub>2</sub>, pH 7.0] in different ratios; 1:10 or 1:20. Cultures were then incubated at 18 °C for 16 h with vigorous shaking (250-300 rpm). The bacterial culture that has the OD<sub>600</sub> reading closest to but under 0.55 were then continued to grow to OD<sub>600</sub> reading of 0.55. Bacterial cells were then collected by centrifugation (4,500 ×g, 4 °C, 10 min), and washed with 80 mL of precooled transformation buffer [55 mM MnCl<sub>2</sub>, 15 mM CaCl<sub>2</sub>, 250 mM KCl, 10 mM PIPES, pH 6.7], followed by centrifugation as above. The bacterial pellet was then resuspended in 20 mL of ice cold transformation buffer, and 1.5 mL of DMSO was added and 200 µL aliquots were stored at -80 °C (Inoue et al. 1990). These competent cells could be used with heat-shock transformation.

#### **2.4.5 Heat-shock transformation**

Chemically competent cells were thawed on ice before 2 µL plasmid DNA or ligation mixture was added and the mixture was incubated on ice for 20 minutes. Bacteria was then heat-shocked at 42 °C for 90 sec, then immediately transferred to ice for 5 minutes, before being added to 900 µL of SOC recovery media, cells were then incubated at 37 °C for 1 hr and then plated and grown at 37 °C O/N.

### **2.5 LPS techniques**

---

#### **2.5.1 Preparation of LPS samples**

LPS samples were prepared as described by Hitchcock and Brown (1983). In short, following 4 hrs subculture a volume of culture was standardised to an OD<sub>600</sub> of 2.00 (1×10<sup>9</sup> cells/mL) and centrifuged (16,250×g, 1 min) then resuspended in 50 µL of lysis buffer. After a 10 min incubation at 100°C, 10 µL of 2.5 mg/mL proteinase K solution was added and samples were incubated at 56°C for 16 hrs. LPS samples were then stored at -20°C.

#### **2.5.2 Analysis of LPS by silver-stained SDS-PAGE**

LPS samples were heated at 100°C for 5 min before loading onto SDS 15% (w/v) polyacrylamide gels as described by Macpherson et al. (1991). Gels were then electrophoresed at 12 mA for 13 hrs in LPS running buffer. Silver-staining was performed as described by Tsai and Frasch (1982). In short, gels were incubated for 2 hrs in fixing solution, oxidized for 5 min in

oxidizing solution, washed in MQ water 6×15 min, stained for 10 min in staining solution, and washed in MQ water 5×10 min. Gels were then developed in pre-warmed (42°C) developing solution, and the development reaction was stopped using stopping solution.

## **2.6 Protein techniques**

---

### **2.6.1 Whole cell lysate protein samples**

5 x 10<sup>8</sup> Mid-exponential phase (OD<sub>600</sub> ~0.5) bacteria were grown in 10mL LB broths and harvested via centrifugation (2,200 x g, 10 min, 4 °C). Cells were then resuspended in 100 µL of sample buffer [2 % (w/v) SDS, 10 % (v/v) glycerol, 5 % (v/v) β-mercaptoethanol, 0.02 % (w/v) bromophenol blue, 62.5 mM Tris pH 7]. Samples were heated to 100 °C for 5 minutes before use in SDS-PAGE or stored at -20 °C. If Wzy was the protein of interest then samples were heated for 5 min at 42 °C.

### **2.6.2 Purification of WzyB**

Cells were harvested from 100 ml induced culture by centrifugation (Beckman Coulter Avanti J-26XPI centrifuge; 9600 g, 10 min, 4 °C) and the cell pellet was resuspended in sonication buffer [50 mM Tris/HCl, 150 mM NaCl, pH 7.5] followed by cell lysis via sonication (Branson B15). Cell debris was removed by centrifugation (Thermo Scientific Labofuge 400 R centrifuge; 3500 g, 10 min, 4 °C) and the whole membrane (WM) fraction was collected by ultracentrifugation (Beckman Coulter Optima L-100 XP ultra-centrifuge; 250 000 g, 1 h, 4 °C). WM fraction was then resuspended in 500 µl of MQ water, and 500 µl of 2× solubilisation buffer [100 mM Tris/HCl, 300 mM NaCl, 2% (w/v) n-dodecyl-β-D-maltopyranoside (DDM) (Anatrace)] and was mixed for 16 h, 4 °C. Insolubilized material was removed by ultracentrifugation (Beckman Coulter Optima Max-XP tabletop ultracentrifuge; 160000 g, 1 h, 4 °C) and the solubilized supernatant was incubated with 100 µl of anti-DYKDDDDK G (FLAG) affinity resin (GenScript) pre-equilibrated with equilibration buffer [50 mM Tris/HCl, 150 mM NaCl, 0.02% (w/v) DDM, pH 8] for 1.5 h at room temperature (RT). The loaded FLAG affinity resin was then washed with wash buffer [50 mM Tris/HCl, 150 mM NaCl, 0.02% (w/v) DDM, pH 8] several times (10 ml total). Finally, WzyB-FLAG was eluted in 100 µl of elution buffer [0.1 M glycine, 0.02% (w/v) DDM, pH 3.5] at RT and neutralised with 1 M Tris, pH 9.0.

### **2.6.3 *In vivo* protein crosslinking**

*In vivo* crosslinking with DSP was performed prior to cell lysis via sonication as above. Cells were harvested from 100 ml induced culture by centrifugation (Beckman Coulter Avanti J-26XPI centrifuge; 9600 g, 10 min, 4 °C) and the cell pellet was washed in DSP crosslinking buffer [20 mM sodium phosphate buffer (Na<sub>2</sub>PO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub>), 150 mM NaCl, pH 7.5]. The pellets were

then resuspended in 5 ml DSP crosslinking buffer followed by incubation with 1 mM DSP (Thermo Fischer Scientific) [treated samples (T)] for 30 min at 37 °C. A duplicate sample was also incubated without DSP [untreated samples (UT)]. Excess DSP was quenched with 20 mM Tris/HCl, pH 7.5 and left for 10 min at RT, before resuspension in sonication buffer and purification (as described above).

#### **2.6.4 SDS-PAGE for Western transfer**

Whole cell samples were heated at 100 °C (42 °C for WzyB samples) for 5 min, and separated on SDS 15% polyacrylamide gels in protein running buffer at 200V (Biorad MiniProtean) for 1 hr. As a molecular size maker, BenchMark Prestained molecular weight marker (Invitrogen) was used.

#### **2.6.5 Western transfer**

SDS-PAGE separated protein samples were transferred to a nitrocellulose membranes (NitroBind, Pure nitrocellulose, 0.45 µm, GE Water & Process Technologies) for 1 hr at 250 mA in transfer buffer.

#### **2.6.6 Immunoblotting**

Following transfer, nitrocellulose membranes were blocked for 1 hr in TTBS containing 5% (w/v) skim milk, followed by incubation with primary antibody in TTBS containing 2.5% skim milk for 16 hrs at RT with rocking. The membrane was then washed 3 times in TTBS (10 min), followed by incubation with secondary antibody in TTBS containing 2.5% (w/v) skim milk for 2 hrs at RT with rocking. The membrane was then washed 3 times in TTBS (5 min), then finally 3 times in TBS (5 min). For detection of bound secondary antibody, Chemiluminescent Peroxidase substrate-3 (Sigma) was used as described by the manufacturer's instructions. The fluorescent signals were detected by the BioRad ChemiDoc imaging system.

#### **2.6.7 Analysis of ECA by SDS-PAGE**

ECA samples for SDS-PAGE and Immunoblotting were generated identically to LPS samples 2.5.1. SDS-PAGE, Western transfer and immunoblotting were performed as mentioned above using polyclonal rabbit anti-ECA antibody (Morona Lab).

#### **2.6.8 Coomassie Blue staining**

SDS-PAGE separated proteins were stained by incubating the gel in Coomassie blue stain solution [0.01 % (w/v) Coomassie blue G250, 10 % (v/v) acetic acid, 50 % (v/v) methanol] at room temperature with shaking overnight. Gels were destained with multiple washes of Destain solution [10 % (v/v) acetic acid, 50 % (v/v) methanol].

### **2.6.9 Colloidal Blue staining**

SDS-PAGE separated proteins were stained by incubating the gel in Colloidal Blue stain solution (ThermoFisher Scientific) at room temperature with shaking overnight. Gels were destained with multiple washes of MQ H<sub>2</sub>O.

## **2.7 Mass spectrometry**

---

For WzyB interaction identification, purified samples from method 2.6.2 were separated via SDS-PAGE using Mini-PROTEAN TGX Precast Gels (BioRad) and stained by Colloidal Blue. Following staining, the gel was handled by Adelaide Proteomics Centre. Regions and bands of interest were excised and destained with 100 mM ammonium bicarbonate (NH<sub>4</sub>HCO<sub>3</sub>) in 30% (v/v) acetonitrile (ACN). Samples were then washed with 50 mM NH<sub>4</sub>HCO<sub>3</sub>, reduced with 0.5 μmol DDT in 50 mM NH<sub>4</sub>HCO<sub>3</sub>, and alkylated with 2.75 μmol iodoacetamide in 100 mM NH<sub>4</sub>HCO<sub>3</sub> followed by digestion with 100 ng trypsin (Promega) in 5 mM NH<sub>4</sub>HCO<sub>3</sub> in 10% (v/v) ACN. Resulting peptides were extracted using three washes of 1% (v/v) formic acid (FA) in water, 1% (v/v) FA in 50% (v/v) ACN, and 100% (v/v) ACN, respectively. The volumes of the resulting peptide extracts were reduced by vacuum centrifugation to approximately 1 μl and resuspended with 0.1% (v/v) formic acid in 2% (v/v) ACN to a total volume of 10 μl prior to analysis. The digested samples were then analysed by Orbitrap LC-MS (University of South Australia). Post-acquisition, acquired spectra were subjected to peak detection and deconvolution using Compass Data Analysis for QTOF. Processed MS/MS spectra were then exported to Mascot generic format and submitted to Mascot for identification.

## **2.8 Anti-bacterial sensitivity assays**

---

### **2.8.1 Colicin E2 spot assay**

For spot sensitivity assays, bacteria (~5×10<sup>8</sup> cells) were spread onto selective 25 ml LB agar plates and plates were spotted with 5 μl purified colicin E2 protein (Tran et al. 2014) in MilliQ (MQ) water at the following concentrations (μg ml<sup>-1</sup>): 0.50, 1.0, 2.0, 4.0, 8.0, 12, 16, 20, 24, 28, 32 and 36. Plates were incubated at 37 °C for 16 h and the minimum inhibitory concentration (MIC), defined here as the lowest concentration that generated a clear zone of inhibition, was recorded.

### **2.8.2 Colicin E2 swab assay**

For swab sensitivity assays, purified colicin E2 (Tran et al. 2014) protein in MilliQ (MQ) water at a concentration of 6 μg ml<sup>-1</sup> was swabbed onto antibiotic LB agar plates containing 1mM IPTG with a cotton swab and left to dry for 30 min RT. Individual 1mM IPTG induced bacteria

( $\sim 5 \times 10^8$  cells) were swabbed perpendicular to the colicin E2 streak, and were left to dry for 30 minutes before being incubated for 16 h at 37 °C.

## **2.9 Bioinformatical techniques**

---

### **2.9.1 Alignment of whole genome sequencing to reference sequence**

Bacterial whole-genome resequencing data from 2.3.18 were analysed using the Geneious Prime software. Using a reference sequence of *E. coli* BW25113 (PMID: 25323716) the individual whole genome sequencing data sets were mapped to reference sequence. Following this analysis Find SNP function was performed to identify any changes from the original reference sequence. All SNP were recorded and exported to Excel with % variation frequency, those with 80% or greater were considered a statistical variation.



# Chapter Three

---

## ARTICLE 1:

**Polysaccharide co-polymerase WzzB/WzzE chimeras reveal transmembrane 2 region of WzzB is important for interaction with WzyB**

Vincenzo Leo, Elizabeth Tran and Renato Morona

---

## Chapter 3: Research Article 1: Polysaccharide co-polymerase WzzB/WzzE chimeras reveal transmembrane 2 region of WzzB is important for interaction with WzyB

---

### 3.1 Statement of Authorship

---

Title of Paper	Polysaccharide co-polymerase WzzB/WzzE chimeras reveal transmembrane 2 region of WzzB is important for interaction with WzyB
Status	Published
Citation	Leo V, Tran E, Morona R. 2020. Polysaccharide co-polymerase WzzB/WzzE chimeras reveal transmembrane 2 region of WzzB is important for interaction with WzyB. J Bacteriol doi:10.1128/JB.00598-20.

Author Contributions: By signing the Statement of Authorship, each author certifies that: (i) the candidate's stated contribution to the publication is accurate, (ii) permission is granted for the candidate to include the publication in the thesis; and (iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Author	Vincenzo Leo		
Contribution	Construction of strains and plasmids, conducted all experiments pertaining to all Figures and constructed these figures, data analysis, conception of model, and writing of manuscript.		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	28/04/20

Author	Elizabeth Ngoc Hoa Tran		
Contribution	Supervised development of work and generation of preliminary data. Data analysis, conception of model, and editing and evaluation of the manuscript.		
Signature		Date	28/04/20

Author	Renato Morona		
Contribution	Supervised development of work, provision of laboratory and materials, manuscript evaluation and editing, acting corresponding author.		

Signature		Date	28/04/20
-----------	--	------	----------

## 3.2 Article Abstract

---

The ability of bacteria to synthesise complex polysaccharide chains at a controlled number of repeating units has wide implications for a range of biological activities that include: symbiosis, biofilm formation and immune system avoidance. Complex polysaccharide chains such as the O antigen (Oag) component of lipopolysaccharide and the enterobacterial common antigen (ECA) are synthesised by the most common polysaccharide synthesis pathway used in bacteria, known as the Wzy-dependent pathway. The Oag and ECA are polymerized into chains via the inner membrane proteins WzyB and WzyE, respectively, while the respective co-polymerases WzzB and WzzE modulate the number of repeat units in the chains or “the modal length” of the polysaccharide via a hypothesised interaction. Our data shows for the first time “cross-talk” between Oag and ECA synthesis in that WzzE is able to partially regulate Oag modal length via a potential interaction with WzyB. To investigate this, one or both of the transmembrane regions (TM1 and TM2) of WzzE and WzzB were swapped creating six chimera proteins. Several chimeric proteins showed significant increases in Oag modal length control, while others reduced control. Additionally, co-purification experiments show an interaction between WzyB and WzzB for the first time without the use of a chemical crosslinker, and a novel interaction between WzyB and WzzE. These results suggest the TM2 region of Wzz proteins plays a critical role in Oag and ECA modal length control, presumably via the interaction with respective Wzy proteins, thus providing insight into the complex mechanism underlying the control of polysaccharide biosynthesis.

### 3.3 Article Introduction

---

*Shigella flexneri* bacteria have the ability to synthesise many complex polysaccharide chains that are critical for virulence and structural stability. Complex polysaccharide chains such as the O antigen (Oag) element of the lipopolysaccharide (LPS), and the unrelated enterobacterial common antigen (ECA), are synthesised by variants of the Wzy-dependent pathway (Rai & Mitchell 2020; Whitfield, Wear, et al. 2020).

*S. flexneri* LPS is comprised of three domains: (1) lipid A, a hydrophobic lipid that anchors the molecule to the outer membrane; (2) core oligosaccharides, a non-repeating domain; (3) Oag, a oligosaccharide repeat unit (RU), that varies in number of repeats per LPS molecule. The Oag repeat units are attached to the lipid A component via the core sugars (Raetz & Whitfield 2002). In *S. flexneri* Y-serotype strains the Oag is comprised of tetrasaccharide RUs which contain *N*-acetyl-glucosamine (GlcNAc) and rhamnose (Rha) (Kenne et al. 1977). ECA is a polysaccharide repeat unit that is comprised of three different sugars, and is conserved among Enterobacterales. There are three forms of ECA, two of which are lipid anchored to the outer membrane (ECA<sub>PG</sub> and ECA<sub>LPS</sub>), while the third form is located in the periplasm (ECA<sub>cyc</sub>). (Dell et al. 1984; Kuhn et al. 1983; Rai & Mitchell 2020; Schmidt et al. 1976).

The Wzy-dependent polysaccharide synthesis mechanism is the most commonly used system in bacteria to produce Oag which takes place in the inner membrane, and requires a set of integral membrane proteins (Whitfield, Williams, et al. 2020). Oag is synthesised by the Oag polymerase, WzyB. The individual RUs bound to the lipid carrier undecaprenyl diphosphate (Und-PP) are transported across the inner membrane from the cytoplasm into the periplasm by the Wzx flippase/transporter. The WaaL protein ligates the Oag to the previously synthesised core + lipid A molecules. Oag chain length is regulated by the co-polymerase protein WzzB. LPS lacking the Oag polysaccharide is termed rough LPS, LPS structures without Oag and outer core sugars are termed deep-rough LPS and the completed structure with all components is termed smooth LPS. In *S. flexneri* the ECA polysaccharide is also synthesised by a unique homologue set of the aforementioned integral membrane proteins named WzyE and WzzE, which function similarly in the ECA biosynthetic pathway.

Despite there being several studies on *S. flexneri* WzyB and its paralogues, there is little known about how WzyB functions in combination with the co-polymerase, WzzB, to polymerise and control Oag length. The *S. flexneri* WzyB protein is approximately 43.7 kDa with 12 transmembrane (TM) domains, 6 periplasmic loops and 5 cytoplasmic loops (Daniels et al. 1998). The proposed “catch and release” model suggests that the Und-PP-Oag repeat unit (RU) binds a site on the third periplasmic loop and is then transferred to a second site on the fifth periplasmic

loop, mediated by the loop differences in pI, basic vs acidic, respectively (Islam et al. 2013). Conversely in *S. flexneri*, the pI of the loops appear to play less of a role as both have a similar basic pI (Nath & Morona, R 2015).

In *S. flexneri* WzzB confers the short-Oag (S-Oag) (~10-17 RU) modal length and is a 36 kDa inner membrane protein that is anchored by two TM domains which are located near the N and C termini of the protein, however, the majority of the protein resides in the periplasmic space. WzzB is a member of the polysaccharide co-polymerase group 1 (PCP1) family of proteins (Morona et al. 2009). Despite low sequence identity among members the PCP1 family, the monomeric structures of the proteins are remarkably similar. Structural studies on PCP1 proteins show that each form a distinct “bell” shaped structure with different numbers of monomers. The number of monomers that make up each protein is controversial as formation of both hexamers and octamers have been reported (Kalynych et al. 2014; Larue et al. 2009). Additionally, *in situ* WzzB has been shown to exist in multiple oligomeric forms at equilibrium with the monomeric form (Papadopoulos & Morona 2010; Tran & Morona 2013). The full length oligomeric structure of WzzB<sup>ST</sup> from *Salmonella enterica* serovar Typhimurium was resolved using cryoelectron microscopy experiments, revealing a potential binding region for Wzy (Collins et al. 2017). Recently, a biochemical interaction between WzzB and WzyB, has been shown through the use of *in vivo* chemical crosslinking using dithiobis (succinimidyl propionate) (DSP) (Nath & Morona 2015). This interaction is thought to be required for modal length control, and although this interaction occurred between WzyB and WzzB, it is hypothesised that WzyE and WzzE also have a similar interaction.

Several studies have revealed genetic interactions between the Oag and ECA pathways. The Oag and ECA biosynthesis pathways share the same initial glycosyl transferase (WecA) which adds GlcNAc to Und-PP, thus disruptions to WecA result in loss of both ECA and Oag (Schmidt et al. 1976). It has also been previously reported that certain mutations in ECA genes (e.g. *wzzE*) had an effect on the Oag of *E. coli* O25 (Phan et al. 2013). However, no other effect of ECA gene mutations on Oag have been reported.

In light of this, and the fact that despite having a low sequence identity, WzzB and WzzE monomeric 3-D structures are remarkably similar (Kalynych et al. 2012), we investigated whether WzzE could function in the Oag system and vice versa, if WzzB could function in the ECA system.

In this study, we found that WzzE partially controlled Oag modal length. We then created chimera proteins of the unrelated PCP proteins WzzB and WzzE by swapping the TM regions. The WzzE chimera with TM2 of WzzB showed a significant increase in activity compared to WT

WzzE, which was determined via LPS analysis by silver-stained SDS-PAGE gels and colicin E2 assays. Pull down experiments involving the purification of FLAG tagged WzyB, revealed an interaction between WzyB and WzzB as well as an interaction between WzyB and WzzE, without the use of a chemical crosslinker.

### 3.4 Article Methods

---

#### 3.4.1 Ethics statement.

The WzzB<sub>SF</sub> and WzzE<sub>SF</sub> antibodies were produced under the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and was approved by the University of Adelaide Animal Ethics Committee.

#### 3.4.2 Bacterial strains, growth media and growth conditions.

The bacterial strains used in this study are shown in Table 3.1. Strains were routinely grown on lysogeny broth (LB) agar (10 g tryptone l<sup>-1</sup>, 5 g yeast extract l<sup>-1</sup>, 5 g NaCl l<sup>-1</sup>, 15 g agar l<sup>-1</sup>) or in LB. Strains carrying pQE30 constructs requiring induction were grown in LB at 37 °C with aeration for 16 h, subcultured 1/20 into fresh broth with 0.01 mM IPTG and grown for another 4 h. Strains carrying pBAD33 constructs requiring induction were grown in LB at 37 °C with aeration for 16 h, subcultured 1/20 into fresh broth with 0.2 % (w/v) arabinose and grown for another 4 h. Antibiotics were used at the following concentrations: 100 mg ampicillin (Ap) ml<sup>-1</sup>, 25 mg chloramphenicol (Cml) ml<sup>-1</sup> and 50 mg kanamycin (Kan) ml<sup>-1</sup>.

#### 3.4.3 Chimera construction by inverse PCR.

The oligonucleotides used to amplify pQE30:wzzB-His<sub>6</sub> without TM1 region, were 5' ACGCCACAACACTGCACTAG 3' and 5' AAGGAGAAATGGACGTCAACA 3' (Table 3.3 [ST1]). The complementary forward and reverse strand of DNA encoding the TM1 region of wzzE were synthesised (IDT) (Table 3.3 [ST1]) and then annealed *in vitro*. First, 0.02 nM of DNA was resuspended in annealing buffer [100 mM potassium acetate, 30 mM HEPES, 2 mM magnesium acetate], heated to 95 °C for 4 min, followed by 70 °C for 10 min, and finally followed by slowly cooling the DNA down to 4 °C. The annealed oligonucleotide's 5' ends were then phosphorylated using T4 polynucleotide kinase (NEB), before being mixed at a 3:1 ratio with previously amplified plasmid. The DNA mix was then ligated using T4 Ligase (NEB) and transformed into competent *E. coli* DH5α strains. This process was repeated to construct all chimeric wzz genes in pQE30 using the oligonucleotides listed in Table 3.3 [ST1] and were all sequencing confirmed via Sanger sequencing (AGRF).

#### 3.4.4 DNA methods.

The plasmids used in this study are shown in Table 3.1. Plasmid constructs were extracted from *E. coli* DH5α strains using a QIAprep Spin Miniprep kit (Qiagen). Preparation of electro-competent cells and the electroporation method were performed as described previously (Purins et al. 2008).



#### **3.4.5 LPS SDS-PAGE and silver staining.**

Bacteria were grown and induced as described above, before  $1 \times 10^9$  cells were harvested by centrifugation, resuspended in  $2 \times$  lysis buffer (Murray et al. 2003), and heated at  $100^\circ\text{C}$  for 10 min, prior to incubation with 2.5 mg/ml proteinase K (Sigma-Aldrich) for 2 h, at  $56^\circ\text{C}$ , followed by electrophoresis on 15% SDS-PAGE gels for 13 h at 12 mA. Silver staining on LPS was performed as described previously (Murray et al. 2003).

#### **3.4.6 Protein SDS-PAGE.**

Bacteria was grown and induced as described above, before  $5 \times 10^8$  cells were harvested by centrifugation, resuspended in  $2 \times$  sample buffer (Lugtenberg et al. 1975) and heated at  $100^\circ\text{C}$  or  $37^\circ\text{C}$  for 5 min, prior to SDS-PAGE on 12% gels for 1 h at 200 V. Purified proteins were mixed 1:1 with  $2 \times$  sample buffer (Lugtenberg et al. 1975) with or without  $\beta$ -mercaptoethanol and heated to  $37^\circ\text{C}$  for 5 min, prior to SDS-PAGE on 15% gels for 1 h at 200 V. ECA samples were prepared as described above (LPS SDS-PAGE), followed by SDS-PAGE on 12% gels for 1 h at 200 V.

#### **3.4.7 Western immunoblotting.**

Western transfers were performed at 400 mA for 1 h. Protein gels were then subjected to Western immunoblotting on nitrocellulose membrane (Bio-Rad) with either polyclonal WzzB<sub>SF</sub> or WzzE<sub>SF</sub> rabbit antibodies (Daniels & Morona 1999) at 1:500 dilution, polyclonal ECA rabbit antibodies (made in house) at 1:500 dilution, monoclonal His<sub>6</sub> mouse antibodies (GenScript) at 1:50000 dilution or polyclonal FLAG rabbit antibodies (Sigma) at 1:2000 dilution in 2.5% (w/v) skim milk. Detection was performed with either goat anti-rabbit horseradish-peroxidase-conjugated antibodies (KPL) or goat anti-mouse horseradish-peroxidase-conjugated antibodies (KPL) and chemiluminescence reagent (Sigma). 5  $\mu\text{L}$  of BenchMark SeeBlue Plus2 pre-Stained Protein Ladder (Invitrogen) was used as the molecular mass standard.

#### **3.4.8 Colicin spot assay.**

For spot sensitivity assays, bacteria ( $\sim 5 \times 10^8$  cells) were spread onto selective 25 ml LB agar plates and plates were spotted with 5  $\mu\text{l}$  purified colicin E2 protein (Tran et al. 2014) in MilliQ (MQ) water at the following concentrations ( $\mu\text{g ml}^{-1}$ ): 0.50, 1.0, 2.0, 4.0, 8.0, 12, 16, 20, 24, 28, 32 and 36. Plates were incubated at  $37^\circ\text{C}$  for 16 h and the minimum inhibitory concentration (MIC), defined here as the lowest concentration that generated a clear zone of inhibition, was recorded.

### 3.4.9 Purification of WzyB from *S. flexneri* strains.

Cells were harvested from 100 ml induced culture by centrifugation (Beckman Coulter Avanti J-26XPI centrifuge; 9600 g, 10 min, 4 °C) and the cell pellet was resuspended in sonication buffer [50 mM Tris/HCl, 150 mM NaCl, pH 7.5] followed by cell lysis via sonication (Branson B15). Cell debris was removed by centrifugation (Thermo Scientific Labofuge 400 R centrifuge; 3500 g, 10 min, 4 °C) and the whole membrane (WM) fraction was collected by ultracentrifugation (Beckman Coulter Optima L-100 XP ultra-centrifuge; 250 000 g, 1 h, 4 °C). WM fraction was then resuspended in 500 µl of MQ water, and 500 µl of 2× solubilisation buffer [100 mM Tris/HCl, 300 mM NaCl, 2% (w/v) n-dodecyl-b-D-maltopyranoside (DDM) (Anatrace)] and was mixed for 16 h, 4 °C. Insolubilized material was removed by ultracentrifugation (Beckman Coulter Optima Max-XP tabletop ultracentrifuge; 160000 g, 1 h, 4 °C) and the solubilized supernatant was incubated with 100 µl of anti-DYKDDDDK G (FLAG) affinity resin (GenScript) pre-equilibrated with equilibration buffer [50 mM Tris/HCl, 150 mM NaCl, 0.02% (w/v) DDM, pH 8] for 1.5 h at room temperature (RT). The loaded FLAG affinity resin was then washed with wash buffer [50 mM Tris/HCl, 150 mM NaCl, 0.02% (w/v) DDM, pH 8] several times (10 ml total). Finally, WzyB-FLAG was eluted in 100 µl of elution buffer [0.1 M glycine, 0.02% (w/v) DDM, pH 3.5] at RT and neutralised with 1 M Tris, pH 9.0.

### 3.4.10 *In vivo* protein crosslinking.

*In vivo* crosslinking with DSP was performed prior to cell lysis via sonication as above. Cells were harvested from 100 ml induced culture by centrifugation (Beckman Coulter Avanti J-26XPI centrifuge; 9600 g, 10 min, 4 °C) and the cell pellet was washed in DSP crosslinking buffer [20 mM sodium phosphate buffer (Na<sub>2</sub>PO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub>), 150 mM NaCl, pH 7.5]. The pellets were then resuspended in 5 ml DSP crosslinking buffer followed by incubation with 1 mM DSP (Thermo Fischer Scientific) [treated samples (T)] for 30 min at 37 °C. A duplicate sample was also incubated without DSP [untreated samples (UT)]. Excess DSP was quenched with 20 mM Tris/HCl, pH 7.5 and left for 10 min at RT, before resuspension in sonication buffer and purification (as described above).

**Table 3.1: Bacterial strains and plasmids**

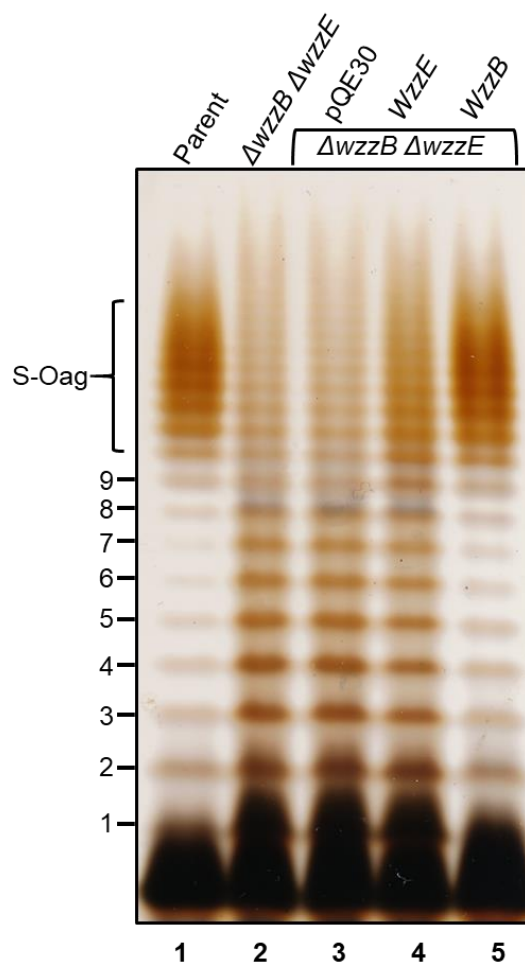
Strains and plasmids	Description	Reference
<b>Strains</b>		
RMA2162	<i>S. flexneri</i> PE860 Y serotype, this strain lacks the virulence plasmid and pHS-2 (parent)	Laboratory stock
RMA4662	<i>S. flexneri</i> PE860 Y serotype <i>wzzB::kan<sup>r</sup></i> , $\Delta$ <i>wzzE</i> ,	Laboratory stock
<b>Plasmids</b>		
pQE30	IPTG inducible, expression vector; Amp <sup>R</sup>	Qiagen
pQE30-His <sub>6</sub> - <i>wzzB</i>	pQE30 with <i>Shigella flexneri wzzB</i> gene; Amp <sup>R</sup>	GenScript
pQE30-His <sub>6</sub> - <i>wzzE</i>	pQE30 with <i>Shigella flexneri wzzE</i> gene; Amp <sup>R</sup>	GenScript
pVLRM18	pQE30 encoding WzzE <sup>(WzzBTM1)</sup> -His <sub>6</sub> , Amp <sup>R</sup>	This study
pVLRM16	pQE30 encoding WzzE <sup>(WzzBTM2)</sup> -His <sub>6</sub> , Amp <sup>R</sup>	This study
pVLRM20	pQE30 encoding WzzE <sup>(WzzBTM1 &amp; 2)</sup> -His <sub>6</sub> , Amp <sup>R</sup>	This study
pVLRM19	pQE30 encoding WzzB <sup>(WzzETM1)</sup> -His <sub>6</sub> , Amp <sup>R</sup>	This study
pVLRM17	pQE30 encoding WzzB <sup>(WzzETM2)</sup> -His <sub>6</sub> , Amp <sup>R</sup>	This study
pVLRM21	pQE30 encoding WzzB <sup>(WzzETM1 &amp; 2)</sup> -His <sub>6</sub> , Amp <sup>R</sup>	This study
pBAD33	Arabinose inducible, expression vector; Cml <sup>R</sup>	
pVLRM11	pBAD33 encoding WzyB-Flag	This study

## 3.5 Article Results

---

### 3.5.1 WzzE is able to partially complement a *wzzB* mutant.

Due to the previously reported links between the biosynthetic pathways of Oag and ECA, as well as the monomeric 3-D structural similarities between WzzE and WzzB, we investigated if the ECA co-polymerase, WzzE, was able to function in the modal length control of Oag. The LPS of parent RMA2162 (*S. flexneri* Y  $\Delta_{wzzpHS2}$ ) and RMA4662 (*S. flexneri* Y  $\Delta_{wzzE}/\Delta_{wzzpHS2}/wzzB::kan^R$ ), transformed with pQE30, pQE30-*wzzE*-His<sub>6</sub> or pQE30-*wzzB*-His<sub>6</sub> were analysed via SDS-PAGE and silver staining (Figure 3.1). As expected when His<sub>6</sub>-WzzB (referred to hereafter as WzzB) was expressed, the LPS profile was comparable to that of the parent strain with a pronounced S-Oag modal length (10-17 Oag RUs) (Figure 3.1, lane 5 and 1, respectively). Interestingly, when His<sub>6</sub>-WzzE (referred to hereafter as WzzE) was expressed, there was a distinct increase in the level of S-Oag when compared to the mutant and vector only controls (Figure 3.1, lanes 4, 2 and 3, respectively). These results suggested that WzzE was able to partially act on the Oag Wzy-dependent system by regulating the length of the Oag chains of LPS molecules.

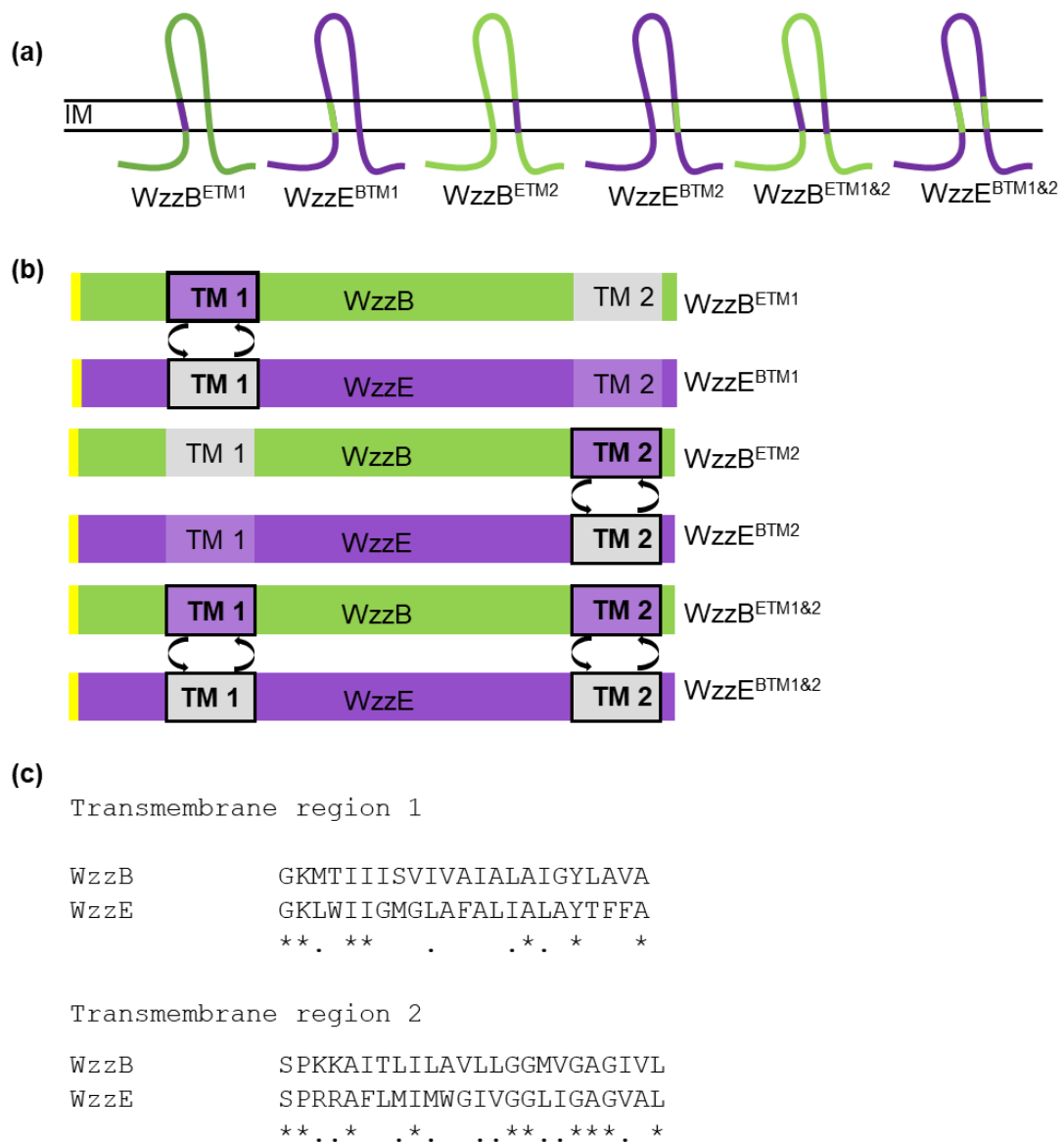


**Figure 3.1: Analysis of LPS profile conferred by triple co-polymerase mutant in the presence of either WzzB or WzzE.**

*S. flexneri* Y strains were grown and the LPS was isolated and detected from whole-cell lysates of parent *S. flexneri* Y  $\Delta wzz_{pHS2}$  (lane 1), *S. flexneri* Y  $\Delta wzzE/\Delta wzz_{pHS2}/wzzB::kan^R$  (lane 2) harbouring pQE30 control (lane 3) or expressing WzzE protein (lane 4) or WzzB protein (lane 5). Strains grown to log-phase were collected ( $1 \times 10^9$  cells) and lysed in lysis buffer in the presence of proteinase K. Samples were then electrophoresed on a SDS-15% (w/v) PAGE gel, and silver-stained (see methods). Number of Oag RUs are shown on the left-hand side.

### 3.5.2 Functional analysis of the transmembrane regions within the co-polymerases.

To further investigate which regions of the co-polymerases may be interacting with WzyB, the TM regions of the WzzE and WzzB were selected for mutagenesis, on the basis that the majority of WzyB exists in the membrane and the conserved GGxxG motifs of the Wzz proteins are present in TM region 2. Six unique co-polymerase chimeric proteins ( $WzzB^{ETM1}$ ,  $WzzB^{ETM2}$ ,  $WzzB^{ETM1\&2}$ ,  $WzzE^{BTM1}$ ,  $WzzE^{BTM2}$  and  $WzzE^{BTM1\&2}$ ) were designed to exchange either TM region 1, 2 or both, between WzzB and WzzE; these were generated via inverse-PCR and *in vitro* oligonucleotide annealing (as described in methods 3.4.4) (Figure 3.2, Figure 3.9 [S1], Figure 3.10 [S2] and Table 3.1). The aligned amino acid sequence of the TM regions of WzzB and WzzE are shown in Figure 3.2c.



### Figure 3.2: Graphical representation of the polysaccharide co-polymerase chimera proteins.

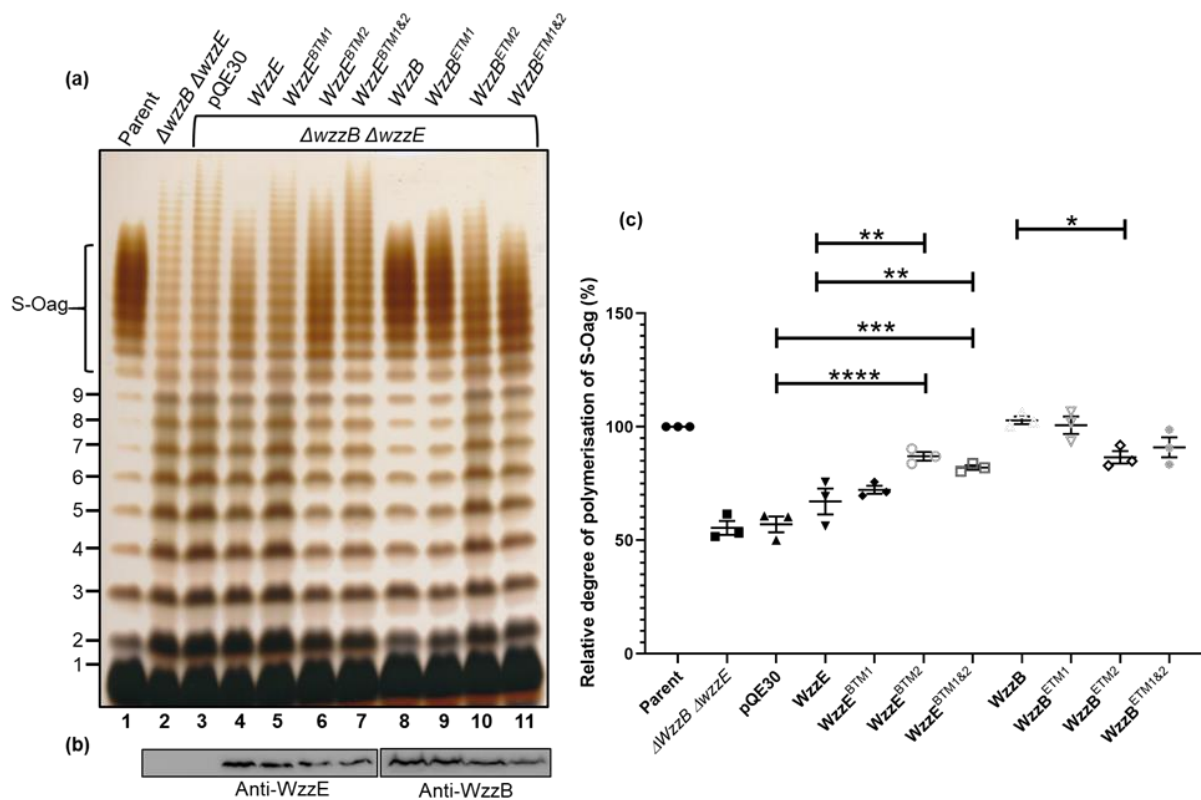
Schematic representing 6 chimeric polysaccharide co-polymerase proteins generated via inverse PCR cloning. (a) Topology representation. (b) Graphical representation of protein maps compositions (IM=Inner membrane, TM=Transmembrane) yellow box indicates a His<sub>6</sub> tag. (c) Amino acid sequence alignment of WzzB and WzzE transmembrane region 1 and 2. An asterisk (\*) indicates a fully conserved residue and a period (.) indicates conservation between groups of weakly similar properties.

The LPS profile of *S. flexneri* Y  $\Delta wzzE/\Delta wzz_{pHS2}/wzzB::kan^R$ , transformed with pQE30 vectors expressing each of these chimera proteins (pVLRM16-21, Table 3.1), were analysed via silver stained SDS-PAGE (Figure 3.3a). The presence of WzzE and additionally the presence of WzzE<sup>BTM1</sup> increased the level of S-Oag when compared to the empty vector and controls (Figure 3.3a, lanes 4, 5, 3 and 2 respectively). Strains with WzzE<sup>BTM2</sup> and WzzE<sup>BTM1&2</sup> had LPS with a further increase of modal length control when compared to the vector control (Figure 3.3a, lanes 6, 7 and 3, respectively), suggesting the presence of the WzzB TM region 2 is important for modal length control and potentially interaction with WzyB. Reciprocally, the addition of WzzE TM2

region to WzzB (i.e. WzzB<sup>ETM2</sup>) reduced the modal length control of Oag when comparing to the parent strain and wild-type WzzB, further supporting the importance of WzzB's TM2 region for function/interaction (Figure 3.3a, lanes 10, 1 and 8, respectively). The replacement of WzzB TM1 (i.e. WzzB<sup>ETM1</sup>) with WzzE TM1 had little effect of the LPS profile (Figure 3.3a, lane 9).

We then investigated the expression of the chimeras. Western immunoblotting with either anti-WzzE<sub>SF</sub> or anti-WzzB<sub>SF</sub> (Daniels & Morona 1999) detected bands consistent with the size of WzzE (~40 kDa) and of WzzB (~38) respectively, for all strains expressing each of the WT and chimera co-polymerase proteins (Figure 3.3b).

To quantify the relative degree of polymerisation of S-Oag produced by strains expressing the chimera proteins, densitometry was performed on three biological replicates (Figure 3.3a and Figure 3.11 [S3]) of silver stained SDS-PAGE gels (Figure 3.3c). The degree of polymerisation is presented as the densitometry of S-Oag LPS molecules normalised to the parent as a percentage. This analysis provides insight into a particular strain's ability to regulate Oag length, whereby strains without the presence of WzzB have a lower proportion of overall LPS with 10-17 RUs. In the presence of either WzzE or WzzE<sup>TM1</sup> the relative degree of polymerisation of S-Oag appeared higher than that of the vector control. Additionally, in the presence of either WzzE<sup>BTM2</sup> or WzzE<sup>BTM1&2</sup> the relative degree of polymerisation of S-Oag was significantly higher than that of both the vector control and to WzzE. Furthermore, LPS from the strain expressing WzzB<sup>ETM2</sup> had a significant decrease in S-Oag when compared to the WzzB expressing strain. Collectively these results further support the importance of WzzB TM2 in Oag modal length control and presumably the interaction with WzyB.



**Figure 3.3: Analysis of the LPS profiles of strains expressing chimeric Wzz proteins.**

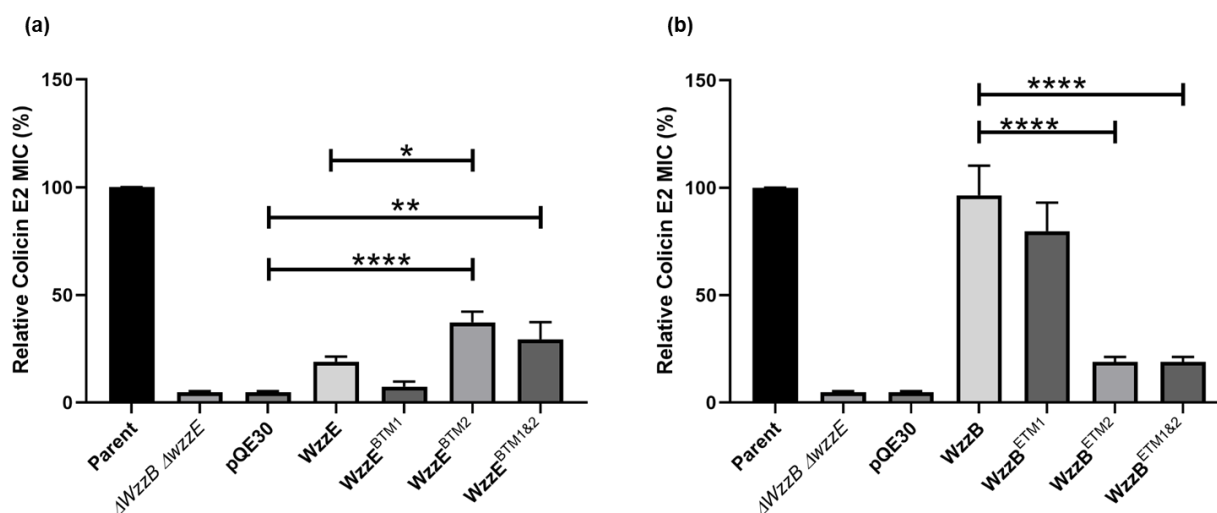
a) Silver-stained PAGE separation of the LPS from parent *S. flexneri* Y  $\Delta wzzE/wzzB::kan^R$  (lane 1) and  $\Delta wzzE/wzzB::kan^R$  (lane 2), harbouring pQE30 (lane 3) encoding either WT Wzz proteins (lanes 4 or 8) or a chimeric Wzz (lanes 5-7 or 9-11). Strains grown to log-phase were collected ( $1 \times 10^9$  cells) and lysed in lysis buffer in the presence of proteinase K. Samples were electrophoresed on a SDS-15% (w/v) PAGE gel, and silver-stained. b) Whole-cell lysates ( $5 \times 10^8$  cells) of indicated strains, were separated by SDS-PAGE, transferred onto a nitrocellulose membrane and probed with either polyclonal rabbit WzzE<sub>SF</sub> or WzzB<sub>SF</sub> antibodies. c) Analysis of polymerisation of S-Oag via densitometry (Image Lab). The degree of polymerisation of S-Oag with is represented as the densitometry of Oag RUs 10-17 (indicated by bracket) as a percentage relative to the parent. Data represents 3 independent experiments with SEM shown, and significance is calculated with a one-way ANOVA, \*  $P < 0.033$ , \*\*  $P < 0.0021$ , \*\*\*  $P < 0.0002$ , and \*\*\*\*  $P < 0.0001$ .

### 3.5.3 The effect of the chimeric proteins on resistance to colicin E2.

A colicin E2 spot sensitivity assay was used as an indirect measure to determine the S-Oag production of the strains expressing the chimera proteins (Tran et al. 2014). Log-phase cultures of *S. flexneri* Y  $\Delta wzzE/\Delta wzzE/pHS2/wzzB::kan^R$  transformed with pQE30 vectors encoding each of WT or chimeric Wzz proteins (pVLRM16-21) were tested against decreasing concentrations of purified His<sub>6</sub>-Colicin E2 (Figure 3.4). As anticipated, when WzzE was expressed the strain was more resistant to colicin E2 when compared to the vector control. When WzzE<sup>BTM2</sup> was expressed, the strain was significantly more resistant to colicin E2 than both the vector control and WzzE. When WzzE<sup>BTM1&2</sup> was expressed the strain was significantly more resistant when compared to the vector control (Figure 3.4a). Furthermore, when WzzB<sup>ETM1</sup> was expressed, the strain was slightly less resistant to colicin E2 when compared to the WzzB control strain, however this was



non-significant. When either  $WzzB^{ETM2}$  or  $WzzB^{ETM1\&2}$  was expressed, the strains showed a significant reduction in colicin E2 resistance when compared to the  $WzzB$  control strain. Combined, these results suggested that the TM1 region of  $Wzz$  proteins was not likely to be important for function and/or interaction with  $WzyB$ , while TM2 was likely critical for function and/or interaction with  $WzyB$ .



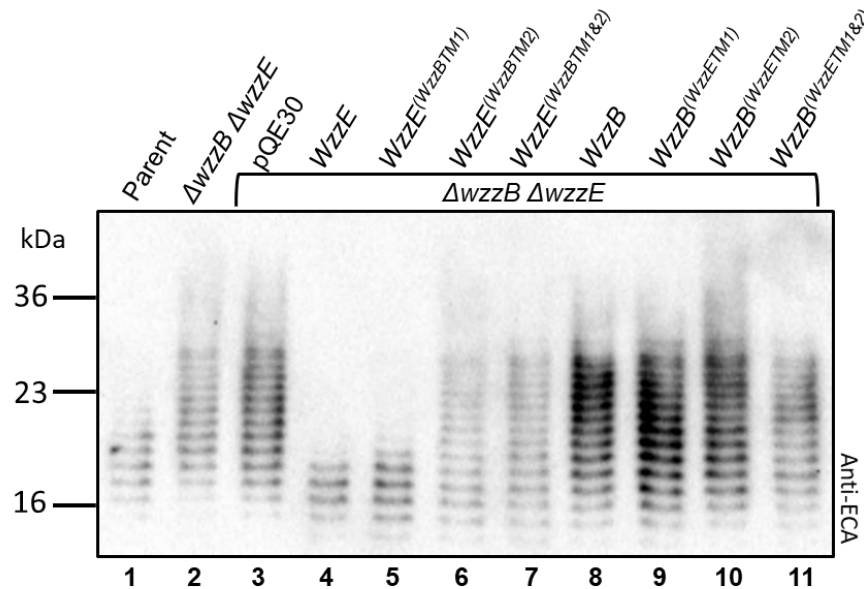
**Figure 3.4: Colicin E2 sensitivity of strains producing  $Wzz$  chimeric proteins.**

*S. flexneri* Y strains expressing the indicated proteins from pQE30 are indicated on the x-axis. The MIC of colicin E2 required to generate a clear zone of bacterial inhibition for each strain was normalised (%) to the parent strain MIC and is shown on the Y-axis. a)  $WzzE/WzzE$  chimeric protein expressing strains. b)  $WzzB/WzzB$  chimeric protein expressing strains. Data represents 4 independent experiments with SEM shown, and significance is calculated with a one-way ANOVA, \*  $P < 0.033$ , \*\*  $P < 0.0021$ , \*\*\*  $P < 0.0002$ , and \*\*\*\*  $P < 0.0001$ .

### 3.5.4 The effect of the chimeric proteins on ECA production and modal length control.

We then investigated the effect of the  $Wzz$  chimeric proteins ability to produce ECA, by Western immunoblotting. ECA modal length is controlled by  $WzzE$  (Barr et al. 1999b) and as such when *S. flexneri* Y ECA was analysed, banding could be seen between approximately 16-23 kDa (Figure 3.5, lane 1), while the mutant *S. flexneri* Y  $\Delta wzzE/\Delta wzz_{pHS2}/wzzB::kan^R$  and vector control strains produced unregulated ECA chains ranging from 16-40 kDa (Figure 3.5, lane 2). When WT  $WzzE$  was expressed the modal length of ECA was controlled, however it was shorter compared to that of the parent strain (Figure 3.5, lanes 4 and 1, respectively), suggesting that the absence of  $WzzB$  or  $Wzz_{pHS2}$  may be altering ECA production and/or modal length control. Similarly, when  $WzzE^{BTM1}$  was expressed, the ECA modal length was controlled, however again it was shorter when compared to the parent strain (Figure 3.5, lanes 5 and 1, respectively). Despite the relatively shorter modal length,  $WzzE^{BTM1}$  was still able to control the ECA modal length, suggesting that the presence of  $WzzB$  TM1 was equivalent to  $WzzE$  TM1 as no negative effect on ECA production or control was detected. When either  $WzzE^{BTM2}$  or  $WzzE^{BTM1\&2}$  were expressed, the ECA banding

size was less controlled and comparable to the mutant and vector control (Figure 3.5 lanes, 6, 7, 2 and 3, respectively). Protein expression of these samples can be seen in Figure 3b, as the same samples were used in LPS and ECA analysis. These results suggest that the WzzE TM2 region was likely to be critical for function and/or interaction with WzyE. Strains expressing WzzB and all the WzzB chimeras did not have any effect on ECA modal length control (Figure 3.5, lanes 8-10).



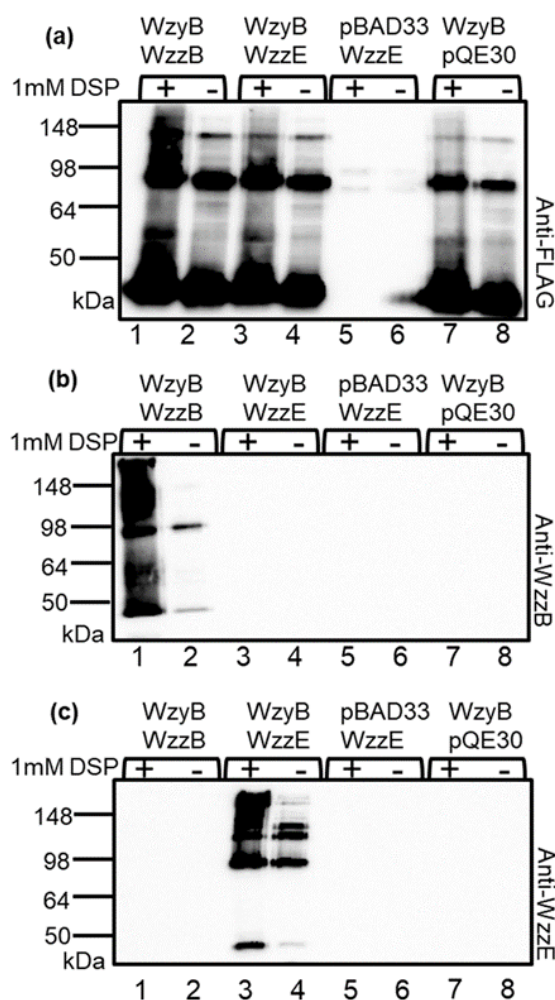
**Figure 3.5: Functional analysis of the transmembrane regions within the co-polymerases via Western immunoblotting.**

Silver-stained PAGE separation and Western immunoblot of ECA from parent *S. flexneri* Y  $\Delta_{wzz,pHS2}$  (lane 1) and  $\Delta_{wzzE/wzzB::kan^R}$  (lane 2), harbouring pQE30 (lane3) encoding either WT Wzz proteins (lanes 4 or 8) or a chimeric Wzz protein (lanes 5-7 or 9-11). Whole-cell lysates ( $1 \times 10^9$  cells) of the indicated strains were treated with proteinase K, separated by SDS-PAGE, transferred onto a nitrocellulose membrane and probed with polyclonal rabbit ECA antibodies. The migration positions of the protein molecular mass standards (in kDa) is indicated on the left-hand side.

### 3.5.5 Interaction of the Wzz chimera proteins with WzyB.

To investigate the ability of the Wzz chimeric proteins to physically interact with WzyB, a new WzyB expression construct was made. The *S. flexneri* *wzyB* coding region was PCR amplified from pRMPN1 (Nath et al. 2015) to include a FLAG tag and cloned into pBAD33, creating a WzyB-FLAG expression construct which possesses a strong arabinose promoter for protein expression, named pVLRM11 (Table 3.1). *S. flexneri* Y  $\Delta_{wzzE/\Delta_{wzz,pHS2}} /wzzB::kan^R$  strains expressing the WT and chimeric Wzz proteins from pQE30 plasmids were also transformed with pVLRM11 (pBAD33-*wzyB*-FLAG) and the vector controls. WzzB WT and WzzE WT expressing strains, co-expressing WzyB, were grown and whole cells were incubated in the presence or absence of DSP, followed by co-purification experiments using anti-DYKDDDDK G

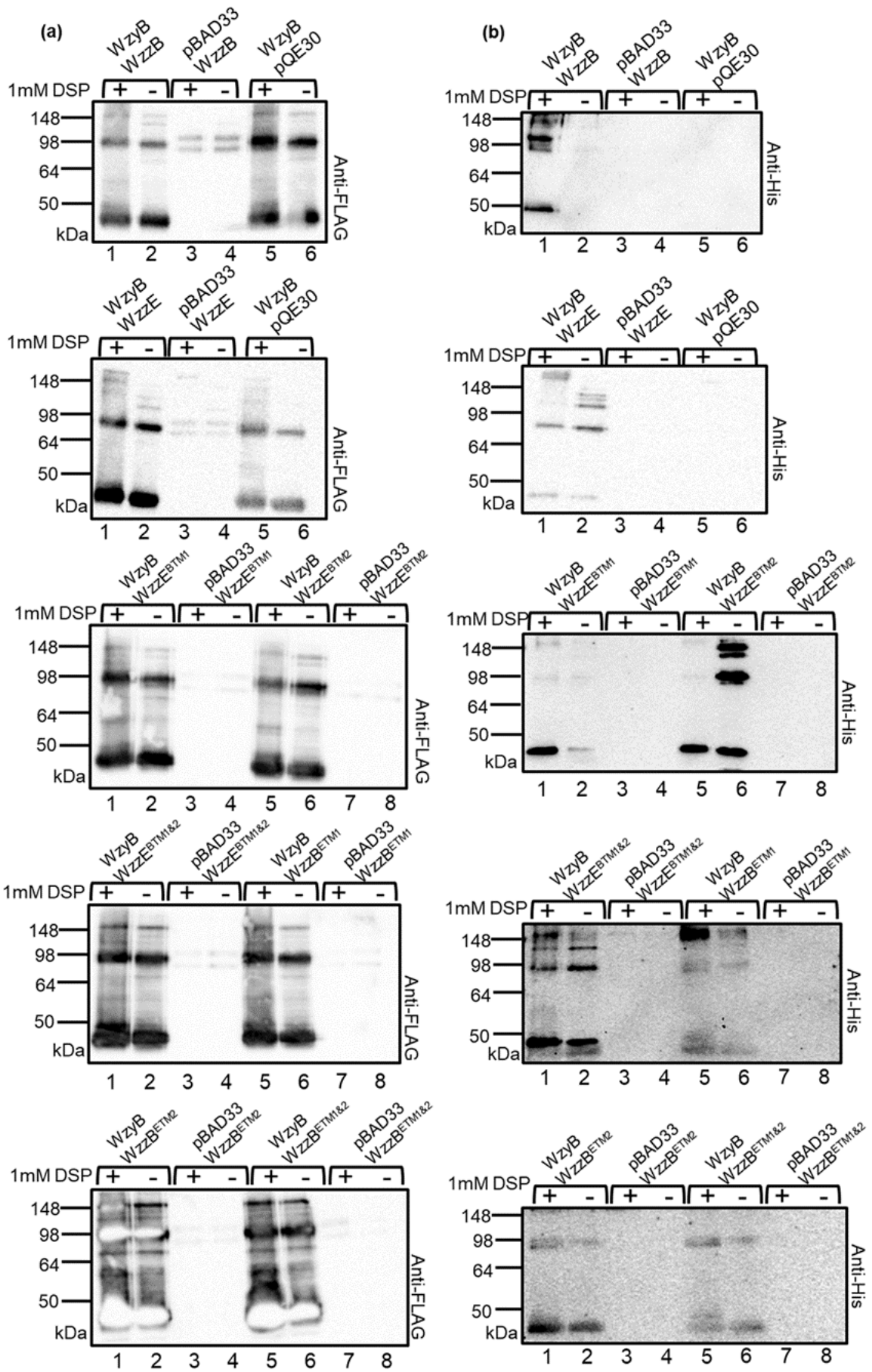
(FLAG) affinity resin (Methods). The purified samples were separated on a SDS-12% (w/v) polyacrylamide gel and subjected to Western immunoblotting using either anti-FLAG, anti-WzzB<sub>SF</sub> or anti-WzzE<sub>SF</sub> antibodies (Figure 3.6). WzyB was purified at comparable levels in all the separate co-purifications in the presence of the different Wzz proteins, and 3 bands at ~47 to ~148 kDa were detected (Figure 3.6a). As expected when probing with anti-WzzB, WzzB was detected in both the presence and absence of DSP (Figure 3.6b). Similarly, WzzE was detected via anti-WzzE in both the presence of and absence of DSP (Figure 3.6c). As, expected no bands were detected when probing with either WzzB or WzzE when, either WzyB or either Wzz protein was absent from the co-purification experiment. Previously co-purification experiments of WzzB and WzyB without the presence of DSP were unable to detect co-purification of WzzB (Nath & Morona 2015).



**Figure 3.6: Co-purification of WzyB and Wzz/Wzz chimeric proteins using anti-FLAG resin.**

Purification of WzyB-FLAG from pVLRM11 (Table 3.1) from *S. flexneri* Y  $\Delta wzzE/wzzB::kan^R$  expressing either WzzB or WzzE proteins. Whole-cell treatment with (+) and without (-) 1 mM DSP (as described in methods). Samples were electrophoresed on SDS-12% (w/v) polyacrylamide gels followed by Western immunoblotting with (a) polyclonal FLAG antibody, (b) polyclonal WzzB<sub>SF</sub> antibody, or (c) polyclonal WzzE<sub>SF</sub> antibody. The migration positions of the molecular mass standards (in kDa) are indicated on the left-hand side.

Next, we performed co-purification experiments in strains expressing each of the Wzz chimeric proteins followed by Western immunoblotting using either anti-FLAG or anti-His antibodies. Anti-His antibodies were used so WzzB and WzzE chimeric proteins could be detected equally. WzyB was purified at comparable levels in all the separate co-purifications in the presence of the different Wzz chimeric proteins, as bands ranging from ~47 to ~148 kDa were detected (Figure 3.7a). All chimeric proteins were detected using anti-His in both the presence and absence of DSP treatment. Co-purifications performed with no WzyB expressed (vector control) produced no WzyB bands in both anti-FLAG and anti-His Western immunoblots as expected, (two non-specific bands can be seen at ~110 and ~90 kDa) (Figure 3.7). Furthermore, co-purifications performed in the presence of WzyB and pQE30 vector control did not detect any bands via anti-WzzE and anti-WzzB Western immunoblotting, as expected (Figure 3.7). Most notably, co-purifications in the presence of WzzE<sup>BTM2</sup> and absence of DSP show markedly more intense bands when compared to all other the other WzzE chimeric proteins (Figure 3.7b).



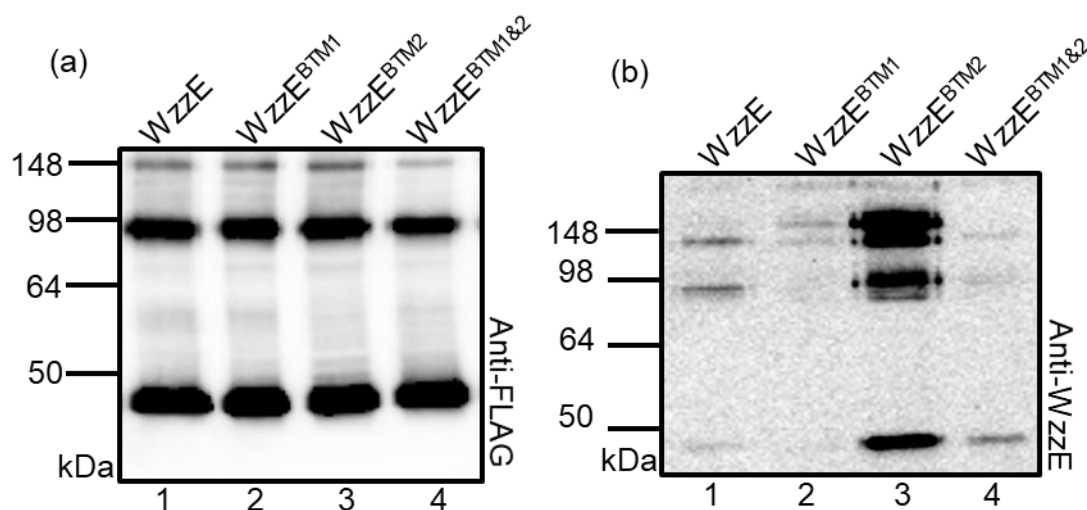
---

**Figure 3.7: Co-purification of WzyB and Wzz chimeric proteins using anti-FLAG resin.**

Purification of WzyB-FLAG expressed by pVLRM11 (Table 3.1) from *S. flexneri* Y  $\Delta wzzE/wzzB::kan^R$  expressing WT or chimeric WzzE or WzzB proteins. Whole-cell treatment with 1 mM DSP indicated with +/- (as described in methods). Samples were electrophoresed on SDS-12% (w/v) polyacrylamide gels followed by Western immunoblotting with (a) polyclonal FLAG antibody, or (b) monoclonal His-Tag antibody. The migration positions of the molecular mass standards (in kDa) are indicated on the left-hand side.

---

Co-purification experiments in strains expressing WzzE and all WzzE chimeric proteins in the absence of DSP were further subjected to Western immunoblotting using anti-WzzE antibodies for direct comparison of relative interaction with WzyB (Figure 3.8). The Strain expressing WzzE<sup>TM2</sup> produced significantly darker banding when compared to the WT WzzE and all other WzzE chimeric proteins, suggesting that the TM2 region of WzzB increased the interaction between WzzE and WzyB. Solubilised membrane fractions prior to purification were also analysed by Western immunoblotting and confirmed that WzyB and all Wzz proteins were expressed at comparable levels before co-purification Figure 3.12 [S4].



---

**Figure 3.8: Comparison of co-purification of WzyB and WzzE chimeric proteins using anti-FLAG resin comparison using anti-WzzE antibodies.**

Purification of WzyB-FLAG expressed by pVLRM11 (Table 3.1) from *S. flexneri* Y  $\Delta wzzE/wzzB::kan^R$  expressing WT or chimeric WzzE proteins (as described in methods). Samples were electrophoresed on SDS-12% (w/v) polyacrylamide gels followed by Western immunoblotting with (a) polyclonal FLAG antibody, or (b) polyclonal WzzE antibody. The migration positions of the molecular mass standards (in kDa) are indicated on the left-hand side.

---

### 3.6 Article Discussion

---

In this study we characterised a series of WzzB/WzzE TM chimeric proteins, and analysis of their activity indicated that the TM2 region of Wzz proteins is likely to be involved in a direct interaction with their specific cognate Wzy partner. Previous mutagenesis studies suggested that the WzzB<sub>SF</sub> TM2 region, particularly the GGXXG motif, was important for function as a G306A/G311A double mutant conferred a very short chain Oag (Daniels & Morona 1999; Papadopoulos et al. 2016). However this mutation altered modal length, but did not decrease modal length control. Additionally, structural studies have also pointed towards the TM region being the likely region in which WzyB and WzzB interact (Collins et al. 2017). In light of this, the TM regions of the two Wzz proteins were targeted to be swapped to investigate their roles in function and interaction with WzyB.

The 3-D structures of WzzB and WzzE, are remarkably similar despite only sharing approximately 22% overall amino acid identity. The conserved GGxxG motif is present in both, and is located in the TM2 region. The chimeras were designed to substitute both the TM regions of the Wzz proteins, and particular care was taken with the TM2 region (23 aa) to ensure the GGxxG motifs were in the same position within the TM2 region of the chimera proteins. In fact, the sequences of the TM1 regions (23 aa) from WzzB and WzzE share approximately 30% amino acid identity while the TM2 regions share approximately 42% identity, thus these regions both share a higher average identity when compared to the overall identity of the full length protein. In the case of TM2 only 14 amino acids were substituted (out of 23), which did not include the conserved GGxxG motif. These surrounding amino acids were able to increase the WzzE proteins function in Oag modal length control which suggests that the non-motif amino acids are either directly involved with protein interaction with WzyB, or the amino acids aid in correctly positioning the important GGxxG motif for full activity and/or interaction.

The results from this study can be found summarised in Table 3.2. Interestingly, WzzE alone was able to partially complement a *wzzB* mutant as it partially controlled Oag modal length as seen by SDS-PAGE and silver staining, and also by an increase in the strains resistance to colicin E2 (Figure 3.1 and Figure 3.4). The addition of the TM2 region of WzzB into WzzE produced the most interesting result, as the presence of the WzzB TM2 region was able to significantly increase the modal length control of Oag, while also significantly increasing colicin E2 resistance compared to the WT WzzE protein (Figure 3.3a, lanes 6 and 4 respectively and Figure 3.4).

**Table 3.2: Summary of the results.**

<b>Protein</b>	<b>LPS Profile<sup>1</sup></b>	<b>S-Oag densitometry (Mean) normalised to parent<sup>2</sup></b>	<b>Colicin E2 MIC (mean) normalised to parent<sup>3</sup></b>	<b>ECA Profile<sup>4</sup></b>	<b>WzyB interaction (-) DSP<sup>5</sup></b>
WzzB	Regulated Oag	102%	97%	Unregulated ECA, darker banding	+
WzzE	Partially regulated Oag	69%	18%	Regulated ECA, lower modal length	++
WzzE <sup>BTM1</sup>	Partially regulated Oag	71%	6%	Regulated ECA, lower modal length	+
WzzE <sup>BTM2</sup>	Moderately regulated Oag	87%	35%	Unregulated ECA	++++
WzzE <sup>ETM1&amp;2</sup>	Partially regulated Oag	82%	27%	Unregulated ECA	++
WzzB <sup>ETM1</sup>	Regulated Oag	102%	78%	Unregulated ECA, darker banding	+
WzzB <sup>ETM2</sup>	Partially regulated Oag	85%	17%	Unregulated ECA, darker banding	+
WzzB <sup>ETM1&amp;2</sup>	Moderately regulated Oag	90%	18%	Unregulated ECA	+
pQE30 ctrl.	Unregulated Oag	30%	5%	Unregulated ECA	-

Strains expressing the WzzB proteins with the WzzE TM2 region produced LPS with decreased levels of S-Oag when compared to the WT WzzB expressing strain, resulting in a 50% increase in colicin E2 sensitivity (Figure 3.3a, lanes 10 and 8 respectively and Figure 3.4). These strains were still able to partially control Oag modal length when compared to the mutant and the empty vector strains, highlighting that these proteins were still functional. This data again suggests that the TM2 region of WzzB is critical for Oag modal length control.

The WzzE TM2 region was shown to be important in ECA modal length control. When WzzB TM2 was present in the WzzE protein (WzzE<sup>BTM2</sup> or WzzE<sup>BTM1&2</sup>), the strains expressing these proteins lost the ability to control the modal length of ECA. Since the 3-D structure of WzzB and WzzE are highly conserved (Kalynych et al. 2012), it is likely that the TM2 region of WzzE, may interact with WzyE (ECA polymerase). Although not investigated, we speculate that WzzE<sup>BTM2</sup> has decreased interaction with WzyE. WzzB chimeras with WzzE TM regions were not able to restore modal length control of ECA, suggesting that the TM2 region may not be the sole interacting region in the case of ECA. This was expected as the WT WzzB protein was unable to partially control the modal length of ECA, unlike WT WzzE, which was able to partially regulate



Oag modal length. Interestingly, when either WzzE or WzzE<sup>BTM1</sup> was used in complementation, the ECA modal length was controlled but this was shorter when compared to the parent strain (Figure 3.5). This is likely a result of increased *wzzE* copy numbers in this strain compared to the single copy in the parent strain. Furthermore, when WzzB or its chimeric variants were expressed, there was increased ECA banding intensity as detected in the anti-ECA Western immunoblot, suggesting increased ECA production was occurring which may be tied with the Oag system. We are unable to explain this phenomenon.

Many groups have hypothesised a potential interaction between WzyB and WzzB. However our group was able to show through the use of the crosslinker DSP, followed by mass spectrometry, that indeed an interaction between these proteins does occur (Nath & Morona 2015). We hypothesised that this interaction is required for function and thus further investigation into this interaction is essential. In this study not only were we able to recreate this interaction, via the use of co-purification and Western immunoblotting, we did so in the absence of the crosslinker DSP, proving a native interaction. We feel the new WzyB-FLAG construct which lacks GFP (Nath & Morona 2015) contributed to detecting this interaction. Additionally, WzzE was shown for the first time to be able to interact with WzyB, however this may be detectable as a result of the absence of other PCP1 proteins in the strains used. The results show that the addition of the WzzB TM2 region, not only increased the activity of the protein *in vivo* but was shown to increase WzzE interaction with WzyB greater than the baseline levels of WzzE interaction. We speculate that Wzz proteins may bind to WzyB in a competitive nature, in that WzzB binds stronger than Wzz<sub>pHS2</sub> which binds stronger than WzzE. Previous studies suggest that decreasing the level of WzyB removed the presence of very long-Oag (90 RU) (controlled by Wzz<sub>pHS2</sub>), suggesting WzzB is able to out compete Wzz<sub>pHS2</sub> for WzyB interaction (Carter et al. 2009).

In conclusion, our findings show that the TM2 region of WzzB and WzzE are important for function in regulating their respective polysaccharide chains, and that WzzE is able to partially act on the Oag pathway by controlling modal length. Furthermore, we have shown that WzzE with only the WzzB's TM2 region is able to increase Oag modal length control significantly compared to the WT WzzE. We have also shown a direct interaction between WzzB without the use of a crosslinker, and a novel interaction of WzzE with WzyB detected in the absence of other PCP proteins and this interaction was increased when the TM2 of WzzB was substituted into the protein. These findings pave the way for future studies that focus on the TM2 region in order to fully elucidate which amino acids are involved in binding to Wzy proteins.

### 3.7 Article References

---

- Barr K, Klena J, Rick PD. 1999. The modality of enterobacterial common antigen polysaccharide chain lengths is regulated by o349 of the *wec* gene cluster of *Escherichia coli* K-12. *J Bacteriol* 181:6564-8.
- Carter JA, Jimenez JC, Zaldivar M, Alvarez SA, Marolda CL, Valvano MA, Contreras I. 2009. The cellular level of O-antigen polymerase Wzy determines chain length regulation by WzzB and WzzpHS-2 in *Shigella flexneri* 2a. *Microbiology* 155:3260-9.
- Collins RF, Kargas V, Clarke BR, Siebert CA, Clare DK, Bond PJ, Whitfield C, Ford RC. 2017. Full-length, oligomeric structure of Wzz determined by cryo-electron microscopy reveals insights into membrane-bound states. *Structure* 25:806-815 e3.
- Daniels C, Morona R. 1999. Analysis of *Shigella flexneri* wzz (Rol) function by mutagenesis and cross-linking: Wzz is able to oligomerize. *Mol Microbiol* 34:181-94.
- Daniels C, Vindurampulle C, Morona R. 1998. Overexpression and topology of the *Shigella flexneri* O-antigen polymerase (Rfc/Wzy). *Molecular Microbiology* 28:1211-1222.
- Dell A, Oates J, Lugowski C, Romanowska E, Kenne L, Lindberg B. 1984. The enterobacterial common-antigen, a cyclic polysaccharide. *Carbohydr Res* 133:95-104.
- Islam ST, Huszczyński SM, Nugent T, Gold AC, Lam JS. 2013. Conserved-residue mutations in Wzy affect O-antigen polymerization and Wzz-mediated chain-length regulation in *Pseudomonas aeruginosa* PAO1. *Sci Rep* 3:3441.
- Kalynyč S, Morona R, Cygler M. 2014. Progress in understanding the assembly process of bacterial O-antigen. *FEMS Microbiol Rev* 38:1048-65.
- Kalynyč S, Valvano MA, Cygler M. 2012. Polysaccharide co-polymerases: the enigmatic conductors of the O-antigen assembly orchestra. *Protein Eng Des Sel* 25:797-802.
- Kenne L, Lindberg B, Petersson K. 1977. Basic structure of the oligosaccharide repeating-unit of the *Shigella flexneri* O-antigens. *Carbohydr Res* 56:363-70.
- Kuhn HM, Neter E, Mayer H. 1983. Modification of the lipid moiety of the enterobacterial common antigen by the "Pseudomonas factor". *Infect Immun* 40:696-700.
- Larue K, Kimber MS, Ford R, Whitfield C. 2009. Biochemical and structural analysis of bacterial O-antigen chain length regulator proteins reveals a conserved quaternary structure. *Journal of Biological Chemistry* 284:7395-7403.

- Lugtenberg B, Meijers J, Peters R, van der Hoek P, van Alphen L. 1975. Electrophoretic resolution of the "major outer membrane protein" of *Escherichia coli* K12 into four bands. FEBS Lett 58:254-8.
- Morona R, Purins L, Tocilj A, Matte A, Cygler M. 2009. Sequence-structure relationships in polysaccharide co-polymerase (PCP) proteins. Trends Biochem Sci 34:78-84.
- Murray GL, Attridge SR, Morona R. 2003. Regulation of *Salmonella typhimurium* lipopolysaccharide O antigen chain length is required for virulence; identification of FepE as a second Wzz. Mol Microbiol 47:1395-406.
- Nath P, Morona R. 2015. Mutational analysis of the major periplasmic loops of *Shigella flexneri* Wzy: identification of the residues affecting O-antigen modal chain length control, and Wzz-dependent polymerization activity. Microbiology 161:774-85.
- Nath P, Tran ENH, Morona R. 2015. Mutational analysis of the *Shigella flexneri* O-antigen polymerase Wzy: identification of Wzz-dependent Wzy mutants. Journal of Bacteriology 197:108-119.
- Nath, Morona. 2015. Detection of Wzy/Wzz interaction in *Shigella flexneri*. Microbiology 161:1797-1805.
- Papadopoulos M, Morona R. 2010. Mutagenesis and chemical cross-linking suggest that Wzz dimer stability and oligomerisation affect lipopolysaccharide O-antigen modal chain length control. Journal of Bacteriology 192:3385-3393.
- Papadopoulos M, Tran EN, Murray GL, Morona R. 2016. Conserved transmembrane glycine residues in the *Shigella flexneri* polysaccharide co-polymerase protein WzzB influence protein-protein interactions. Microbiology 162:921-9.
- Phan MD, Peters KM, Sarkar S, Lukowski SW, Allsopp LP, Gomes Moriel D, Achard ME, Totsika M, Marshall VM, Upton M, Beatson SA, Schembri MA. 2013. The serum resistome of a globally disseminated multidrug resistant uropathogenic *Escherichia coli* clone. PLoS Genet 9:e1003834.
- Purins L, Van Den Bosch L, Richardson V, Morona R. 2008. Coiled-coil regions play a role in the function of the *Shigella flexneri* O-antigen chain length regulator Wzz<sub>pHS2</sub>. Microbiology 154:1104-1116.
- Raetz CR, Whitfield C. 2002. Lipopolysaccharide endotoxins. Annu Rev Biochem 71:635-700.
- Rai AK, Mitchell AM. 2020. Enterobacterial common antigen: synthesis and function of an enigmatic molecule. mBio 11.

Schmidt G, Mannel D, Mayer H, Whang HY, Neter E. 1976. Role of a lipopolysaccharide gene for immunogenicity of the enterobacterial common antigen. *J Bacteriol* 126:579-86.

Tran EN, Morona R. 2013. Residues located inside the *Escherichia coli* FepE protein oligomer are essential for lipopolysaccharide O-antigen modal chain length regulation. *Microbiology* 159:701-14.

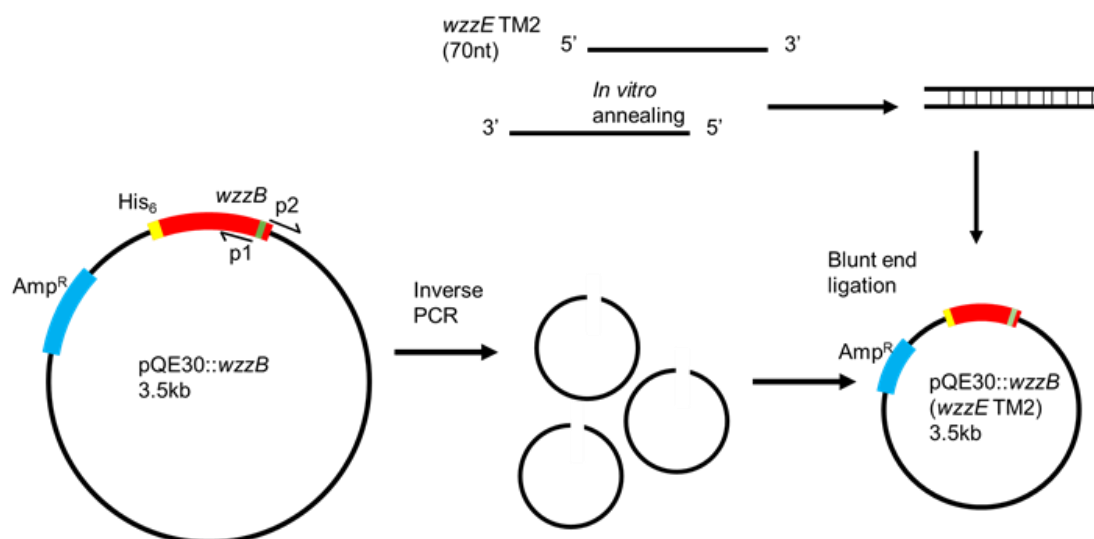
Tran EN, Papadopoulos M, Morona R. 2014. Relationship between O-antigen chain length and resistance to colicin E2 in *Shigella flexneri*. *Microbiology-Sgm* 160:589-601.

Whitfield C, Wear SS, Sande C. 2020. Assembly of bacterial capsular polysaccharides and exopolysaccharides. *Annu Rev Microbiol* doi:10.1146/annurev-micro-011420-075607.

Whitfield C, Williams DM, Kelly SD. 2020. Lipopolysaccharide O-antigens-bacterial glycans made to measure. *J Biol Chem* 295:10593-10609.

## 3.8 Article Supporting Information

### 3.8.1 Supporting Figures



**Figure 3.9 [S1]: Overview of Inverse PCR cloning.**

Inverse PCR and *in vitro* oligonucleotide annealing cloning used to make the chimeric constructs in pQE30

(a) WzzB *S. flexneri* 2457T

```
1      MRTWKFPSVR VMMRVENNNV SGQNHDPQEI DLIDLLVQLW RGKMTIIISV IVAIALAIGY
61     LAVAKEKWTS TAIITQPDVG QIAGYNNAMN VIYGQAAPKV SDLQETLIGR FSSAFSALAE
121    TLDNQEEPEK LTIEPSVKNQ QLPLTVSYVG QTAEGAQMKL AQYIQQVDDK VNQELEKDLK
181    DNIALGRKNL QDSLRTQEVV AQEQKDLRIR QIQEALQYAN QAQVTKPQVQ QTEDVTQDTL
241    FLLGSEALES MIKHEATRPL VFSPNYYQTR QNLLDIEKLN FDDLDIHAYR YVMKPTLPIR
301    RDSPKKAITL ILAVLLGGMV GAGIVLGRNA LRNYNAK
```

(b) WzzE *S. flexneri* 2457T

```
1      MTQPMPGKPA EDAENELDIR GLFRTLWAGK LWIIGMGLAF ALIALAYTFF ARQEWSSTAI
61     TDRPTVNMLG GYYSQQQFLR NLDVRSNMAS ADQPSVMDEA YKEFVMQLAS WDTRREFWLQ
121    TDYYKQRMVG NSKADAALLD EMINNIQFIP GDFTRAVNDS VKLIAETAPD ANNLLRQYVA
181    FASQRAASHL NDELKGAWAA RTIQMKAQVK RQEEVAKAIY DRRMNSIEQA LKIAEQHNIS
241    RSATDVPAAE LPDSEMFLG RPMLQARLEN LQAVGPAFDL DYDQNRAMLN TLNVGPTLDP
301    RFQTYRYLRT PEEPVKRDSP RRAFLMIMWG IVGGLIGAGV ALTRRCSK
```

(c) WzzB<sup>ETM1</sup>

```
1      MRTWKFPSVR VMMRVENNNV SGQNHDPQEI DLIDLLVQLW RGKLWIIGMG LAFALIALAY
61     TFFAKEKWTS TAIITQPDVG QIAGYNNAMN VIYGQAAPKV SDLQETLIGR FSSAFSALAE
121    TLDNQEEPEK LTIEPSVKNQ QLPLTVSYVG QTAEGAQMKL AQYIQQVDDK VNQELEKDLK
181    DNIALGRKNL QDSLRTQEVV AQEQKDLRIR QIQEALQYAN QAQVTKPQVQ QTEDVTQDTL
241    FLLGSEALES MIKHEATRPL VFSPNYYQTR QNLLDIEKLN FDDLDIHAYR YVMKPTLPIR
301    RDSPKKAITL ILAVLLGGMV GAGIVLGRNA LRNYNAK
```

(d) WzzE<sup>ETM1</sup>

```
1      MTQPMPGKPA EDAENELDIR GLFRTLWAGK MTIIISVIVA IALAIGYLAV ARQEWSSTAI
61     TDRPTVNMLG GYYSQQQFLR NLDVRSNMAS ADQPSVMDEA YKEFVMQLAS WDTRREFWLQ
121    TDYYKQRMVG NSKADAALLD EMINNIQFIP GDFTRAVNDS VKLIAETAPD ANNLLRQYVA
181    FASQRAASHL NDELKGAWAA RTIQMKAQVK RQEEVAKAIY DRRMNSIEQA LKIAEQHNIS
241    RSATDVPAAE LPDSEMFLG RPMLQARLEN LQAVGPAFDL DYDQNRAMLN TLNVGPTLDP
301    RFQTYRYLRT PEEPVKRDSP RRAFLMIMWG IVGGLIGAGV ALTRRCSK
```

(e) WzzB<sup>ETM2</sup>

```
1      MRTWKFPSVR VMMRVENNNV SGQNHDPQEI DLIDLLVQLW RGKMTIIISV IVAIALAIGY
61     LAVAKEKWTS TAIITQPDVG QIAGYNNAMN VIYGQAAPKV SDLQETLIGR FSSAFSALAE
121    TLDNQEEPEK LTIEPSVKNQ QLPLTVSYVG QTAEGAQMKL AQYIQQVDDK VNQELEKDLK
181    DNIALGRKNL QDSLRTQEVV AQEQKDLRIR QIQEALQYAN QAQVTKPQVQ QTEDVTQDTL
241    FLLGSEALES MIKHEATRPL VFSPNYYQTR QNLLDIEKLN FDDLDIHAYR YVMKPTLPIR
301    SPRRAFLMIM WG IVGGLIG AGVALGRNA LRNYNAK
```

(f) WzzE<sup>ETM2</sup>

```
1      MTQPMPGKPA EDAENELDIR GLFRTLWAGK LWIIGMGLAF ALIALAYTFF ARQEWSSTAI
61     TDRPTVNMLG GYYSQQQFLR NLDVRSNMAS ADQPSVMDEA YKEFVMQLAS WDTRREFWLQ
121    TDYYKQRMVG NSKADAALLD EMINNIQFIP GDFTRAVNDS VKLIAETAPD ANNLLRQYVA
181    FASQRAASHL NDELKGAWAA RTIQMKAQVK RQEEVAKAIY DRRMNSIEQA LKIAEQHNIS
241    RSATDVPAAE LPDSEMFLG RPMLQARLEN LQAVGPAFDL DYDQNRAMLN TLNVGPTLDP
301    RFQTYRYLRT PEEPVKRDSP KKAITLILAV LLGGMVGAGI VLTRRCSK
```

(g) WzzB<sup>ETM1&2</sup>

```
1      MRTWKFPSVR VMMRVENNNV SGQNHDPQEI DLIDLLVQLW RGKLWIIGMG LAFALIALAY
61     TFFAKEKWTS TAIITQPDVG QIAGYNNAMN VIYGQAAPKV SDLQETLIGR FSSAFSALAE
121    TLDNQEEPEK LTIEPSVKNQ QLPLTVSYVG QTAEGAQMKL AQYIQQVDDK VNQELEKDLK
181    DNIALGRKNL QDSLRTQEVV AQEQKDLRIR QIQEALQYAN QAQVTKPQVQ QTEDVTQDTL
241    FLLGSEALES MIKHEATRPL VFSPNYYQTR QNLLDIEKLN FDDLDIHAYR YVMKPTLPIR
```

301 RDSPRRAFLM IMWGIVGGLI GAGVALGRNA LRNYNAK

(h) WzzE<sup>BTM1&2</sup>

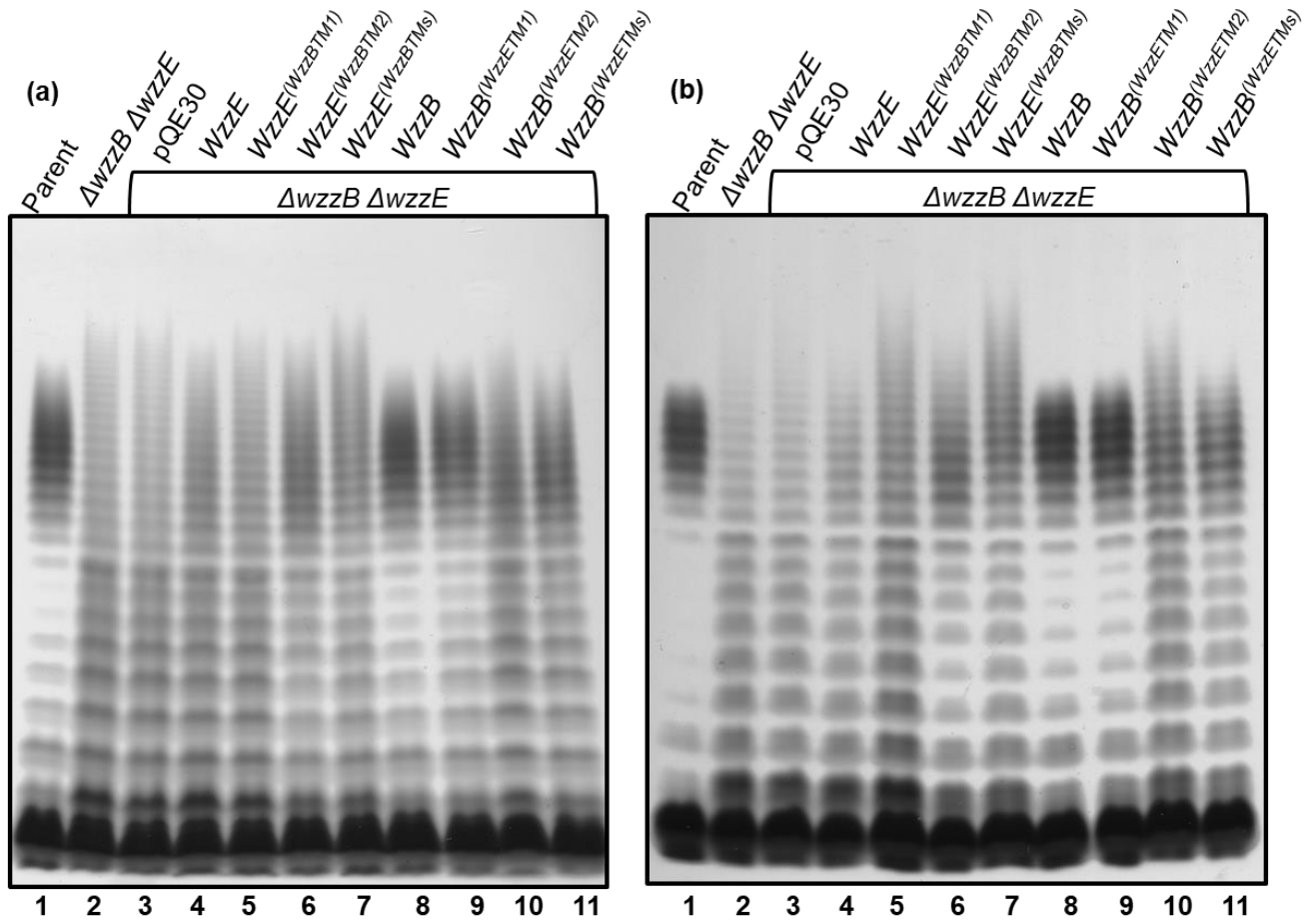
1 MTQPMPGKPA EDAENELDIR GLFRTLWAGK MTIIISVIVA IALAIGYLAV ARQEWSSTAI  
61 TDRPTVNMLG GYYSQQQFLR NLDVRSNMAS ADQPSVMDEA YKEFVMQLAS WDTRREFWLQ  
121 TDYKQRMVG NSKADAALLD EMINNIQFIP GDFTRAVNDS VKLIAETAPD ANNLLRQYVA  
181 FASQRAASHL NDELKGAWAA RTIQMKAQVK RQEEVAKAIY DRRMNSIEQA LKIAEQHNIS  
241 RSATDVPAAE LPDSEMFLLG RPMLQARLEN LQAVGPAFDL DYDQNRAMLN TLNVGPTLDP  
301 RFQTYRYLRT PEEPVKRDSPP KKAITLILAV LLGGMVGAGI VLTRRCSK

---

**Figure 3.10 [S2]: Amino acid sequences of Wzz chimeras.**

Amino acid sequences of the WT Wzz, and Wzz chimera proteins and of the chimeric proteins generated in this study (changed regions are highlighted in grey). (a) WzzB, (b) WzzE, (c) WzzB<sup>ETM1</sup>, (d) WzzB<sup>ETM2</sup>, (e) WzzB<sup>ETM1&2</sup>, (f) WzzE<sup>BTM1</sup>, (g) WzzE<sup>BTM2</sup>, (h) WzzE<sup>BTM1&2</sup>

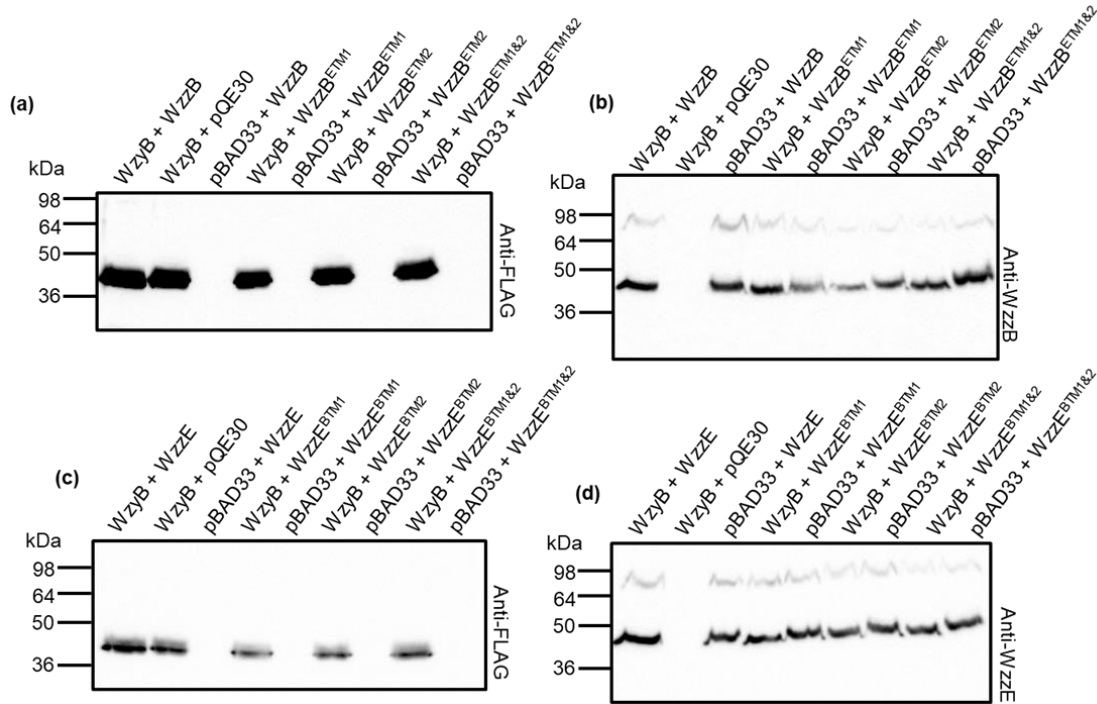
---



**Figure 3.11 [S3]: Analysis of the LPS profiles of strains expressing chimeric Wzz proteins replicates for densitometry.**

Silver-stained PAGE separation of the LPS from parent *S. flexneri* Y  $\Delta W_{zzpHS2}$  and  $\Delta wzzE/wzzB::kan^R$ , complemented with co-polymerase chimeras. Strains grown to log-phase were collected ( $1 \times 10^9$  cells) and lysed in lysis buffer in the presence of proteinase K. Samples were electrophoresed on a SDS-15% (w/v) PAGE gel, and silver-stained (a) replicate 2 (b) replicate 3.





**Figure 3.12 [S4]: Solubilised samples containing WzyB and Wzz protein prior to purification**

*S. flexneri* Y  $\Delta wzzE/wzzB::kan^R$  expressing WzyB and Wzz or chimeric proteins, solubilised whole membrane fractions prior to purification analysed by Western immune blotting (see materials and methods). Samples were electrophoresed on SDS-12% (w/v) polyacrylamide gels followed by Western immunoblotting with (a and c) polyclonal FLAG antibody, or (b) polyclonal WzzB antibody or (d) polyclonal WzzE antibody. The migration positions of the molecular mass standards (in kDa) are indicated on the left-hand side.

### 3.8.2 Supporting Table

**Table 3.3 [ST1]: Oligonucleotides used in this study**

Oligonucleotide	Description
5' acgccacaactgcactag 3'	Forward PCR primer used for inverse PCR used to create WzzB <sup>ETM1</sup>
5' aaggagaaatggacgtcaaca 3'	Reverse PCR primer used for inverse PCR used to create WzzB <sup>ETM1</sup>
5' gggaagctatggattattggcatggggctggcgttgcgtaatcgcgctggcgtatacttttttgc 3'	WzzE TM1 coding DNA strand
5' agcaaaaaagtatacggcagcgcgattaacgcaaacgccagcccatgccaataatccatagcttccc 3	WzzE TM1 complementary DNA strand
5' agcccacaaggtacgaaac 3'	Forward PCR primer used for inverse PCR used to create WzzE <sup>BTM1</sup>
5' cgtcaggagtggagctcg 3'	Reverse PCR primer used for inverse PCR used to create WzzE <sup>BTM1</sup>
5' ggcaagatgacaattatcatttccgtcattgtggctattgcctggctattggtatttggcagtagcg 3'	WzzB TM1 coding DNA strand
5' cgctactgccaataaccaatagccagggcaatagccacaatgacggaaatgataattgcatcttggc 3'	WzzB TM1 complementary DNA strand
5' atcgcgacgaatagtaacg 3'	Forward PCR primer used for inverse PCR used to create WzzB <sup>ETM2</sup>
5' gggcgtaacgctctgc 3'	Reverse PCR primer used for inverse PCR used to create WzzB <sup>ETM2</sup>
5' agcccacgtcgtgccttctgatgattatgtggggcattgtcgggggctgatcggggctggtgtcgcatta 3'	WzzE TM2 coding DNA strand
5' taatgcgacaccagccccgatcagcccccgacaatgccccacataatcatcaggaaggcacgacgtgggct 3'	WzzE TM2 complementary DNA strand
5' atcgcgtttaccggttcttc 3'	Forward PCR primer used for inverse PCR used to create WzzE <sup>BTM2</sup>
5' acccgccgttgcctcg 3'	Reverse PCR primer used for inverse PCR used to create WzzE <sup>BTM1</sup>
5' agtccgaaaaaggcaatcaccttgattctggcagtgcttctggcgggcatggttggcgcggggattgtgttg 3'	WzzB TM2 coding DNA strand
5' caacacaatccccgcgccaaccatgccgcccagaagcactgccagaatcaaggtgattgccttttcggact 3	WzzB TM2 complementary DNA strand

### **3.9 Article Acknowledgements**

---

Funding for this work is provided by a Discovery Project Grant to Renato Morona from the Australian Research Council (PROJECT ID: DP160103903). V. Leo is the recipient of a Research Training Program Stipend Research Scholarship from the University of Adelaide.

# Chapter Four

---

## ARTICLE 2:

**Identification of a region in *Shigella flexneri* WzyB disrupting the interaction with Wzz<sub>pHS2</sub>.**

**Vincenzo Leo, Min Yan Teh, Elizabeth Tran and Renato Morona**

---

## Chapter 4: Research Article 2: Identification of a region in *Shigella flexneri* WzyB disrupting the interaction with WZZ<sub>pHS2</sub>.

---

### 4.1 Statement of Authorship

---

Title of Paper	Identification of a region in <i>Shigella flexneri</i> WzyB disrupting the interaction with WZZ <sub>pHS2</sub> .
Status	Under review

Author Contributions: By signing the Statement of Authorship, each author certifies that: (i) the candidate's stated contribution to the publication is accurate, (ii) permission is granted for the candidate to include the publication in the thesis; and (iii) the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Author	Vincenzo Leo		
Contribution	Construction of strains and plasmids, conducted all experiments pertaining to all Figures and constructed these figures, data analysis, conception of model, and writing of manuscript.		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	28/04/20

Author	Min Yan Teh		
Contribution	Generation of main expression construct from this study and generation of preliminary data.		
Signature		Date	28/04/20

Author	Elizabeth Ngoc Hoa Tran		
Contribution	Supervised development of work and generation of preliminary data. Data analysis, conception of model, and editing and evaluation of the manuscript.		
Signature		Date	28/04/20

Author	Renato Morona		
Contribution	Supervised development of work, provision of laboratory and materials, manuscript evaluation and editing, acting corresponding author.		
Signature		Date	28/04/20

## 4.2 Article Abstract

---

*Shigella flexneri* possess the ability to synthesise complex polysaccharide chains at a controlled number of repeating units. The O antigen (Oag) component of *S. flexneri* lipopolysaccharide which is synthesised by the most common polysaccharide synthesis pathway used in bacteria, known as the Wzy-dependent pathway, has implications for a range of biological activities that include: antibiotic resistance, immune system avoidance and cell to cell spread. The Oag is polymerized into chains via the inner membrane protein WzyB, while the polysaccharide co-polymerases WzzB and Wzz<sub>pHS2</sub> modulate the number of repeat units in the chains or “the modal length” of the polysaccharide into two groups, termed short-type and very long-type. Our data shows for the first time a direct interaction between WzyB and Wzz<sub>pHS2</sub> with the presence and absence of a crosslinker dithiobis (succinimidyl propionate) (DSP). Additionally a novel set of mutants generated via random and site directed mutagenesis identify a region of WzyB that diminishes function and significantly decreases very-long Oag chain polymerisation, and that affected the aforementioned interaction. These results provide insight into the complex mechanism underlying the control of Oag biosynthesis.

### 4.3 Article Introduction

---

*Shigella flexneri* bacteria have the ability to synthesise many complex polysaccharides chains that are critical for virulence, bacterial structural stability and environmental interaction. Complex polysaccharide chains such as the O antigen (Oag) element of lipopolysaccharide (LPS), and the chemically unrelated Enterobacterial Common Antigen (ECA), are synthesised by variants of the Wzy-dependent pathway (Rai & Mitchell 2020; Whitfield, Wear, et al. 2020).

*S. flexneri* LPS is comprised of three domains: (1) lipid A, a hydrophobic lipid that anchors the molecule to the outer membrane; (2) core oligosaccharide, a non-repeating domain; (3) Oag, a polysaccharide that varies in number of repeat units (RU) per LPS molecule. The Oag RUs are attached to the lipid A component via the core sugars (Raetz & Whitfield 2002). In *S. flexneri* Y-serotype strains, the Oag is comprised of tetrasaccharide RUs which contain N-acetyl-glucosamine (GlcNAc), and rhamnose (Rha) (Kenne et al. 1977).

The Wzy-dependent polysaccharide synthesis mechanism is the most commonly used system in bacteria to produce Oag. It takes place in the inner membrane, and requires a set of integral membrane proteins (Whitfield, Williams, et al. 2020). In short, the individual RUs bound to the lipid carrier undecaprenyl diphosphate (Und-PP) are transported across the inner membrane from the cytoplasm into the periplasm by the WzxB flippase/transporter, and Oag is then polymerised by the Oag polymerase, WzyB. Finally, The WaaL protein then ligates the Oag chain to the previously synthesised core + lipid A molecules. *S. flexneri* LPS has a bimodal distribution of the Oag chains consisting of short-type Oag (S-Oag) and the very-long-type Oag (VL-Oag) and these are determined by the polysaccharide co-polymerase proteins (PCPs) WzzB and Wzz<sub>pHS2</sub>, respectively (Stevenson et al. 1995).

Despite several studies on *S. flexneri* WzyB and its paralogues, little is known about how WzyB functions in combination with the PCPs, WzzB and Wzz<sub>pHS2</sub>, to polymerise and control Oag length. The *S. flexneri* WzyB protein is approximately 43.7 kDa with 12 transmembrane (TM) domains, 6 periplasmic loops and 5 cytoplasmic loops (Daniels et al. 1998). The proposed “catch and release” model suggests that the Und-PP-Oag RU binds a site on the third periplasmic loop and is then transferred to a second site on the fifth periplasmic loop, mediated by the loop differences in pI, basic vs acidic, respectively (Islam et al. 2013). Conversely in *S. flexneri* WzyB, the pI of the loops appear to play less of a role as both have a similar basic pI (Nath & Morona, R 2015).

In *S. flexneri*, WzzB confers the S-Oag (~10-17 RU) modal length and is a 36 kDa inner membrane protein that is anchored by two TM domains which are located near the N and C termini



of the protein, however, the majority of the protein resides in the periplasmic space. WzzB is a member of the polysaccharide co-polymerase group 1 (PCP1) family of proteins (Morona et al. 2009). Wzz<sub>pHS2</sub>, encoded on a small plasmid called pHS-2, confers the VL-Oag (~90-100 RU) and is a ~ 41 kDa inner membrane protein, that is also a member of the PCP1 family (Morona et al. 2009). The VL-Oag chains are critical for *S. flexneri* as they provide resistance to the host's complement system (Hong & Payne 1997). The S-Oag has been shown to be important for colicin E2 resistance (Tran et al. 2014). Additionally in the absence of S-Oag, the surface expressed IcsA protein can be masked by the VL-Oag leading to defective actin based motility (Van den Bosch & Morona 2003). It is therefore critical for *S. flexneri* to maintain the presence of both S-Oag and VL-Oag to be virulent.

Despite low sequence identity among members the PCP1 family, the monomeric structures of the proteins are remarkably similar. Structural studies on PCP1 proteins show that each form a distinct “bell” shaped structure with different numbers of monomers. The number of monomers that make up each protein is controversial as formation of both hexamers and octamers have been reported (Kalynych et al. 2014; Larue et al. 2009; Tran & Morona 2013). Additionally, *in situ* WzzB has been shown to exist in multiple oligomeric forms at equilibrium with the monomeric form (Papadopoulos & Morona 2010). The full length oligomeric structure of WzzB from *Escherichia coli* and *Salmonella enterica* serovar Typhimurium was resolved using cryo electron microscopy experiments, revealing a potential binding region/pocket for Wzy (Collins et al. 2017; Wiseman et al. 2021). Recently, a biochemical interaction between WzzB and WzyB was shown through co-purification experiments (Leo et al. 2020), thus we hypothesize that an interaction between WzyB and Wzz<sub>pHS2</sub> is also likely.

In this study, we initially performed random mutagenesis and screening of *wzyB* in the absence of WzzB with the aim of finding mutations of WzyB that affected Oag polymerisation. The random mutagenesis led to a region of WzyB (aa 351-357) which appeared to be involved with the production of VL-Oag chains. Upon further investigation residues 352 to 354 appeared to be of most interest leading to further mutagenesis that resulted in several mutants unable to function in Oag polymerisation. Additionally, WzyB interaction with Wzz<sub>pHS2</sub> was detected for the first time via pull down experiments involving the purification of FLAG tagged WzyB, and this interaction was also detected without the aid of a chemical crosslinker. The pull down experiments were repeated using the aforementioned WzyB mutants and a number of these affected the ability of WzyB to interact with Wzz<sub>pHS2</sub> but had no effect on interaction with WzzB. These results identify a region in WzyB that is either directly or indirectly involved with Wzz<sub>pHS2</sub> interaction, and that controls the production of VL-Oag chains that are important for *S. flexneri* virulence.

## 4.4 Article Methods

---

### 4.4.1 Ethics statement.

The WzzB antibodies were produced under the National Health and Medical Research Council Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, and was approved by the University of Adelaide Animal Ethics Committee.

### 4.4.2 Bacterial strains, growth media and growth conditions.

The bacterial strains used in this study are shown in Table 1. Strains were routinely grown on lysogeny broth (LB) agar (10 g Tryptone l<sup>-1</sup>, 5 g yeast extract l<sup>-1</sup>, 5 g NaCl l<sup>-1</sup>, 15 g agar l<sup>-1</sup>) or in LB. Strains carrying pBCKS<sup>+</sup> constructs requiring induction were grown in LB at 37 °C with aeration for 16 h, subcultured 1/20 into fresh broth with 0.01 mM isopropyl β- D -1-thiogalactopyranoside (IPTG) and grown for another 4 h. Antibiotics were used at the following concentrations: 10 mg tetracycline (Tet) ml<sup>-1</sup>, 25 mg chloramphenicol (Cml) ml<sup>-1</sup> and 25 mg rifampicin (Rif) ml<sup>-1</sup>.

### 4.4.3 DNA methods.

The plasmids used in this study are shown in Table 1. Plasmid constructs were extracted from *E. coli* DH5α strains using a QIAprep Spin Miniprep kit (Qiagen). Preparation of electro-competent cells and the electroporation method were performed as described previously (Purins et al. 2008).

### 4.4.4 Construction of WzyB-FLAG-LacZα vector.

Primers were designed to amplify the *wzyB* coding region from pCDRM6 (Daniels et al. 1998), while adding a C-terminal FLAG tag. The amplified region was cloned into pBCKS<sup>(+)</sup> introducing a C-terminal LacZα peptide for blue white screening, and named pRMA5118.

### 4.4.5 Random mutagenesis.

Random mutagenesis of *wzyB* coding region was performed using the GeneMorph EZ-Clone Domain Mutagenesis kit (catalogue number: 200552; Agilent) according to the manufacturer's instructions with primers VL105 and VL106 (Table 4.2 [ST1]). The mutagenized plasmids were transformed into competent *E. coli* DH5α cells. Plasmid DNA was isolated from random colonies, and transformed into RMA4337 (Table 1). Colicin swab assays (as described below) were used to screen for potential sensitive mutants of interest. Plasmid DNA isolated from putative mutants was transformed into DH5α cells for blue-white colony screening to ensure WzyB was being expressed, and subjected to DNA sequencing by AGRF (Australian Genome Research Facility).

#### **4.4.6 Colicin swab assay.**

For swab sensitivity assays, purified colicin E2 protein (Tran et al. 2014) in MilliQ (MQ) water at a concentration of  $6 \mu\text{g ml}^{-1}$  was swabbed onto antibiotic LB agar plates containing 1mM IPTG with a cotton swab and left to dry for 30 min at room temperature (RT). Individual cultures of 1mM IPTG induced bacteria ( $\sim 5 \times 10^8$  cells) were swabbed perpendicular to the colicin E2 streak, and left to dry for 30 min before being incubated for 16 h at 37 °C.

#### **4.4.7 LPS SDS-PAGE and silver staining.**

Bacteria were grown and induced as described above, before  $1 \times 10^9$  cells were harvested by centrifugation, resuspended in 2× lysis buffer (Murray et al. 2003), and heated at 100 °C for 10 min. Lysed cells were then incubated with 2.5 mg/ml proteinase K (Sigma-Aldrich) for 2 h at 56 °C, followed by electrophoresis on 15% SDS-PAGE gels for 13 h at 12 mA. Silver staining of LPS was performed as described previously (Murray et al. 2003).

#### **4.4.8 Protein SDS-PAGE.**

Bacteria were grown and induced as described above, before  $5 \times 10^8$  cells were harvested by centrifugation, resuspended in 2× sample buffer (Lugtenberg et al. 1975) and heated at 100 °C or 37°C (WzyB samples) for 5 min, prior to SDS-PAGE on 12% gels for 1 h at 200 V. Purified WzyB samples were mixed 1:1 with 2× sample buffer (Lugtenberg et al. 1975) with or without  $\beta$ -mercaptoethanol and heated to 37 °C for 5 min, prior to SDS-PAGE on 15% gels for 1 h at 200 V. ECA samples were prepared as described above (LPS SDS-PAGE), followed by SDS-PAGE on 12% gels for 1 h at 200 V.

#### **4.4.9 Western immunoblotting.**

Western transfers were performed at 400 mA for 1 h. Protein gels were then subjected to Western immunoblotting on nitrocellulose membrane (Bio-Rad) with either polyclonal WzzB or Wzz<sub>pHS2</sub> rabbit antibodies (Daniels & Morona 1999) at 1:500 dilution, polyclonal ECA rabbit antibodies (made in house) at 1:500 dilution, or polyclonal FLAG rabbit antibodies (Sigma) at 1:2000 dilution in 2.5% (w/v) skim milk. Detection was performed with either goat anti-rabbit horseradish-peroxidase-conjugated antibodies (KPL) or goat anti-mouse horseradish-peroxidase-conjugated antibodies (KPL) and chemiluminescence reagent (Sigma). 5  $\mu\text{L}$  of BenchMark SeeBlue Plus2 pre-Stained Protein Ladder (Invitrogen) was used as the molecular mass standard.

#### **4.4.10 Purification of WzyB from *S. flexneri* strains.**

Cells were harvested from 100 ml induced culture by centrifugation (Beckman Coulter Avanti J-26XPI centrifuge; 9600 g, 10 min, 4 C°) and the cell pellet was resuspended in sonication

buffer [50 mM Tris/HCl, 150 mM NaCl, pH 7.5] followed by cell lysis via sonication (Branson B15). Cell debris was removed by centrifugation (Thermo Scientific Labofuge 400 R centrifuge; 3500 g, 10 min, 4 °C) and the whole membrane (WM) fraction was collected by ultracentrifugation (Beckman Coulter Optima L-100 XP ultra-centrifuge; 250 000 g, 1 h, 4 °C). WM fraction was then resuspended in 500 µl of MQ water, and 500 µl of 2× solubilisation buffer [100 mM Tris/HCl, 300 mM NaCl, 2% (w/v) n-dodecyl-b-D-maltopyranoside (DDM) (Anatrace)] and mixed for 16 h at 4 °C. Insoluble material was removed by ultracentrifugation (Beckman Coulter Optima Max-XP tabletop ultracentrifuge; 160000 g, 1 h, 4 °C) and the solubilized supernatant was incubated with 100 µl of anti-DYKDDDDK G (FLAG) affinity resin (GenScript) pre-equilibrated with equilibration buffer [50 mM Tris/HCl, 150 mM NaCl, 0.02% (w/v) DDM, pH 8] for 1.5 h at RT. The loaded FLAG affinity resin was then washed with wash buffer [50 mM Tris/HCl, 150 mM NaCl, 0.02% (w/v) DDM, pH 8] several times (10 ml total). Finally, WzyB-FLAG was eluted in 100 µl of elution buffer [0.1 M glycine, 0.02% (w/v) DDM, pH 3.5] at RT and neutralised with 1 M Tris, pH 9.0.

#### **4.4.11 *In vivo* protein crosslinking.**

Cells were harvested from 100 ml induced culture by centrifugation (Beckman Coulter Avanti J-26XPI centrifuge; 9600 g, 10 min, 4 °C) and the cell pellet was washed in DSP crosslinking buffer [20 mM sodium phosphate buffer (Na<sub>2</sub>PO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub>), 150 mM NaCl, pH 7.5]. The pellets were then resuspended in 5 ml DSP crosslinking buffer followed by incubation with 1 mM DSP (Thermo Fischer Scientific) [treated samples (T)] for 30 min at 37 °C. A duplicate sample was also incubated without DSP [untreated samples (UT)]. Excess DSP was quenched with 20 mM Tris/HCl, pH 7.5 and left for 10 min at RT, before resuspension in sonication buffer and purification (as described above).

## 4.5 Article Results

---

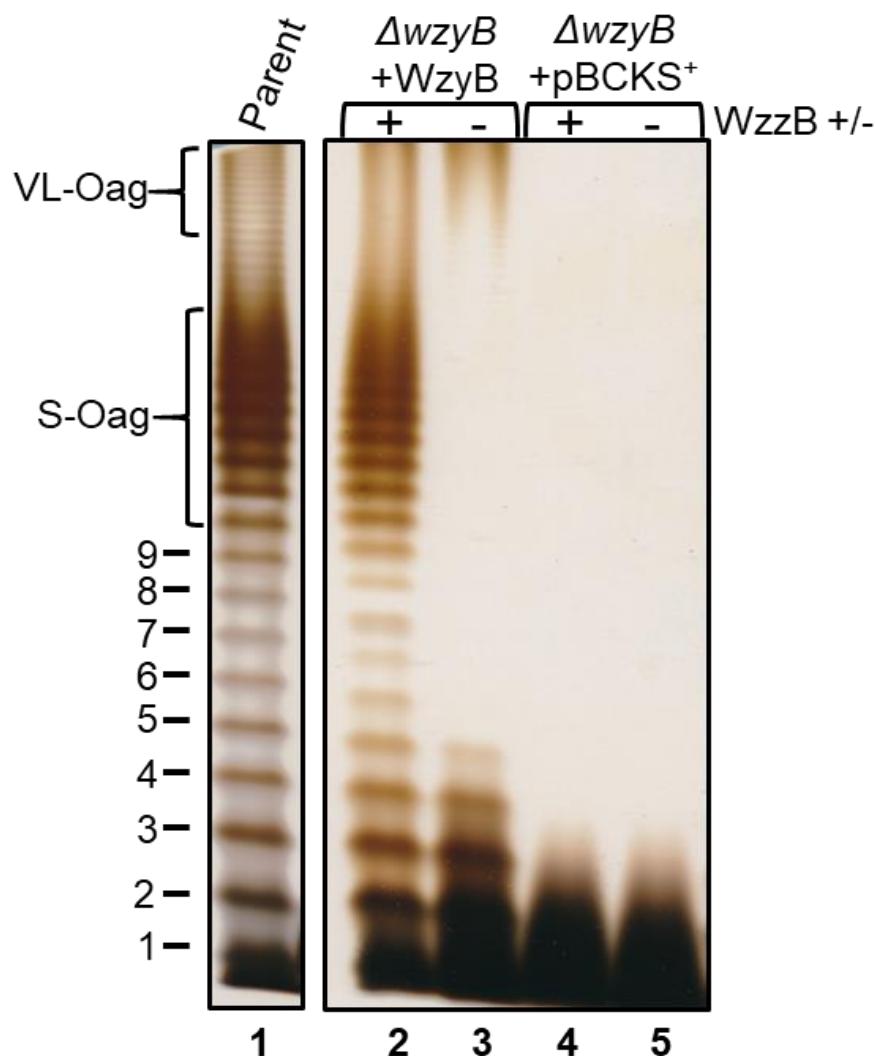
### 4.5.1 Construction and complementation analysis of a WzyB-FLAG-LacZ expression construct.

A suitable WzyB expression system for eventual random mutagenesis and purification was constructed. The *S. flexneri* *wzyB* coding region was amplified from pCDRM6 (Daniels et al. 1998) to include a FLAG tag and cloned into pBCKS<sup>(+)</sup> introducing a LacZ $\alpha$  peptide coding region at the C terminus, for blue-white colony screening, and was named pRMA5118 (Table 4.1). To investigate if the new WzyB construct was able to complement a *wzyB* mutant in the presence and absence of WzzB, pRMA5118, along with the vector control pBCKS<sup>(+)</sup>, were transformed into both RMM109 (*S. flexneri* Y  $\Delta$ *wzyB*) and RMA4337 (*S. flexneri* Y  $\Delta$ *wzyB* *wzzB::tet<sup>R</sup>*) and the LPS was analysed via SDS-PAGE and silver staining (Figure 4.1). As expected when WzyB-FLAG-LacZ (referred to hereafter as WzyB) was expressed, the LPS profile was comparable to the parent strain (Figure 4.1, lanes 2 and 1 respectively). Additionally, when WzyB was expressed in the absence of WzzB, only LPS with VL-Oag was detected (Figure 4.1, lane 3). Strains harbouring only the pBCKS<sup>(+)</sup> vector control, did not produced polymerised Oag, as expected (Figure 4.1, Lanes 4 and 5).

**Table 4.1: Bacterial strains and plasmids**

Strains and plasmids	Description	Reference
<b>Strains</b>		
RMA4199	<i>S. flexneri</i> PE638 Y serotype, this strain lacks the virulence plasmid (parent)	Laboratory stock
RMM109	<i>S. flexneri</i> PE638 Y serotype <i>wzyB</i>	(Morona et al. 1994)
RMA4337	<i>S. flexneri</i> PE638 Y serotype <i>wzyB wzzB:tet<sup>R</sup></i>	Laboratory stock
VLRM253	RMM109 + pVLRM23 (pBCKS <sup>(+)</sup> encoding WzyB <sup>I351A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM254	RMA4337 + pVLRM23 (pBCKS <sup>(+)</sup> encoding WzyB <sup>I351A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM255	RMM109 + pVLRM24 (pBCKS <sup>(+)</sup> encoding WzyB <sup>P352A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM256	RMA4337 + pVLRM24 (pBCKS <sup>(+)</sup> encoding WzyB <sup>P352A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM257	RMM109 + pVLRM25 (pBCKS <sup>(+)</sup> encoding WzyB <sup>M353A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM258	RMA4337 + pVLRM25 (pBCKS <sup>(+)</sup> encoding WzyB <sup>M353A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM259	RMM109 + pVLRM26 (pBCKS <sup>(+)</sup> encoding WzyB <sup>G354A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM260	RMA4337 + pVLRM26 (pBCKS <sup>(+)</sup> encoding WzyB <sup>G354A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM261	RMM109 + pVLRM27 (pBCKS <sup>(+)</sup> encoding WzyB <sup>I355A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM262	RMA4337 + pVLRM27 (pBCKS <sup>(+)</sup> encoding WzyB <sup>I355A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM263	RMM109 + pVLRM28 (pBCKS <sup>(+)</sup> encoding WzyB <sup>F356A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM264	RMA4337 + pVLRM28 (pBCKS <sup>(+)</sup> encoding WzyB <sup>F356A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM265	RMM109 + pVLRM29 (pBCKS <sup>(+)</sup> encoding WzyB <sup>I357A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM266	RMA4337 + pVLRM29 (pBCKS <sup>(+)</sup> encoding WzyB <sup>I357A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM274	RMM109 + pVLRM30 (pBCKS <sup>(+)</sup> encoding WzyB <sup>P352K</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM275	RMA4337 + pVLRM30 (pBCKS <sup>(+)</sup> encoding WzyB <sup>P352K</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM276	RMM109 + pVLRM31 (pBCKS <sup>(+)</sup> encoding WzyB <sup>M353K</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM277	RMA4337 + pVLRM31 (pBCKS <sup>(+)</sup> encoding WzyB <sup>M353K</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM278	RMM109 + pVLRM32 (pBCKS <sup>(+)</sup> encoding WzyB <sup>G354K</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM279	RMA4337 + pVLRM32 (pBCKS <sup>(+)</sup> encoding WzyB <sup>G354K</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM280	RMM109 + pVLRM33 (pBCKS <sup>(+)</sup> encoding WzyB <sup>P352D</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM281	RMA4337 + pVLRM33 (pBCKS <sup>(+)</sup> encoding WzyB <sup>P352D</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM282	RMM109 + pVLRM34 (pBCKS <sup>(+)</sup> encoding WzyB <sup>M353D</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM283	RMA4337 + pVLRM34 (pBCKS <sup>(+)</sup> encoding WzyB <sup>M353D</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM284	RMM109 + pVLRM35 (pBCKS <sup>(+)</sup> encoding WzyB <sup>G354D</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM285	RMA4337 + pVLRM35 (pBCKS <sup>(+)</sup> encoding WzyB <sup>G354D</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM286	RMM109 + pVLRM36 (pBCKS <sup>(+)</sup> encoding WzyB <sup>P352A M353A G354A</sup> -FLAG-LacZ $\alpha$ )	This study
VLRM287	RMA4337 + pVLRM36 (pBCKS <sup>(+)</sup> encoding WzyB <sup>P352A M353A G354A</sup> -FLAG-LacZ $\alpha$ )	This study
<b>Plasmids</b>		
pRMA5118	pBCKS <sup>(+)</sup> encoding WzyB-FLAG-LacZ $\alpha$	This study
pVLRM23	pBCKS <sup>(+)</sup> encoding WzyB <sup>I351A</sup> -FLAG-LacZ $\alpha$	This study
pVLRM24	pBCKS <sup>(+)</sup> encoding WzyB <sup>P352A</sup> -FLAG-LacZ $\alpha$	This study
pVLRM25	pBCKS <sup>(+)</sup> encoding WzyB <sup>M353A</sup> -FLAG-LacZ $\alpha$	This study
pVLRM26	pBCKS <sup>(+)</sup> encoding WzyB <sup>G354A</sup> -FLAG-LacZ $\alpha$	This study
pVLRM27	pBCKS <sup>(+)</sup> encoding WzyB <sup>I355A</sup> -FLAG-LacZ $\alpha$	This study
pVLRM28	pBCKS <sup>(+)</sup> encoding WzyB <sup>F356A</sup> -FLAG-LacZ $\alpha$	This study
pVLRM29	pBCKS <sup>(+)</sup> encoding WzyB <sup>I357A</sup> -FLAG-LacZ $\alpha$	This study
pVLRM30	pBCKS <sup>(+)</sup> encoding WzyB <sup>P352K</sup> -FLAG-LacZ $\alpha$	This study

pVLRM31	pBCKS <sup>(+)</sup> encoding WzyB <sup>M353K</sup> -FLAG-LacZ $\alpha$	This study
pVLRM32	pBCKS <sup>(+)</sup> encoding WzyB <sup>G354K</sup> -FLAG-LacZ $\alpha$	This study
pVLRM33	pBCKS <sup>(+)</sup> encoding WzyB <sup>P352D</sup> -FLAG-LacZ $\alpha$	This study
pVLRM34	pBCKS <sup>(+)</sup> encoding WzyB <sup>M353D</sup> -FLAG-LacZ $\alpha$	This study
pVLRM35	pBCKS <sup>(+)</sup> encoding WzyB <sup>G354D</sup> -FLAG-LacZ $\alpha$	This study
pVLRM36	pBCKS <sup>(+)</sup> encoding WzyB <sup>P352A M353A G354A</sup> -FLAG-LacZ $\alpha$	This study



**Figure 4.1: Complementation of *wzyB* mutant by WzyB-FLAG-LacZ $\alpha$ .**

*S. flexneri* Y strains were grown to log-phase and  $1 \times 10^9$  collected cells were lysed in lysis buffer in the presence of proteinase K. Samples were then electrophoresed on a SDS-15% PAGE gel, and silver-stained (see methods). Number of Oag RUs and Oag modal lengths are shown on the left-hand side. Strains are as follows: Parent *S. flexneri* Y (lane 1), *S. flexneri* Y  $\Delta wzyB$  expressing WzyB (lane 2), or harbouring pBCKS<sup>(+)</sup> control (lane 4), *S. flexneri* Y  $\Delta wzyB$  *wzzB:ter<sup>R</sup>* expressing WzyB (lane 3) or harbouring pBCKS<sup>(+)</sup> control (lane 5).

#### 4.5.2 Random mutagenesis of the *wzyB* coding region.

Due to the lack of 3-D structural data on WzyB, there is relatively little known about how WzyB and Wzz proteins act to regulate the number of RU of Oag. Wzz<sub>pHS2</sub> has been shown to be required for the VL-Oag modal length, however little is known about the interaction between

WzyB and Wzz<sub>pHS2</sub>. To investigate what WzyB residues are needed for function and/or Oag polymerisation, the *wzyB* coding region in plasmid pRMA5118 was subjected to random mutagenesis via an error-prone DNA polymerase, transformed into DH5 $\alpha$  for storage. The mutagenized plasmid library was isolated and transformed into *S. flexneri* Y  $\Delta wzy$  *wzzB:tet<sup>R</sup>* for colicin E2 sensitivity screening (as described in methods). The colicin E2 sensitivity assay was performed in a *wzzB:tet<sup>R</sup>* mutant background to potentially isolate WzyB mutants with a decreased Oag polymerisation activity irrespective of the presence of WzzB, as it has been shown that WzzB can affect WzyB mutant phenotypes (Nath et al. 2015). The colicin E2 screen resulted in ~30 unique strains that were considered to be more sensitive to colicin E2 compared to the WT WzyB expressing strains at the tested concentration (data not shown). The plasmid DNA from the sensitive mutants was then also transformed into *S. flexneri* Y  $\Delta wzy$  for LPS analysis. LPS from *S. flexneri* Y  $\Delta wzy$  or *S. flexneri* Y  $\Delta wzy$  *wzzB:tet<sup>R</sup>* expressing the mutant WzyB proteins were then analysed via SDS-PAGE and silver staining, and the results are summarised in Table 4.3 [ST2] and Figure 4.7 [S1]. The majority of the mutants were classified as WT or comparable to their equivalent strain expressing WT WzyB (class A), or showed no polymerisation activity suggesting an inactivated WzyB (class B). Interestingly, there were mutants that showed decreased levels of Oag polymerisation, (low (class E), extremely low (class D)) and finally mutants that showed decreased VL-Oag chain control (class C) (Table 4.3 [ST2] and Figure 4.7 [S1]). Isolates in class A that appeared to show a WT LPS profile, were disregarded from further experiments. The remaining mutants' plasmid DNA was subjected to DNA sequencing. The amino acid changes observed are listed in Table 4.3 (non-sequenced plasmids are marked with N/A). A number of mutant plasmids expressed WzyB proteins with multiple amino acid changes, resulting in an inability to conclude which amino acid (aa) caused the observed LPS profile, and would require further intensive site directed mutagenesis. However, mutant M353I (#62) was of particular interest as a previous independent screen from our group also identified aa 352 to be important for polymerisation (Nath et al. 2015). As such, aa region 351-357 was selected for further analysis via site directed mutagenesis, as mutant M353I showed a unique profile of decreased modal length control of VL-Oag chains, potentially due to a lessened interaction with Wzz<sub>pHS2</sub> (Figure 4.7 [S1]).

#### **4.5.3 Alanine scanning mutagenesis of WzyB aa 351 to 357**

In order to identify which amino acids in the WzyB region aa 351-357 are important for polymerisation and/or potentially interaction with Wzz<sub>pHS2</sub>, site directed alanine mutagenesis was performed. A universal forward primer and 7 reverse primers with subsequent alanine codon substitutions at each position, were designed (Table 4.2 [ST1]) and used in inverse PCR amplification of pRMA5118 (pBCKS<sup>(+)</sup> *wzyB*-FLAG-LacZ). The resulting mutant plasmids were

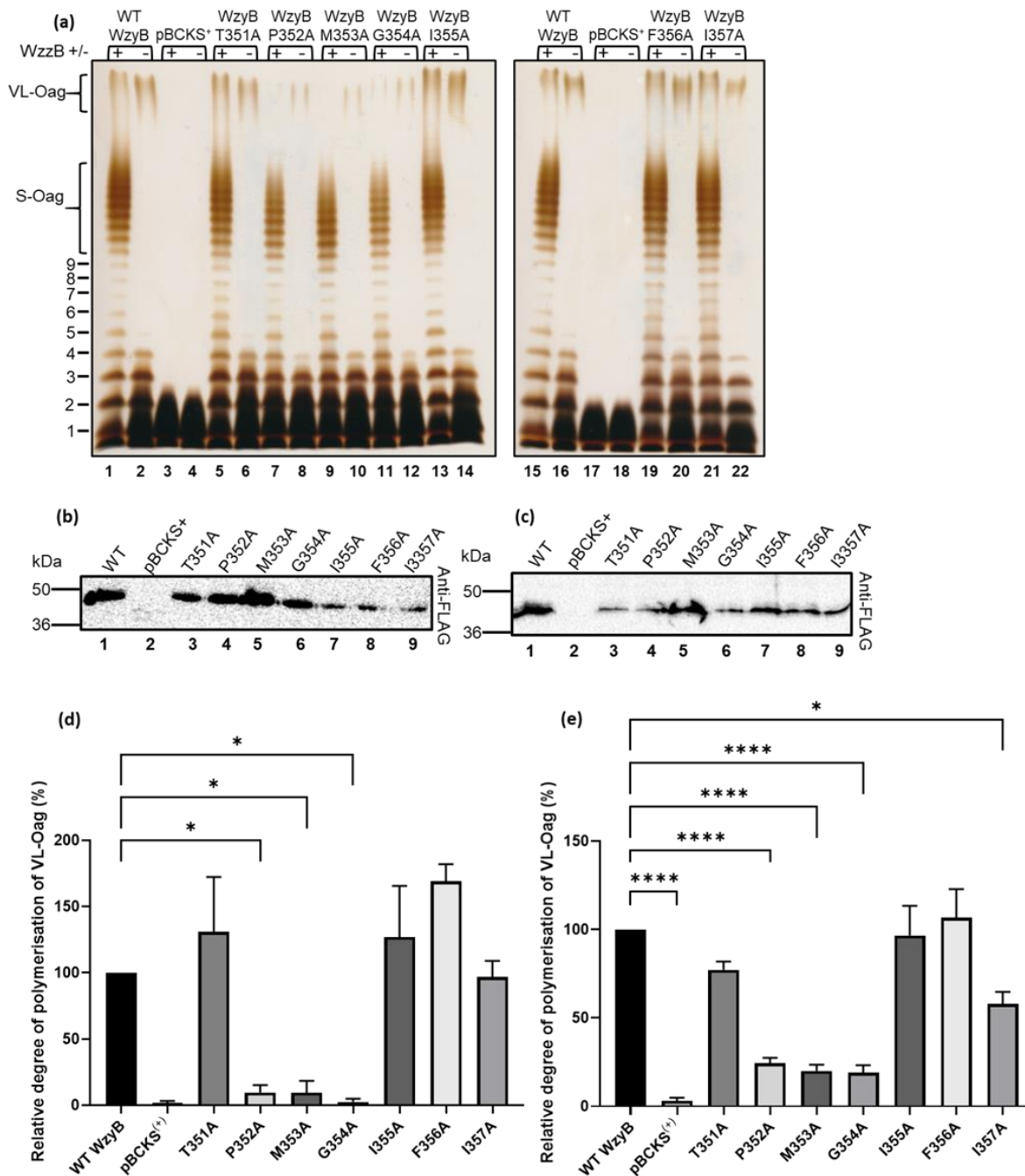


recovered, confirmed with DNA Sanger sequencing and named pVLRM23-29 (Table 4.1) before being transformed into RMM109 and RMA4337, and the resulting strains named VLRM253-266 (Table 4.1). Their LPS profiles were determined via SDS-PAGE and silver staining (Figure 4.2a). As expected, strains harbouring the vector control pBCKS<sup>(+)</sup>, showed no polymerisation of Oag (1 RU) or a semi-rough LPS profile (Figure 4.2a, lanes 3 and 4). Strains expressing WzyB T351A, I355A and F356A all had LPS profiles similar to WzyB WT expressing control strains (Figure 4.2a, lanes 1 and 2), indicating that these residues have little impact on Oag polymerisation / VL-Oag modal length control (Figure 4.2a, lanes 5, 6, 13, 14, 19, 20, 21 and 22). Interestingly, strains expressing WzyB P352A, M353A and G354A, showed LPS profiles comparable to that of the originally identified WzyB M353I mutant (Figure 4.2a, lanes 7-12 and Figure 4.7 [S1] lanes 32 and 33, respectively). In the presence of WzzB, the LPS profile of each of these mutants showed almost no VL-Oag chains and an overall decrease in Oag polymerisation (Figure 4.2a, lanes 7, 9 and 11). In the absence of WzzB, the LPS profiles of these WzyB mutants had decreased levels of VL-Oag chains (Figure 4.2a, lanes 8, 10 and 12) when compared to the WzyB WT expressing control strain (Figure 4.2a, lane 2). Overall in the absence of WzzB these mutants had a lower proportion of LPS with VL-Oag when compared to strains expressing WzzB. In the absence of WzzB, mutant I357A (Figure 4.2a, lane 22) appeared to show a small decrease in LPS with VL-Oag when compared to the respective WzyB WT expressing strain (Figure 4.2a, lane 2).

We then confirmed the production of the mutant WzyB proteins in both the WzzB (+) and (-) backgrounds. As expected, in the WzzB (+) background (Figure 4.2b), Western immunoblotting with anti-FLAG detected bands consistent with the size of WzyB-FLAG-LacZ $\alpha$  (~47 kDa) of varying intensities in all lanes (Figure 4.2b, lanes 1, 3-9) except the pBCKS<sup>(+)</sup> vector control (Figure 4.2b, lane 2). In the WzzB (-) background, again as expected bands were detected in all lanes except the pBCKS<sup>(+)</sup> vector control (Figure 4.2c). The varying protein levels detected indicate that these mutations may alter Wzy stability, however this did not impact the overall level of Oag production as comparable levels of S-Oag was produced.

In order to quantify the relative degree of VL-Oag polymerisation of all strains expressing WzyB alanine substitutions, densitometry was performed on three independent biological replicates of silver stained SDS-PAGE gels (Figure 4.2a and Figure 4.8 [S2] a and b). The degree of polymerisation is presented as the densitometry of VL-Oag LPS molecules normalised to the WzyB WT expressing strain as a percentage. This analysis provides insight into the strains ability to regulate the Oag chain length of the VL variety, thus strains with relatively lower VL-Oag may have either impaired WzyB function or an altered interaction with Wzz<sub>pHS2</sub>. Analysis was performed on both strains with the presence or absence of chromosomal WzzB (Figure 4.2d and

e, respectively). In the presence of WzzB (Figure 4.2d), when WzyB P352A, M353A and G354A were expressed, the relative degree of polymerisation of VL-Oag was significantly lower when compared to the WzyB WT expressing control. When WzyB T351A, I355A, F356A and I357A was expressed, VL-Oag polymerisation was comparable to the WzyB WT expressing strain (Figure 4.2d). In the absence of chromosomal WzzB (Figure 4.2e), when WzyB P352A, M353A, G354A and I357A was expressed, VL-Oag polymerisation was significantly less than the WzyB WT expressing control strain. These results mimic what was detected in the presence of WzzB, with the exception of WzyB I357A. In the absence of WzzB, it appears WzyB I357A strain is producing significantly less VL-Oag when compared to the WzyB WT expressing strain, suggesting that WzzB may be having an effect of the WzyB stability/function as similarly reported previously (Nath et al. 2015). As expected, strains expressing either WzyB T351A, I355A or F356A, produce VL-Oag at a comparable level to the WzyB WT expressing control. Collectively, these results suggest that mutational alteration of aa 352-354 causes a significant decrease in VL-Oag polymerisation, potentially via decreased WzyB protein activity and/or hindered interaction with Wzz<sub>pHS2</sub>.



**Figure 4.2: Alanine scanning of WzyB aa region 351 to 357.**

(a) *S. flexneri* Y strains were grown to log-phase and  $1 \times 10^9$  collected cells were lysed in lysis buffer in the presence of proteinase K. Samples were then electrophoresed on a SDS-15% (w/v) PAGE gel, and silver-stained (see methods). Parent *S. flexneri* Y  $\Delta wzyB$  (indicated with WzzB +) expressing WT WzyB (lane 1), expressing WzyB T351A, P352A, M353A, G354A, I355A, F356A or I357A (lanes 5, 7, 9, 11, 13, 19, 21, respectively) or harbouring pBCKS<sup>(+)</sup> vector control (lane 3). *S. flexneri* Y  $\Delta wzyB wzzB: tet^R$  (indicated with WzzB -) expressing WT WzyB (lane 2), expressing WzyB T351A, P352A, M353A, G354A, I355A, F356A or I357A (lanes 6, 8, 10, 12, 14, 20, 22, respectively) or harbouring pBCKS<sup>(+)</sup> vector control (lane 4). Whole-cell lysates ( $5 \times 10^8$  cells) of *S. flexneri* Y  $\Delta wzyB$  (b), or *S. flexneri* Y  $\Delta wzyB wzzB: tet^R$  (c), expressing the indicated proteins were separated by SDS-PAGE, transferred onto a nitrocellulose membrane and probed with monoclonal mouse FLAG antibodies. Analysis of polymerisation of VL-Oag via densitometry of WzzB +ve strains (d) and WzzB -ve strains (e). The degree of polymerisation of VL-Oag is represented as the densitometry of the VL-Oag (indicated by bracket) as a percentage relative to the WT. Data represents 3 independent experiments with SEM shown, and significance is calculated with a one-way ANOVA, \*  $P < 0.033$ , \*\*  $P < 0.0021$ , \*\*\*  $P < 0.0002$ , and \*\*\*\*  $P < 0.0001$ .

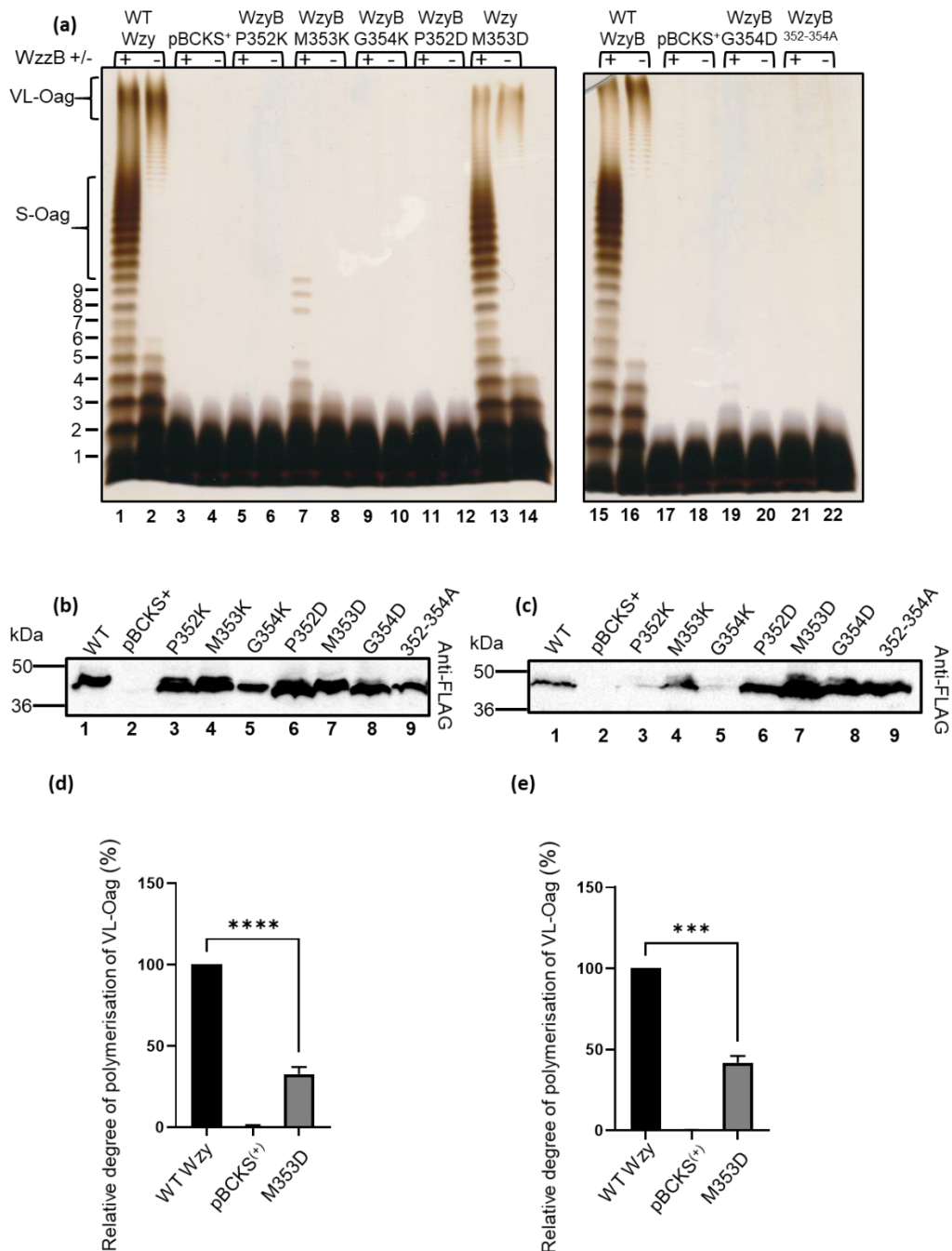
#### 4.5.4 Further site-directed mutagenesis of WzyB aa 352-354

To further investigate the WzyB region of interest, site directed mutagenesis was performed on pRMA5118 to substitute each of the three aa (P352, M353 and G354) to either a lysine or an aspartate, or a triple alanine mutation. The aforementioned universal primer and 7 reverse primers with subsequent lysine or aspartate codon substitutions, as well as a triple alanine substitution were designed (Table 4.2 [ST1]) and used in inverse PCR amplification of the pRMA5118. The resulting mutant plasmids were generated and confirmed by DNA Sanger sequencing and named VLRM30-36 (Table 4.1) before being transformed into RMM109 and RMA4337 and named VLRM274-287 (Table 4.1). The LPS profiles of the strains expressing the WzyB mutants, was then determined via SDS-PAGE and silver staining (Figure 4.3a). The substitution to either a lysine or an aspartate had a more pronounced impact of the WzyB function; substitutions P352K, G354K, P352D as well as the triple alanine substitution all resulted in a non-functional WzyB protein as no polymerised Oag was detected in both WzzB (+/-) backgrounds (Figure 4.3a, lanes 5, 6, 9, 10, 11, 12, 21 and 22, respectively). Mutant M353K showed a low level of Oag polymerisation, as only LPS with very short chains (Oag RU bands 2-9) was detectable by silver staining in the WzzB (+) background (Figure 4.3a, lanes 7). Similarly G354D, showed extremely low WzyB function with only a single band at ~3 Oag RU detected in the WzzB (+) background (Figure 4.3a, lane 19). Mutant M353D showed a decreased level of VL-Oag chains (Fig 3a, lanes 13 and 14) compared to the WzyB WT (Figure 4.3a, lanes 1 and 2) expressing strains in both WzzB (+/-) backgrounds. As to be expected the vector control strains did not show any polymerisation of Oag (Figure 4.3a, lanes 3 and 4). Collectively, these results suggest that aa 352-354 of WzyB are critical to its function.

The protein expression of the mutant WzyB proteins in both WzzB (+ or -) was investigated. In the WzzB (+) background all WT and mutant WzyB expressing strains showed bands at the expected size (~47 kDa) via Western immunoblotting with anti-FLAG antibodies (Figure 4.3b, lanes 1, 3-9). All bands appeared to be relatively similar in intensity, with the exception of G354K, which appeared slightly less intense (Figure 4.3b, lane 5). As expected, no bands were detected in the vector control strain (Figure 4.3b, lane 2). In the WzzB (-) background the WT and all mutant WzyB expressing strains showed bands at the expected size (~47 kDa) with varying intensities (Figure 4.3c). Mutants P352K and G354K showed bands that were relatively lower in intensity compared to the WzyB WT expressing control, (this was not seen in the WzzB (+) background) suggesting that the presence of WzzB potentially impacts these particular mutants' stability (Figure 4.3c, lanes 3 and 5, respectively). As expected no band was detected in

the pBCKS<sup>(+)</sup> control strain (Figure 4.3c, lane 2). The varying protein levels detected indicate that several of the mutational alterations may alter Wzy stability.

As WzyB M353D was the only mutant that produced LPS with detectable VL-Oag (Figure 4.3, lane 13 and 14), the relative degree of polymerisation of VL-Oag was determined via densitometry on three biological replicates (Figure 4.3a and Figure 4.9 [S3]). In both the WzzB (+) and (-) backgrounds the proportion of VL-Oag were significantly lower compared to the respective WzyB WT expressing controls (Figure 4.3d and e). The decreased level of VL-Oag may be due to a decreased interaction with Wzz<sub>pHS2</sub> as proposed above for the other mutants.

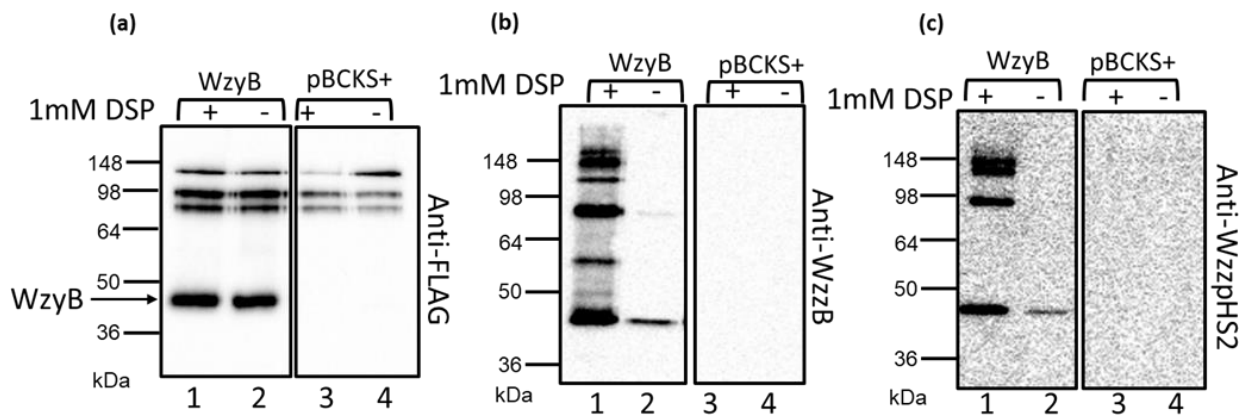


**Figure 4.3: Analysis of WzyB aa region 352 to 354 site-directed mutants.**

(a) *S. flexneri* Y strains were grown to log-phase and  $1 \times 10^9$  collected cells were lysed in lysis buffer in the presence of proteinase K. Samples were then electrophoresed on a SDS-15% (w/v) PAGE gel, and silver-stained (see methods). Parent *S. flexneri* Y  $\Delta wzyB$  (indicated with WzzB +) expressing WT WzyB (lane 1), expressing WzyB P352K, M353K, G354K, P352D, M353D, G354D or 352-354A (lanes 5, 7, 9, 11, 13, 19, 21, respectively) or harbouring pBCKS<sup>(+)</sup> vector control (lane 3). *S. flexneri* Y  $\Delta wzyB$  wzzB:*tet*<sup>R</sup> (indicated with WzzB -) expressing WT WzyB (lane 2), expressing WzyB P352K, M353K, G354K, P352D, M353D, G354D or 352-354A (lanes 6, 8, 10, 12, 14, 20, 22, respectively) or harbouring pBCKS<sup>(+)</sup> vector control (lane 4). Whole-cell lysates ( $5 \times 10^8$  cells) of *S. flexneri* Y  $\Delta wzyB$  (b), or *S. flexneri* Y  $\Delta wzyB$  wzzB:*tet*<sup>R</sup> (c), expressing the indicated proteins were separated by SDS-PAGE, transferred onto a nitrocellulose membrane and probed with monoclonal mouse FLAG antibodies. Analysis of polymerisation of VL-Oag via densitometry of WzzB +ve strains (d) and WzzB -ve strains (e) (Image Lab). The degree of polymerisation of VL-Oag is represented as the densitometry of the VL-Oag (indicated by bracket) as a percentage relative to the WT. Data represents 3 independent experiments with SEM shown, and significance is calculated with a student t-test, \*\*\* P < 0.001, and \*\*\*\* P < 0.0001.

#### 4.5.5 Interaction of the WzyB mutant proteins with WzzB and Wzz<sub>pHS2</sub>

As it appeared that some of the WzyB mutants had a decreased level of VL-Oag we initially investigated if WzyB and Wzz<sub>pHS2</sub> could interact, and if any of the mutations from this study decreased this interaction, and also if any of the mutations disrupted the previously reported interaction of WzyB and WzzB (Leo et al. 2020; Nath & Morona 2015). Previous studies from our group have used the chemical crosslinker DSP to detect the interaction of WzyB and Wzz proteins, thus the same methodology was applied (Leo et al. 2020; Nath & Morona 2015). *S. flexneri* Y  $\Delta$ wzyB strains expressing the WT WzyB-FLAG or harbouring the pBCKS<sup>(+)</sup> vector control, were grown and whole cells were incubated in the presence or absence of DSP, followed by purification using anti-DYKDDDDK G (FLAG) affinity resin. Following purification, samples were separated on a SDS-12% (w/v) polyacrylamide gel and subjected to Western immunoblotting using either anti-FLAG, anti-WzzB or anti-Wzz<sub>pHS2</sub> (Figure 4.4). WzyB was purified at comparable level in the presence or absence of DSP, as bands were detected at the expected size of ~47 kDa (Figure 4.4a, lanes 1 and 2). As expected, no band of this size was detected in the strain harbouring the pBCKS<sup>(+)</sup> vector control in the presence or absence of DSP (Figure 4.4a, lanes 3 and 4). Several non-specific bands were detected when using this background strain when using the anti-FLAG antibodies in all lanes (~70-120 kDa); this was unavoidable but did not impact the result based on the vector only construct control used (Figure 4.4a). When probed with anti-WzzB, WzzB was co-purified with WzyB in both the presence and absence of DSP (Figure 4.4b, lanes 1 and 2). When DSP was present, bands were detected of sizes ranging from ~42-160 kDa, suggesting that WzzB was crosslinked to WzyB and co-purified (Figure 4.4b, lane 1). In the absence of DSP, two bands were detected at ~42 and ~85 kDa, suggesting that WzzB had been co-purified without the aid of a crosslinker (Figure 4.4b, lane 2). No bands were detected in the strain harbouring the pBCKS<sup>(+)</sup> vector control in the presence or absence of DSP (Figure 4.4b, lanes 3 and 4). When probed with anti-Wzz<sub>pHS2</sub>, Wzz<sub>pHS2</sub> was detected in both the presence and absence of DSP (Figure 4.4c, lanes 1 and 2). When DSP was present bands were detected of sizes ranging from ~42-160 kDa, suggesting that Wzz<sub>pHS2</sub> was crosslinked to WzyB and co-purified (Figure 4.4c lane 1). In the absence of DSP, a band was detected at ~47 kDa, suggesting that Wzz<sub>pHS2</sub> was co-purified without the aid of a crosslinker (Figure 4.4c lane 2). No bands were detected in the strain harbouring the pBCKS<sup>(+)</sup> vector control in the presence or absence of DSP (Figure 4.4c, lanes 3 and 4). Additionally, solubilised membrane fractions prior to purification were also analysed by Western immunoblotting and confirmed that WzyB was only present when expressed from pRMA5118 and not the vector control, and that WzzB and Wzz<sub>pHS2</sub> proteins were present at comparable levels before co-purification (Figure 4.10 [S4]). These results show that Wzz<sub>pHS2</sub> can be detected interacting with the WzyB polymerase, as hypothesised, even without the use of a crosslinker



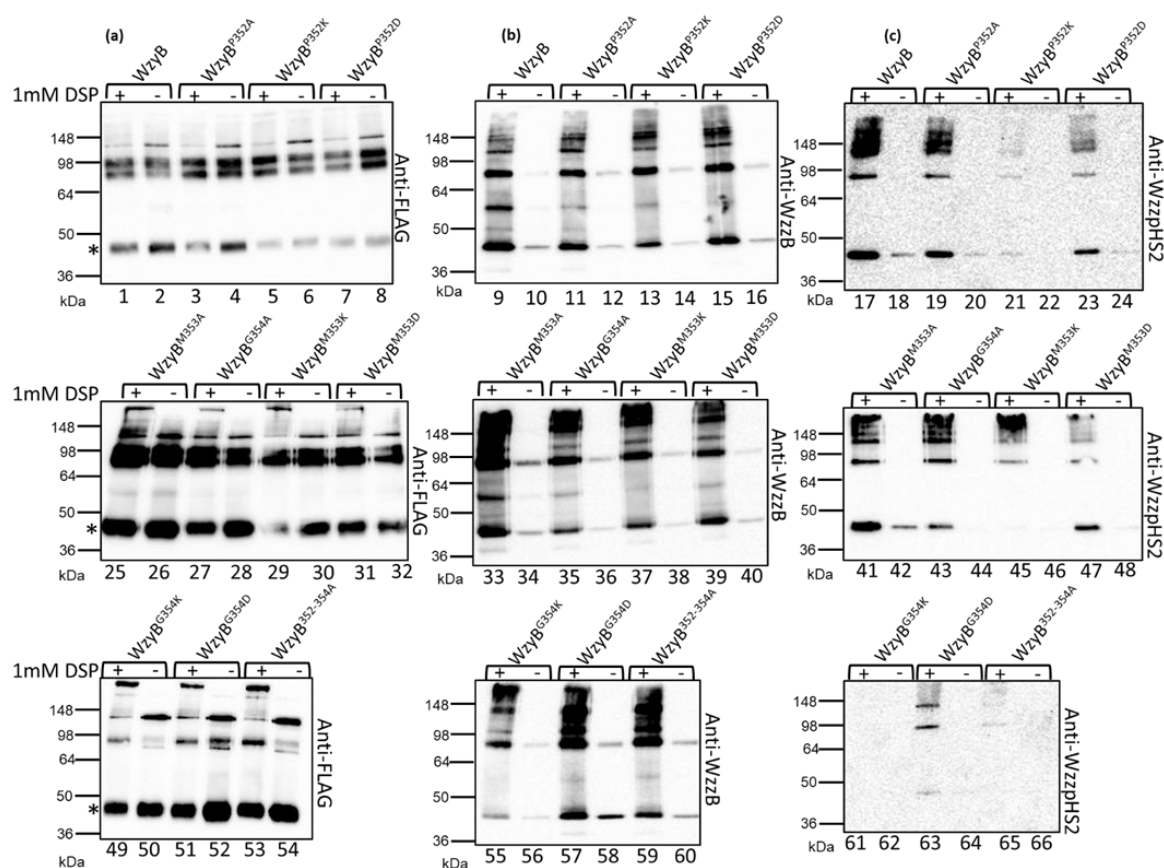
**Figure 4.4: Co-purification of WzyB and WzzB/Wzz<sub>pHS2</sub> using anti-FLAG resin.**

WzyB-FLAG was purified from pRMA5118 (Table 4.1) in *S. flexneri* Y  $\Delta$ wzyB. Whole-cell were treated with (+) and without (-) 1 mM DSP (as described in methods). Samples were separated via 12% SDS-PAGE followed by Western immunoblotting with (a) polyclonal FLAG antibody, (b) polyclonal WzzB antibody, or (c) polyclonal Wzz<sub>pHS2</sub> antibody. The migration positions of the molecular mass standards (in kDa) are indicated on the left-hand side. The location of the WzyB band in (a) is also indicated.

Next we investigated if the interaction of WzyB and Wzz<sub>pHS2</sub> was affected in the WzyB mutant expressing strains. *S. flexneri* Y  $\Delta$ wzyB strains expressing the WT WzyB-FLAG, or a WzyB mutant, or harbouring the pBCKS<sup>(+)</sup> vector control, were grown and whole cells were incubated in the presence or absence of the crosslinker DSP, and purification experiments followed using anti-DYKDDDDK G (FLAG) affinity resin (see methods). Following purification, samples were separated on a SDS-12% (w/v) polyacrylamide gel and subjected to Western immunoblotting using either anti-FLAG, anti WzzB<sub>SF</sub> or Wzz<sub>pHS2</sub> (Figure 4.5). WzyB and all WzyB mutants were purified at a relatively comparable level in all separate co-purification experiments with an expected band at ~47kDa (indicated by \*). Additionally bands were detected at ~70-120 kDa in all lanes (Figure 4.5a) however this is likely the nonspecific bands seen in Figure 4.5a. Interestingly, WzzB co-purified at comparable levels across all co-purification experiments, with bands ranging from 42-160 kDa in the presence of DSP and 2 bands at ~42 and 85 kDa in the absence of DSP (Figure 4.5b). These results suggest the WzyB mutations do not appear to interrupt the interaction with WzzB. Wzz<sub>pHS2</sub> banding patterns and intensities in the presence or absence of DSP was more varied (Figure 4.5c). In the presence of DSP, WzyB P352A, G354A and M353D showed Wzz<sub>pHS2</sub> banding patterns (bands from ~42-160) and band intensity comparable to the WzyB WT, suggesting no impact on interaction (Figure 4.5c, lanes 19, 43, 47 and 17, respectively). WzyB P352K, P352D, G354D and 352-354A, showed a similar Wzz<sub>pHS2</sub> banding pattern to the WzyB WT expressing strain however at a variable lower intensity, suggesting these mutations may have affected the ability of the WzyB and Wzz<sub>pHS2</sub> to be co-purified (Figure 4.5c, lanes, 21, 23, 63, 65 and 17, respectively). WzyB M353K appeared to be missing the Wzz<sub>pHS2</sub> band at ~42 kDa, other Wzz<sub>pHS2</sub> bands were present and at a comparable intensity compared to the



WT WzyB (Figure 4.5c, lanes, 45 and 17, respectively). WzyB G354K, showed no bands, suggesting that this mutation severely impacted the ability of WzyB to co-purify Wzz<sub>pHS2</sub> (Figure 4.5c, lane 61). In the absence of DSP, WzyB P352A, P352D, M353A, M353D and G354D all show a band at ~42 with varying intensities compared to the WzyB WT (Figure 4.5c, lanes, 20, 24, 42, 48, 63 and 18, respectively). WzyB P352K, G345A, M353K, G354K and 352-354A, showed no banding suggesting that the mutations have severely hindered the ability of Wzz<sub>pHS2</sub> to be co-purified in the absence of DSP (Figure 4.5c, lanes, 22, 46, 44, 62, and 66, respectively). Additionally, solubilised membrane fractions prior to purification were also analysed by Western immunoblotting and confirmed that WzyB was expressed and that WzzB and Wzz<sub>pHS2</sub> proteins were present at comparable levels before co-purification (Figure 4.11 [S5]). These results combined suggest that WzyB is able to co-purify Wzz<sub>pHS2</sub> and certain mutations in the WzyB region aa 352-354 affect or in some cases eliminate this interaction without having a detectable effect on WzzB co-purification.



**Figure 4.5: Co-purification of WzyB mutants and WzzB/Wzz<sub>pHS2</sub> using anti-FLAG resin.**

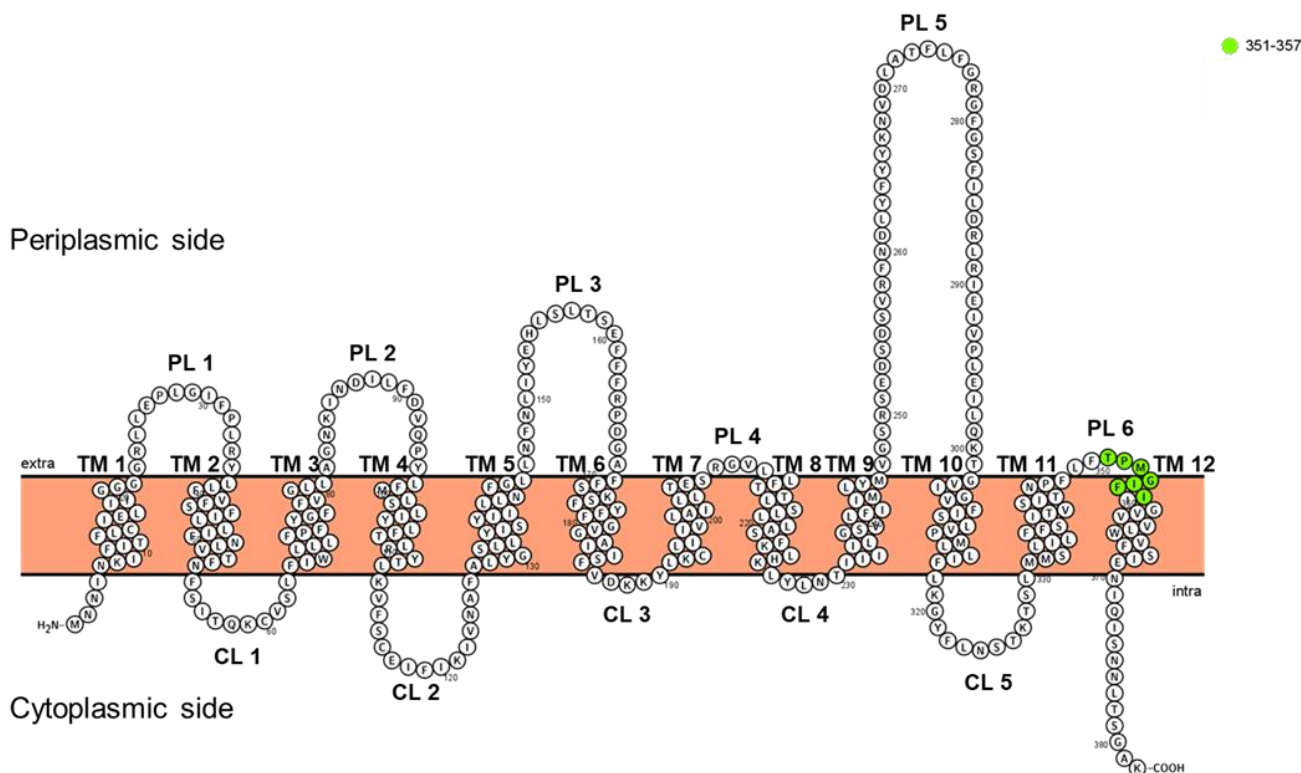
WzyB-FLAG mutants were purified from pVLRM23-36 (Table 1) in *S. flexneri* Y  $\Delta$ wzyB. Whole-cells were treated with (+) and without (-) 1 mM DSP (as described in methods). Samples were separated via 12% SDS-PAGE followed by Western immunoblotting with (a) polyclonal FLAG antibody, (b) polyclonal WzzB antibody, or (c) polyclonal Wzz<sub>pHS2</sub> antibody. The migration positions of the molecular mass standards (in kDa) are indicated on the left-hand side. The location of the WzyB band in (a) is indicated by a \*.

## 4.6 Article Discussion

---

In this study we characterised WzyB mutants affecting Oag polymerisation generated by both random and site-directed mutagenesis. The activity of these WzyB mutants', their expression and ability to interact with different Wzz proteins was investigated and revealed that the WzyB aa region 352 to 354 appeared to be important for WzyB function and potentially be important in a novel interaction with Wzz<sub>pHS2</sub>. WzyB has been shown to interact with its partner protein WzzB via co-purification experiments (Leo et al. 2020; Nath & Morona 2015), thus it was hypothesised that the Wzz<sub>pHS2</sub> protein, of similar function and 3-D structure would also interact with WzyB.

In this study we used random mutagenesis of WzyB in the absence of WzzB to find WzyB mutants that had impaired Oag modal length production. The results are summarised in Table 4.3 [ST2]. The random mutagenesis yielded a variety of mutants but the most interesting WzyB mutant was M353I, as it showed a reduced ability to produce VL-Oag chains and the mutation is adjacent to a previously reported mutant P352H that had affected Oag polymerisation (Nath et al. 2015). Since these mutations were adjacent to one another and were uncovered by separate and unique screening methods, it was of interest to investigate this region further. WzyB aa M353 is on the interface between periplasmic loop 6 and transmembrane region 12 of the WzyB topology map (Figure 4.6) (Daniels et al. 1998). Since it has been proposed that an interaction between WzyB and Wzz proteins most likely occurs in the transmembrane regions (Collins et al. 2017; Leo et al. 2020; Wiseman et al. 2021) this region of WzyB may be involved with interaction with Wzz.



**Figure 4.6: Topology map of WzyB highlighting region mutated in this study.**

The location of amino acids subjected to site-directed mutagenesis is indicated on the topology map of WzyB [generated using “Protter” <https://wlab.ethz.ch/protter/start/>]. Region 351-357 is highlighted in green. The periplasmic loops (PL), cytoplasmic loops (CL) and transmembrane (TM) regions are also indicated.

Further mutagenesis of the region (aa 351-357) by site-directed alanine substitutions showed that amino acids P352, M353, G354 affected WzyB ability to polymerise VL-Oag chains, and this was quantified via silver-stained SDS-PAGE analysis via densitometry (Figure 4.2). Interestingly, mutant I357A showed a significant decrease in VL-Oag chains only seen in the absence of WzzB (Figure 4.2e), suggesting that WzzB may be having a direct/indirect effect of the stability or polymerisation ability of this WzyB mutant. WzyB mutants that appear to be dependent on WzzB has been previously reported (Nath et al. 2015), however, never VL-Oag chain specific.

As certain alanine substitutions caused a significant change to VL-Oag chain polymerisation (Figure 4.2), we next wanted to determine if a substitution to a charged aa (lysine and aspartate) would further decrease WzyB polymerisation activity. The region of interest (352-354) was subjected to site-directed mutagenesis, whereby each aa was replaced with a lysine or an aspartate. Additionally, all three aa were replaced with alanines to generate a triple alanine substitution. Since alanine changes resulted in non-WT Oag polymerisation in this region, drastic aa substitutions (adding a positive or negative charge) was expected to also result in less than WT

Oag polymerisation phenotype. However the majority of these new substitutions (P352K, G354K P352D and 352-354A) caused complete a knockout of WzyB activity (Figure 4.3). Additionally, WzyB M353K and WzyB G354D had extremely low polymerisation activity (Figure 4.3), suggesting that this region of WzyB is critical for its enzymatic function. Interestingly, WzyB M353K showed a unique profile where there appears to be a relatively high level of Oag chains at 8-10, suggesting some form of shifted modal length control. As protein expression appeared mostly unaffected, the shift in charge of this region most likely caused a conformational change resulting in a non-active WzyB. This region (352-354) appears be involved in the active site of WzyB and is found on periplasmic loop (PL) 6 and transmembrane region (TM) 12 (Daniels et al. 1998) (Figure 4.6) adjacent to the known active site of WzyB (PL3 and 5) (Islam et al. 2011; Nath & Morona, R 2015).

As some of these mutants appeared to be producing LPS with significantly fewer VL-Oag chains, we wanted to firstly show that WzyB and Wzz<sub>pHS2</sub> can interact and also determine if any of these mutations impacted this interaction. For the first time, we were able to show that WzyB and Wzz<sub>pHS2</sub> could be crosslinked and co-purified via the use of DSP, and additionally co-purification experiments detected co-purification of Wzz<sub>pHS2</sub> without the use of DSP (Figure 4.4). These results suggest that there is a clear interaction between WzyB and Wzz<sub>pHS2</sub> as hypothesised and it was then hypothesised that this interaction is necessary for Wzz<sub>pHS2</sub> function to regulate VL-Oag chains. These co-purification experiments were repeated using the mutant WzyB proteins to determine if any mutants altered the interaction. In the presence of DSP, mutation G354K showed no Wzz<sub>pHS2</sub> bands suggesting no interaction (Figure 4.5c), while mutations G354D, P352K, M353K and triple alanine mutation 352-354A all showed clear altered/reduced co-purification of Wzz<sub>pHS2</sub> (Figure 4.5c). Mutants P352K, M353K, M353D, G354K and triple alanine mutation 352-354A, only had detectable interactions with Wzz<sub>pHS2</sub> in the presence of DSP (Figure 4.5). WzyB G354K was the only mutant to show no interaction with Wzz<sub>pHS2</sub> in both the presence and absence of DSP. Interestingly none of the mutants had a notable effect on WzzB co-purification with WzyB, hence, non-functional WzyB mutants still interact with WzzB (Figure 4.5).

We have previously hypothesised that members of the PCP1 protein family (WzzB and Wzz<sub>pHS2</sub>) compete for WzyB at a shared interaction site to regulate the Oag chains at different lengths (Leo et al. 2020). However the results from this study suggest otherwise, as a number of WzyB mutations appeared to affect the interaction WzyB/Wzz<sub>pHS2</sub> exclusively. This may be explained by a few possibilities: The WzyB interacting site for WzzB and Wzz<sub>pHS2</sub> are separate and unique, consequently the mutations only affect interactions with Wzz<sub>pHS2</sub>. This is the most likely possibility as these mutations completely abolished the ability of WzyB to interact with

Wzz<sub>pHS2</sub> while having no effect on the interaction with WzzB. Alternatively WzzB and Wzz<sub>pHS2</sub> share the same interacting site on WzyB, however the mutations in this study only affected Wzz<sub>pHS2</sub> interaction with WzyB. Previous studies have shown that decreasing the WzyB expression causes a decrease in VL-Oag chains prior to the loss of S-Oag chains (Carter et al. 2009), which could be explained by WzzB out-competing Wzz<sub>pHS2</sub> for the shared WzyB interacting site. For example, this can be seen in Figure 2a where the absence of WzzB resulted in a larger proportion of Oag chains that were a VL length.

The mutated region of WzyB appears to affect the interaction with only Wzz<sub>pHS2</sub>, however, given that the majority of the more substantial mutations that introduced a charged aa caused either complete loss or near complete loss of WzyB activity, this region is also critical for WzyB function and thus appears to be an active site. We propose that this region may be a “switch” region involved in the enzymatic activity of WzyB that is activated when interacting with Wzz<sub>pHS2</sub>. Once activated, WzyB is able to maintain the Oag chain for a longer period, potentially via increasing its affinity to Oag, leading to larger Oag chains (VL-chains). Assuming WzzB and Wzz<sub>pHS2</sub> form homooligomeric bells of the same number of protomers, this model explains why despite being of similar sizes, WzzB and Wzz<sub>pHS2</sub> produce drastically different size Oag chains. Significant mutations to this “switch” region may result in a break of this switch leading to no polymerisation, potentially completely removing the ability to interact with Oag.

In conclusion, our findings show that WzyB and Wzz<sub>pHS2</sub> can interact using biochemical co-purification experiments without the aid of a crosslinker, and that region 352-354 is important in WzyB polymerisation function and is directly or indirectly involved with Wzz<sub>pHS2</sub> interaction. These findings aid in the understanding of WzyB polymerase activity and its interaction with partner Wzz proteins, and for the first time identifies a region that interacts with a Wzz protein.

## 4.7 Article References

---

- Carter JA, Jimenez JC, Zaldivar M, Alvarez SA, Marolda CL, Valvano MA, Contreras I. 2009. The cellular level of O-antigen polymerase Wzy determines chain length regulation by WzzB and WzzpHS-2 in *Shigella flexneri* 2a. *Microbiology* 155:3260-9.
- Collins RF, Kargas V, Clarke BR, Siebert CA, Clare DK, Bond PJ, Whitfield C, Ford RC. 2017. Full-length, oligomeric structure of Wzz determined by cryoelectron microscopy reveals insights into membrane-bound states. *Structure* 25:806-815 e3.
- Daniels C, Morona R. 1999. Analysis of *Shigella flexneri* wzz (R<sub>ol</sub>) function by mutagenesis and cross-linking: Wzz is able to oligomerize. *Mol Microbiol* 34:181-94.
- Daniels C, Vindurampulle C, Morona R. 1998. Overexpression and topology of the *Shigella flexneri* O-antigen polymerase (R<sub>fc</sub>/Wzy). *Molecular Microbiology* 28:1211-1222.
- Hong M, Payne SM. 1997. Effect of mutations in *Shigella flexneri* chromosomal and plasmid-encoded lipopolysaccharide genes on invasion and serum resistance. *Mol Microbiol* 24:779-91.
- Islam ST, Gold AC, Taylor VL, Anderson EM, Ford RC, Lam JS. 2011. Dual conserved periplasmic loops possess essential charge characteristics that support a catch-and-release mechanism of O-antigen polymerization by Wzy in *Pseudomonas aeruginosa* PAO1. *J Biol Chem* 286:20600-5.
- Islam ST, Huszczyński SM, Nugent T, Gold AC, Lam JS. 2013. Conserved-residue mutations in Wzy affect O-antigen polymerization and Wzz-mediated chain-length regulation in *Pseudomonas aeruginosa* PAO1. *Sci Rep* 3:3441.
- Kalynysh S, Morona R, Cygler M. 2014. Progress in understanding the assembly process of bacterial O-antigen. *FEMS Microbiol Rev* 38:1048-65.
- Kenne L, Lindberg B, Petersson K. 1977. Basic structure of the oligosaccharide repeating-unit of the *Shigella flexneri* O-antigens. *Carbohydr Res* 56:363-70.
- Larue K, Kimber MS, Ford R, Whitfield C. 2009. Biochemical and structural analysis of bacterial O-antigen chain length regulator proteins reveals a conserved quaternary structure. *Journal of Biological Chemistry* 284:7395-7403.
- Leo V, Tran E, Morona R. 2020. Polysaccharide co-polymerase WzzB/WzzE chimeras reveal transmembrane 2 region of WzzB is important for interaction with WzyB. *J Bacteriol* doi:10.1128/JB.00598-20.

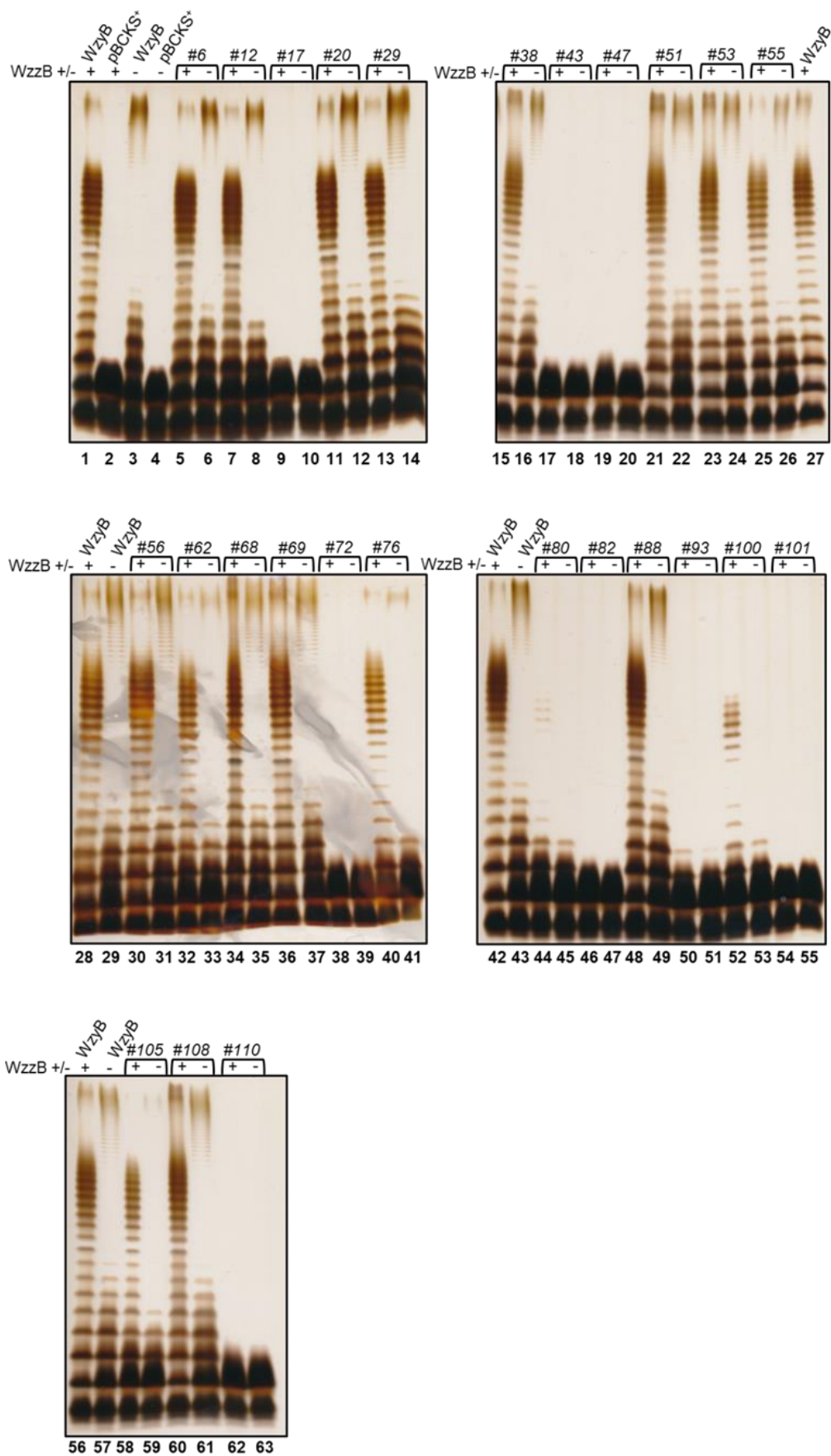
- Lugtenberg B, Meijers J, Peters R, van der Hoek P, van Alphen L. 1975. Electrophoretic resolution of the "major outer membrane protein" of *Escherichia coli* K12 into four bands. FEBS Lett 58:254-8.
- Morona R, Mavris M, Fallarino A, Manning PA. 1994. Characterization of the *rfc* region of *Shigella flexneri*. J Bacteriol 176:733-47.
- Morona R, Purins L, Tocilj A, Matte A, Cygler M. 2009. Sequence-structure relationships in polysaccharide co-polymerase (PCP) proteins. Trends Biochem Sci 34:78-84.
- Murray GL, Attridge SR, Morona R. 2003. Regulation of *Salmonella typhimurium* lipopolysaccharide O antigen chain length is required for virulence; identification of FepE as a second Wzz. Mol Microbiol 47:1395-406.
- Nath P, Morona R. 2015. Mutational analysis of the major periplasmic loops of *Shigella flexneri* Wzy: identification of the residues affecting O-antigen modal chain length control, and Wzz-dependent polymerization activity. Microbiology 161:774-85.
- Nath P, Tran EN, Morona R. 2015. Mutational analysis of the *Shigella flexneri* O-antigen polymerase Wzy: identification of Wzz-dependent Wzy mutants. J Bacteriol 197:108-19.
- Nath, Morona. 2015. Detection of Wzy/Wzz interaction in *Shigella flexneri*. Microbiology 161:1797-1805.
- Papadopoulos M, Morona R. 2010. Mutagenesis and chemical cross-linking suggest that Wzz dimer stability and oligomerization affect lipopolysaccharide O-antigen modal chain length control. Journal of Bacteriology 192:3385-3393.
- Purins L, Van Den Bosch L, Richardson V, Morona R. 2008. Coiled-coil regions play a role in the function of the *Shigella flexneri* O-antigen chain length regulator Wzz<sub>pHS2</sub>. Microbiology 154:1104-1116.
- Raetz CR, Whitfield C. 2002. Lipopolysaccharide endotoxins. Annu Rev Biochem 71:635-700.
- Rai AK, Mitchell AM. 2020. Enterobacterial common antigen: synthesis and function of an enigmatic molecule. mBio 11.
- Stevenson G, Kessler A, Reeves PR. 1995. A plasmid-borne O-antigen chain length determinant and its relationship to other chain length determinants. FEMS Microbiol Lett 125:23-30.

- Tran EN, Morona R. 2013. Residues located inside the *Escherichia coli* FepE protein oligomer are essential for lipopolysaccharide O-antigen modal chain length regulation. *Microbiology* 159:701-14.
- Tran EN, Papadopoulos M, Morona R. 2014. Relationship between O-antigen chain length and resistance to colicin E2 in *Shigella flexneri*. *Microbiology-Sgm* 160:589-601.
- Van den Bosch L, Morona R. 2003. The actin-based motility defect of a *Shigella flexneri* rmlD rough LPS mutant is not due to loss of IcsA polarity. *Microb Pathog* 35:11-8.
- Whitfield C, Wear SS, Sande C. 2020. Assembly of bacterial capsular polysaccharides and exopolysaccharides. *Annu Rev Microbiol* doi:10.1146/annurev-micro-011420-075607.
- Whitfield C, Williams DM, Kelly SD. 2020. Lipopolysaccharide O-antigens-bacterial glycans made to measure. *J Biol Chem* 295:10593-10609.
- Wiseman B, Nitharwal RG, Widmalm G, Hogbom M. 2021. Structure of a full-length bacterial polysaccharide co-polymerase. *Nat Commun* 12:369.



## 4.8 Article Supporting Information

### 4.8.1 Supporting Figures

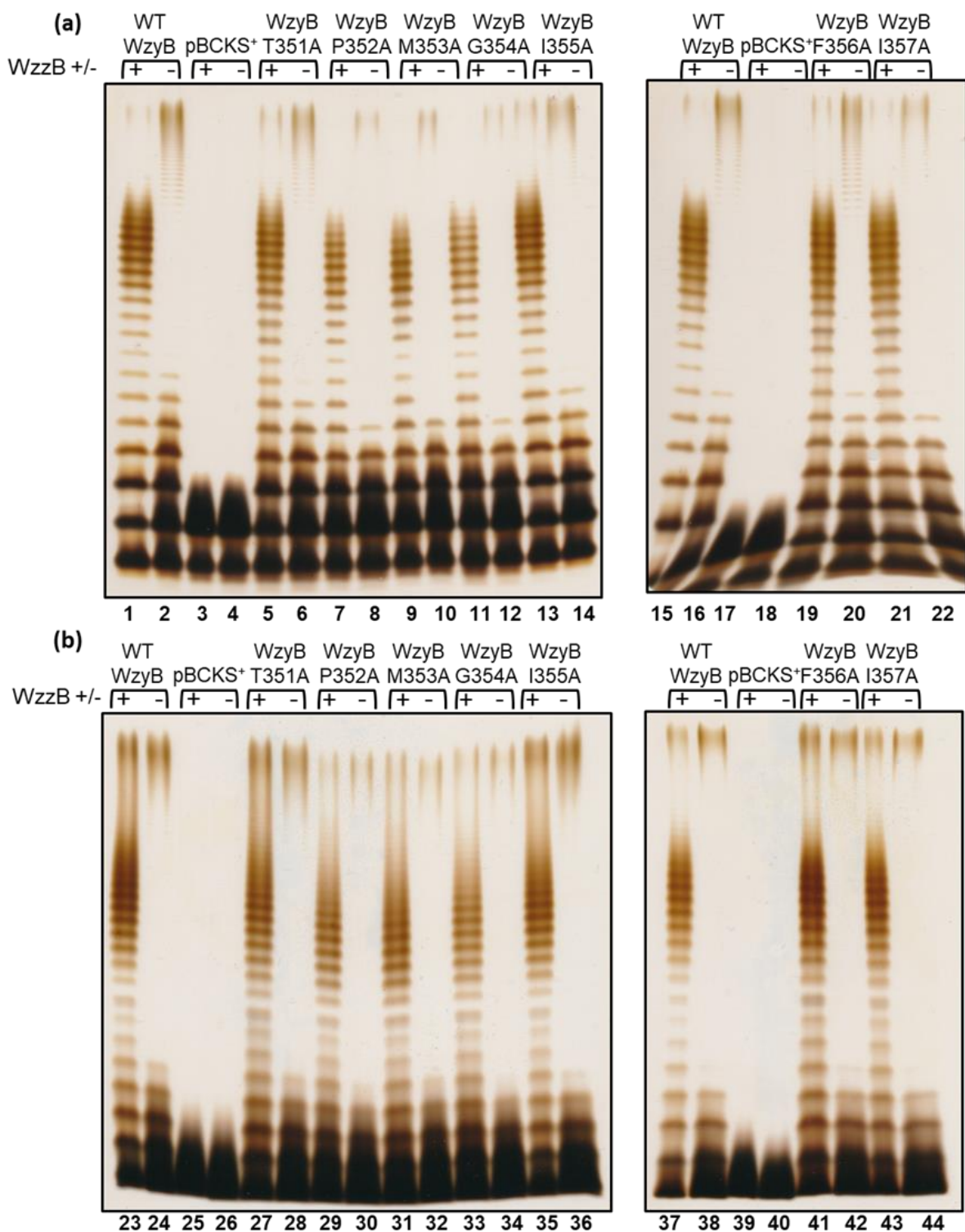


---

**Figure 4.7 [S1]: LPS profile screen of colicin E2 sensitive mutants.**

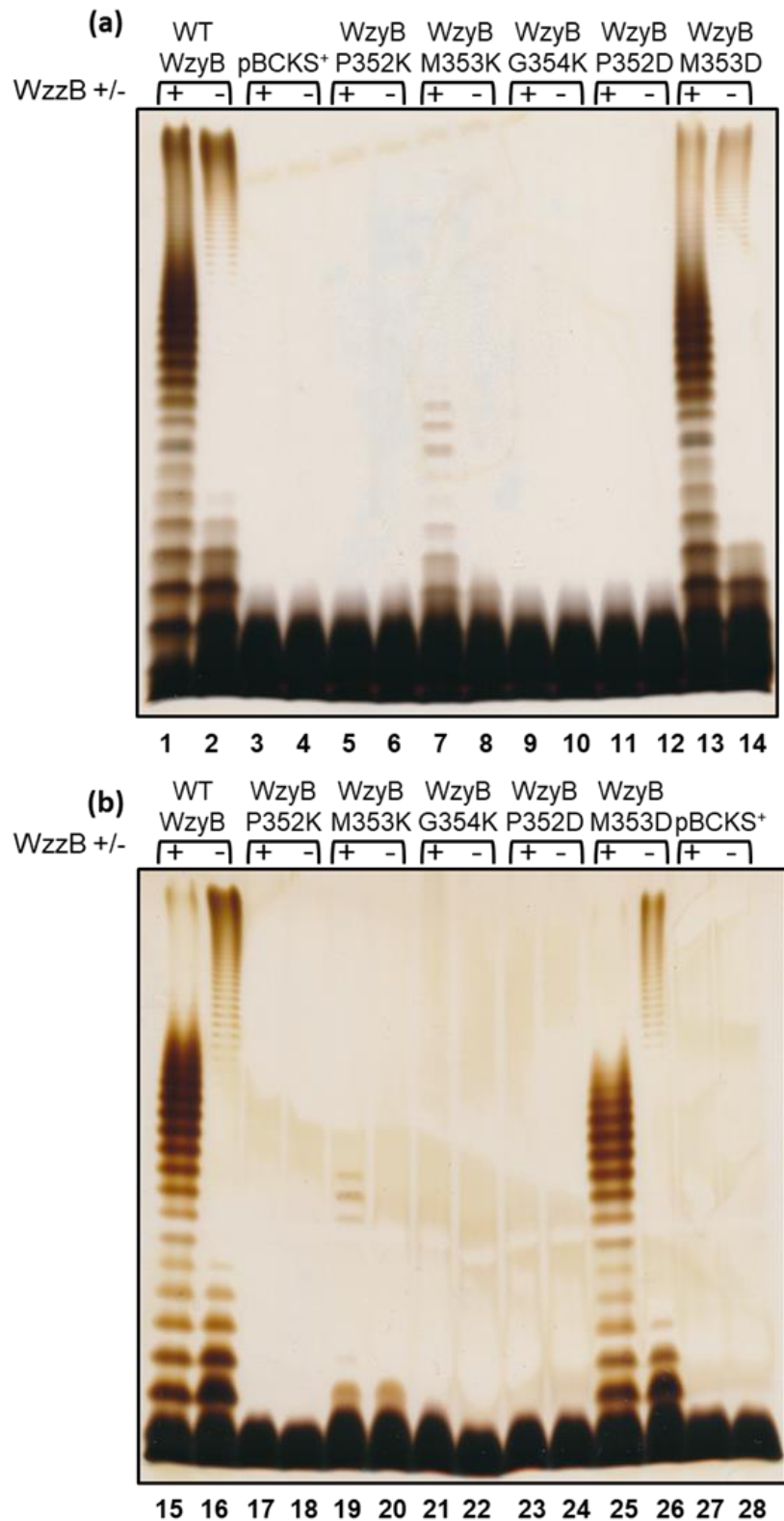
*S. flexneri* Y strains were grown to log-phase and  $1 \times 10^9$  collected cells were lysed in lysis buffer in the presence of proteinase K. Samples were then electrophoresed on a SDS-15% PAGE gel, and silver-stained (see methods). The parent *S. flexneri* Y  $\Delta wzyB$  (indicated with WzzB +) or *S. flexneri* Y  $\Delta wzyB wzzB:ter^R$  (indicated with WzzB -) expressing either WT WzyB (lane 1 or 2), random mutant (# number) WzyB or harbouring pBCKS<sup>(+)</sup> vector control is indicated (lane 3 or 4).

---



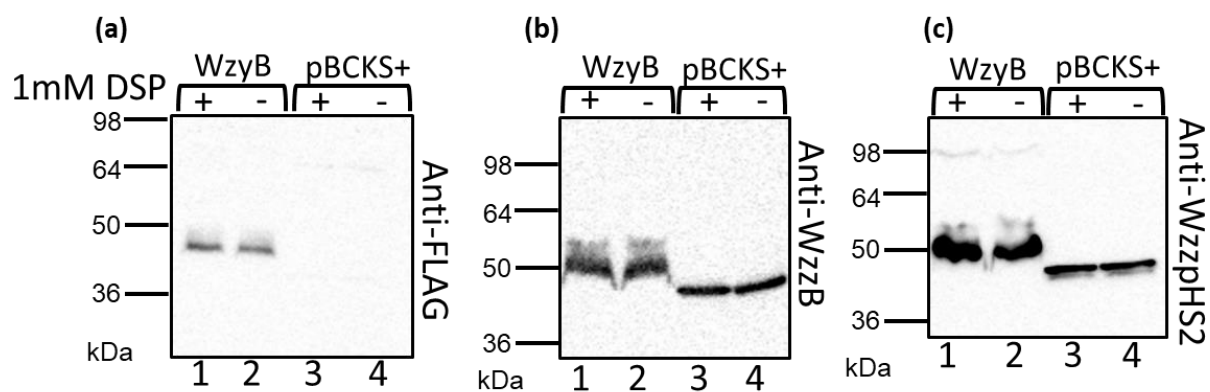
**Figure 4.8 [S2]: LPS gels used for densitometry analysis of VL-Oag chains in WzyB mutants, P352A, M353A and G354A.**

*S. flexneri* Y strains were grown to log-phase and  $1 \times 10^9$  collected cells were lysed in lysis buffer in the presence of proteinase K. Samples were then electrophoresed on a SDS-15% PAGE gel, and silver-stained (a) replicate 2 (b) replicate 3 (see methods). The parent *S. flexneri* Y  $\Delta wzyB$  (indicated with WzzB +) or *S. flexneri* Y  $\Delta wzyB$  *wzzB:tet<sup>R</sup>* (indicated with WzzB -) expressing either WT WzyB (lane 1 or 2), the WzyB aa 351-357 alanine mutations or harbouring pBCKS<sup>(+)</sup> vector control (lane 3 or 4) is indicated.



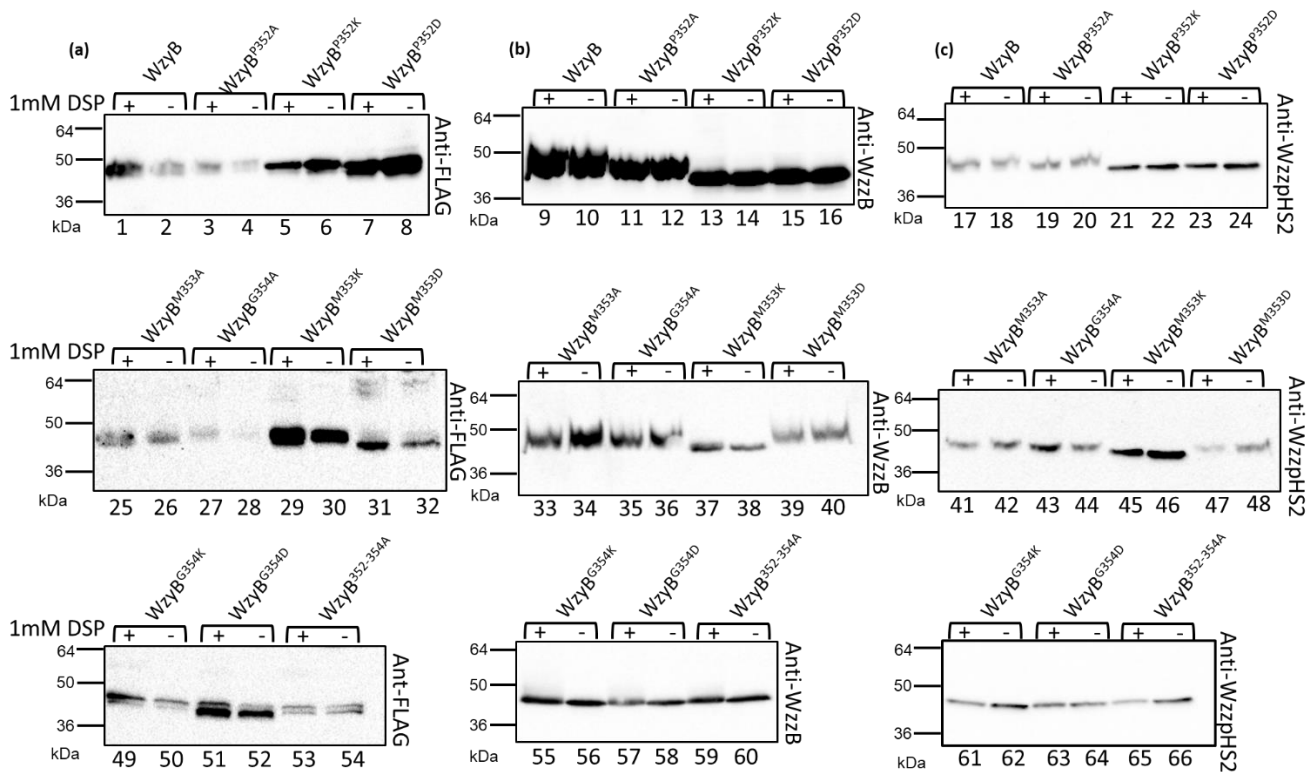
**Figure 4.9 [S3]: LPS gels were used for densitometry analysis of VL-Oag chains in WzyB mutant M353D.**

*S. flexneri* Y strains were grown to log-phase were collected ( $1 \times 10^9$  cells) and lysed in lysis buffer in the presence of proteinase K. Samples were then electrophoresed on a SDS-15% PAGE gel, and silver-stained (a) replicate 2 (b) replicate 3 (see methods). The parent *S. flexneri* Y  $\Delta wzyB$  (indicated with WzzB +) or *S. flexneri* Y  $\Delta wzyB wzzB: tet^R$  (indicated with WzzB -), the indicated WzyB mutations or harbouring pBCKS<sup>(+)</sup> vector control (lane 3 or 4) is indicated.



**Figure 4.10 [S4]: Solubilised samples of WzyB WT prior to purification.**

*S. flexneri* Y *wzyB* expressing WzyB from pRMA5118 or harbouring pBCKS<sup>(+)</sup> were treated with (+) and without (-) 1 mM DSP (as described in methods). Samples were electrophoresed on SDS-12% polyacrylamide gels followed by Western immunoblotting with (a) polyclonal FLAG antibody, (b) polyclonal WzzB antibody, or (c) polyclonal Wzz<sub>pHS2</sub> antibody. The migration positions of the molecular mass standards (in kDa) are indicated on the left-hand side.



**Figure 4.11 [S5]: Solubilised samples of WzyB mutants prior to purification.**

*S. flexneri* Y  $\Delta wzyB$  expressing WzyB from pRMA5118, WzyB mutants from pVLRM23-36 or harbouring pBCKS<sup>(+)</sup> were treated with (+) and without (-) 1 mM DSP (as described in methods). Samples were electrophoresed on SDS-12% (w/v) polyacrylamide gels followed by Western immunoblotting with (a) polyclonal FLAG antibody, (b) polyclonal WzzB antibody, or (c) polyclonal Wzz<sub>pHS2</sub> antibody. The migration positions of the molecular mass standards (in kDa) are indicated on the left-hand side.

## 4.8.2 Supporting Tables

**Table 4.2 [ST1]: Oligonucleotides used in this study.**

Oligonucleotide	Description
5' aaaaaggaatggattcgttatagaaacggttaactgaaaaaagtattaacg 3'	Universal reverse PCR primer used for inverse PCR used to create all site directed substitutions (alanine, lysine, aspartate and triple alanine)
5'-gcacccatgggaatTTTTATTATAGGCgttg 3'	Forward PCR primer used for inverse PCR to create VL23 (pBCKS <sup>(+)</sup> -wzyB <sup>T351A</sup> -FLAG-LacZ $\alpha$ )
5'-acagcaatgggaatTTTTATTATAGGCgttgtag 3'	Forward PCR primer used for inverse PCR to create VL24 (pBCKS <sup>(+)</sup> -wzyB <sup>P352A</sup> -FLAG-LacZ $\alpha$ )
5'-acacccgcaggaatTTTTATTATAGGCgttgtagTTTatgg-3'	Forward PCR primer used for inverse PCR to create VL25 (pBCKS <sup>(+)</sup> -wzyB <sup>M353A</sup> -FLAG-LacZ $\alpha$ )
5'-acacccatggcaatTTTTATTATAGGCgttgtagTTTatgggtatttc-3'	Forward PCR primer used for inverse PCR to create VL26 (pBCKS <sup>(+)</sup> -wzyB <sup>G354A</sup> -FLAG-LacZ $\alpha$ )
5'-acacccatgggagcatttattatagcgttgtagTTTatgggtatttc-3'	Forward PCR primer used for inverse PCR to create VL27 (pBCKS <sup>(+)</sup> -wzyB <sup>I355A</sup> -FLAG-LacZ $\alpha$ )
5'-acacccatgggaattgcaattatagcgttgtagTTTatgggtatttc-3'	Forward PCR primer used for inverse PCR to create VL28 (pBCKS <sup>(+)</sup> -wzyB <sup>P356A</sup> -FLAG-LacZ $\alpha$ )
5'- acacccatgggaatTTTTgcaatagcgttgtagTTTatgggtatttc -3'	Forward PCR primer used for inverse PCR to create VL29 (pBCKS <sup>(+)</sup> -wzyB <sup>I357A</sup> -FLAG-LacZ $\alpha$ )
5'- acaaaaatgggaatTTTTATTATAGGCgttgtag -3'	Forward PCR primer used for inverse PCR to create VL30 (pBCKS <sup>(+)</sup> -wzyB <sup>P352K</sup> -FLAG-LacZ $\alpha$ )
5'- acacccaaaggaatTTTTATTATAGGCgttgtagTTTatgg -3'	Forward PCR primer used for inverse PCR to create VL31 (pBCKS <sup>(+)</sup> -wzyB <sup>M353K</sup> -FLAG-LacZ $\alpha$ )
5'- acacccatgaaaatTTTTATTATAGGCgttgtagTTTatgggtatttc -3'	Forward PCR primer used for inverse PCR to create VL32 (pBCKS <sup>(+)</sup> -wzyB <sup>G354K</sup> -FLAG-LacZ $\alpha$ )
5'- acagatatgggaatTTTTATTATAGGCgttgtag -3'	Forward PCR primer used for inverse PCR to create VL33 (pBCKS <sup>(+)</sup> -wzyB <sup>P352D</sup> -FLAG-LacZ $\alpha$ )
5'- acacccgatggaatTTTTATTATAGGCgttgtagTTTatgg -3'	Forward PCR primer used for inverse PCR to create VL34 (pBCKS <sup>(+)</sup> -wzyB <sup>M353D</sup> -FLAG-LacZ $\alpha$ )
5'- acacccatggatTTTTATTATAGGCgttgtagTTTatgggtatttc -3'	Forward PCR primer used for inverse PCR to create VL35 (pBCKS <sup>(+)</sup> -wzyB <sup>G354D</sup> -FLAG-LacZ $\alpha$ )
5'- acagcagcagcaatTTTTATTATAGGCgttgtagTTTatgggtatttc -3'	Forward PCR primer used for inverse PCR to create VL36 (pBCKS <sup>(+)</sup> -wzyB <sup>P352A M353A G354A</sup> -FLAG-LacZ $\alpha$ )

**Table 4.3 [ST2]: Colicin E2 sensitive mutants.**

<b>Mutant plasmid #</b>	<b>LPS profile WzzB +/- background</b>	<b>Amino acid changes</b>
6	Comparable to WT WzyB expressing control (class A)	N/A
12	Comparable to WT WzyB expressing control (class A)	N/A
17	No polymerisation activity (class B)	(P165T K300I)
20	Comparable to WT WzyB expressing control (class A)	N/A
29	Comparable to WT WzyB expressing control (class A)	N/A
38	Comparable to WT WzyB expressing control (class A)	N/A
43	No polymerisation activity (class B)	N/A
47	No polymerisation activity (class B)	(E15D R23S F162I F185I)
51	Comparable to WT WzyB expressing control (class A)	N/A
53	Comparable to WT WzyB expressing control (class A)	N/A
55	Comparable to WT WzyB expressing control (class A)	N/A
56	Comparable to WT WzyB expressing control (class A)	N/A
62	Decreased modal length control of VL Oag chains (class C)	M353I
68	Comparable to WT WzyB expressing control (class A)	N/A
69	Comparable to WT WzyB expressing control (class A)	N/A
72	No polymerisation activity (class B)	N/A
76	Decreased modal length control of VL Oag chains (class C)	(F185S N260K I316V I373T)
80	Extremely poor polymerisation (class D)	(L63I G74D G82A L202F)
82	No polymerisation activity (class B)	N/A
88	Comparable to WT WzyB expressing control (class A)	N/A
93	Extremely poor polymerisation (class D)	(F38L S309P)
100	Lower polymerisation (class E)	(L24F F170L F356L)
101	No polymerisation activity (class B)	N/A
105	Lower polymerisation (class E)	(L101I F177Y S251R)
108	Comparable to WT WzyB expressing control (class A)	N/A
110	No polymerisation activity (class B)	(N3D D89G F107Y L254S E298G)

N/A= not sequenced



#### **4.9 Article Acknowledgements**

---

Funding for this work is provided by a Discovery Project Grant to R. Morona from the Australian Research Council (PROJECT ID: DP160103903). V. Leo is the recipient of a Research Training Program Stipend Research Scholarship from the University of Adelaide.

# Chapter Five

---

## IDENTIFICATION OF WzyB BINDING PARTNERS

---

## Chapter 5: Identification of WzyB binding partners

---

### 5.1 Introduction

---

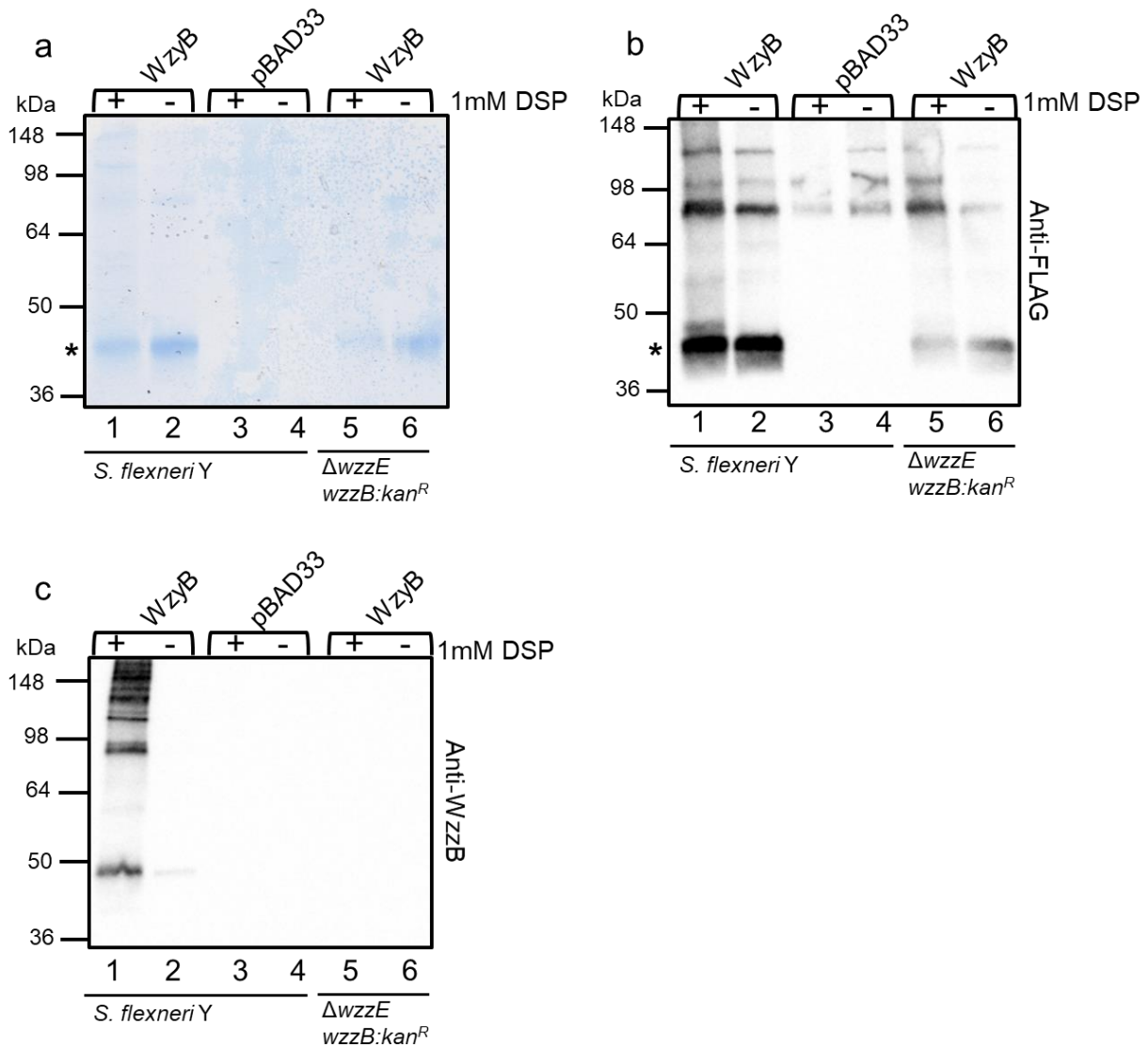
WzyB has been shown to interact with the polysaccharide co-polymerases of the Oag and ECA biosynthetic pathways (WzzB, WzzE and Wzz<sub>pHS2</sub>) (Leo et al. 2020). However WzyB has long been hypothesised to be included in a Wzy-dependent pathway complex made up of other proteins including Wzx (Marolda et al. 2006) (Figure 1.8). Early crosslinking and co-purification attempts to identify a complex were not successful (Carter et al. 2009; Marczak et al. 2013), likely due to the relatively low expression of these proteins. More recent attempts to show direct evidence of WzyB interactions detected WzyB interacting with WzzB with the use of the crosslinker DSP in *S. flexneri* (Nath & Morona 2015). Thus new expression constructs with higher expression were generated and used in co-purification experiments to detect WzyB interactions without the use of a crosslinker (Leo et al. 2020) (Chapter 3). The aim of this Chapter is to build upon the WzyB interactions shown in Chapter 3 using the novel expression system and purification protocols to attempt to detect direct interaction of WzyB protein binding partners through the use of mass spectrometry.

### 5.2 WzyB purification

---

In order to firstly confirm the WzyB and WzzE novel interaction identified in Chapter 3 and to identify any WzyB binding partners, co-purification experiments were performed in *S. flexneri*, using the new expression system and purification protocols (Section 2.6.2) generated in this thesis. The parent strain RMA2162 (*S. flexneri* Y), was transformed with pVL11 (pBAD33-WzyB-FLAG) (generated in 3.5.5) and pBAD33, RMA4662 (*S. flexneri* Y  $\Delta$ wzzE/ $\Delta$ wzz<sub>pHS2</sub>/wzzB::kan<sup>R</sup>) was also transformed with pVL11, and the mutant strains were named VLRM288-290, respectively (Appendix A). These strains were grown and the whole cells were incubated in the presence and absence of DSP (Section 2.6.3), followed by purification experiments using anti-DYKDDDDK G (FLAG) affinity resin (Section 2.6.2). The purified samples were separated on SDS-12% polyacrylamide gels and subjected to Western immunoblotting using anti-FLAG or anti-WzzB antibodies (2.6.5), or colloidal blue staining (Section 2.6.9) (Figure 5.1). WzyB was purified successfully when expressed in both the *S. flexneri* Y parent and the *S. flexneri* Y  $\Delta$ wzzE/ $\Delta$ wzz<sub>pHS2</sub>/wzzB::kan<sup>R</sup> mutant strain, in both the presence and absence of DSP, as major bands were detected at ~44 kDa using colloidal blue stain (Figure 5.1a, lanes 1, 2, 5 and 6). When whole cells were treated with DSP extra bands were detected in the *S. flexneri* Y parent expressing strain at ~98 to ~147 kDa (Figure 5.1a, lane 1). As expected there were no bands at the expected size of WzyB (~45 kDa) when the *S. flexneri* Y parent harboured the vector control (Figure 5.1a, lanes 3 and 4). Following Western immunoblotting probing with

anti-FLAG antibodies, bands were detected in both the *S. flexneri* Y parent and the *S. flexneri* Y  $\Delta wzzE/\Delta wzz_{pHS2}/wzzB::kan^R$  mutant strain, in both the presence and absence of DSP, at sizes from ~44 to ~120 kDa (Figure 5.1b, lanes 1, 2, and 4). When whole cells were treated with DSP an extra band was detected in the *S. flexneri* Y parent WzyB expressing strain at ~47 kDa (Figure 5.1b, lane 1). As expected there were no bands detected at the expected size of WzyB (~45 kDa) when the *S. flexneri* Y parent harboured the vector control pBAD33, in the presence or absence of DSP (Figure 5.1b, lanes 3 and 4). However, several non-specific bands were detected in all lanes when using this background strain when probing with the anti-FLAG antibodies (~70-120 kDa); this was unavoidable but did not impact the result based on the vector only construct control used (Figure 5.1a). When probing with anti-WzzB, WzzB was detected in both the presence and absence of DSP, confirming that chromosomally expressed WzzB was co-purified with WzyB (Figure 5.1c, lanes 1 and 2). As expected, no bands were detected when probing with anti-WzzB in either the vector control harbouring strain or *S. flexneri* Y  $\Delta wzzE/\Delta wzz_{pHS2}/wzzB::kan^R$  expressing WzyB (Figure 5.1c, lanes 3-6).



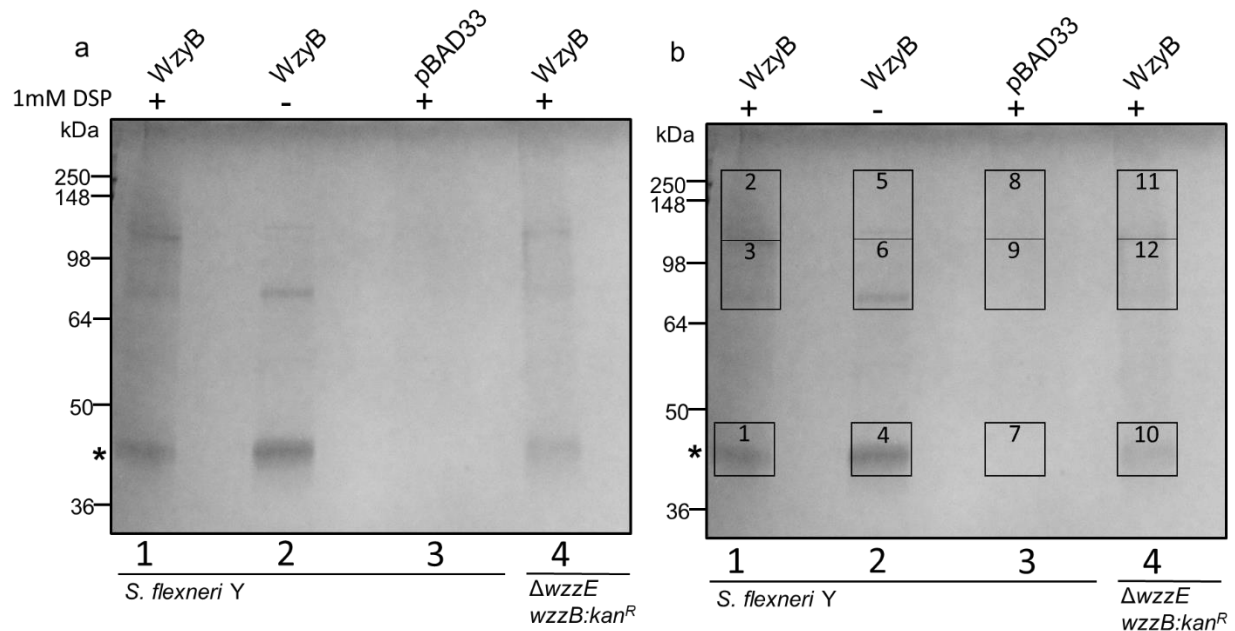
**Figure 5.1: Purification of WzyB from *S. flexneri*.**

Purification of WzyB-FLAG from pVLRM11 (Appendix B) from parent *S. flexneri* Y or *S. flexneri* Y  $\Delta wzzE/\Delta wzzE_{pHS2}wzzB::kan^R$ . Whole-cell treatment with (+) and without (-) 1mM DSP (Section 2.6.3) is indicated. Samples were electrophoresed on SDS-12% polyacrylamide gels followed by direct staining with colloidal blue stain (a) or Western immunoblotting with (b) polyclonal FLAG antibody, (c) polyclonal WzzB antibody. The migration positions of molecular mass standards (in kDa) are indicated on the left hand side. The location of the WzyB band in (a and b) is indicated by a \*.

### 5.3 Upscaled WzyB purification and band excision

In order to identify WzyB binding partners via mass spectrometry and confirm the WzyB/WzzB interaction, the WzyB purification protocol (Section 2.6.2) was repeated with 200 mL induced cultures, followed by whole-cell treatment with DSP. The parent *S. flexneri* Y expressing WzyB was also purified in the absence of DSP for WzyB partner identification comparison and to confirm the interaction of WzyB and WzzB without the use of a crosslinker via mass spectrometry. Following the purifications, the samples were electrophoresed on a pre-cast 4-

12% gradient SDS- AnykDa gel (BioRad) and stained with colloidal blue (Figure 5.2a) (Section 2.7). Sections of the stained gel were then excised and digested with trypsin, each lane of the gel was excised at three places; the first at ~39 to ~48 kDa the second at ~70 and ~110 and the third was between ~110 to ~255 (Figure 5.2b).



**Figure 5.2: Purification of WzyB for band excision for mass spectrometry analysis.**

Purification of WzyB-FLAG from pVLRM11 (Appendix B) from 200 mL of parent *S. flexneri* Y or *S. flexneri* Y  $\Delta wzzE/\Delta wzz_{pHS2}wzzB::kan^R$ . Whole-cell treatment with (+) and without (-) 1mM DSP (Section 2.6.3) is indicated. Samples were electrophoresed on SDS-12% polyacrylamide gels followed by direct staining with colloidal blue stain (a), and the excised bands that were analysed by mass spectrometry are indicated (b). The migration positions of molecular mass standards (in kDa) are indicated on the left hand side. The location of the WzyB band in (a and b) is indicated by a \*.

#### 5.4 Mass spectrometry analysis

Following trypsin digestion the samples were analysed by Q-ToF/Orbitrap at the Mass Spectrometry and Proteomics facility, UniSA. Due to the high sensitivity of the equipment used, a large number of peptides were detected (hits). The highest 20 Peptide Spectrum Matches (PSMs), which is a measure of relative protein abundance, can be seen summarised in Tables 5.1-5.9. The full list of hits, data acquired from the negative control bands (Figure 5.2b, excised bands 7-9), along with additional information can be found in Appendix D.

Hits that were detected from the negative control samples (Figure 5.2b, lane 3, bands 7-9) were removed from the other data sets if there was not at least a 5-fold PSMs increase of that protein in the other equivalent band. The purpose of these controls is to confirm that any positive hit acquired in the other experimental bands are valid, hits with high PSMs in the negative controls bands can all be seen in Appendix D. As expected the WzyB peptides were detected by mass

spectrometry in all bands (Tables 5.1-5.9). WzzB was also detected in excised bands, from purifications of *S. flexneri* Y expressing WzyB, in both the presence and absence of DSP, suggesting that WzyB and WzzB can interact without the aid of a crosslinker (Tables 5.1-5.4). As expected there was no WzzB peptides detected in the control purification, using the *S. flexneri* Y  $\Delta wzzE/\Delta wzz_{pHS2}/wzzB::kan^R$  mutant strain (Tables 5.7-5.9). Additionally as shown previously (Section 3.5.5) WzzE was able to interact with WzyB via co-purification experiments, therefore, as expected, WzzE peptides were detected in excised bands, from purifications of *S. flexneri* Y expressing WzyB, in both the presence and absence of DSP, suggesting that WzyB and WzzE can interact without the aid of a crosslinker (Tables 5.1 & 5.3-5.6). As expected there was no WzzE peptides detected in the control purification, using the *S. flexneri* Y  $\Delta wzzE/\Delta wzz_{pHS2}/wzzB::kan^R$  mutant strain (Tables 5.7-5.9). Interestingly, WzzE was consistently detected more frequently (higher PSMs) than WzzB, suggesting that WzyB and WzzE interact readily. Furthermore they can interact despite the presence of WzzB, which was absent in previous experiments (Section 3.5.5).

Other than the expected WzyB and Wzz proteins of interest, a number of proteins were detected frequently across the excised bands. Most notable was the FtsH/HflB protease protein which was detected consistently in all the higher molecular weight excised bands, including bands from the WzyB purifications without DSP crosslinker (Table 5.2, 5.3, 5.5, 5.6, 5.8 and 5.9). This suggests that WzyB and FtsH are likely interacting, however the implications of this interaction are yet to be investigated. Additionally, HflC was detected in all the lower molecular weight excised bands, including the band from the WzyB purification without DSP (Tables 5.1, 5.4 and 5.7). HflC is known to interact with the aforementioned FtsH (Kihara et al. 1996), again suggesting a complex involving WzyB and FtsH. The OmpC involved with Colicin and toxin CdiA resistance (Beck et al. 2016), was also detected consistently across all excised band with the exception of bands 2 and 11 (Tables 5.1, 5.3, 5.4, 5.5, 5.6, 5.7 and 5.9). The BamA protein was also only detected in all the higher molecular weight excised bands, including bands from the WzyB purifications without DSP crosslinker (Table 5.2, 5.3, 5.5, 5.6, 5.8 and 5.9). Additionally BamC was detected in all the lower molecular weight excised bands, again, including the band from the WzyB purification without DSP (Tables 5.1, 5.4 and 5.7). The BamA and BamC proteins are part of a membrane assembly complex involved in the assembly and insertion of beta-barrel proteins into the outer membrane (Hagan et al. 2010). They have also been shown to be the outer membrane receptor to the CdiA toxin (Aoki et al. 2008). The outer membrane linked proteins from the previously discussed Lpt system were also detected via mass spectrometry, including LptD (Table 5.3, 5.5, 5.6 and 5.9) and LptE (Table 5.6). Interestingly the Lpt proteins were more abundant in the absence of DSP, suggesting native interaction with WzyB directly or indirectly through their

common “substrate”, LPS. The PpiD chaperone which acts as a periplasmic “gatekeeper” to the Sec translocon (Antonoaea et al. 2008; Furst et al. 2018), was detected consistently in the higher molecular weight excised bands (Table 5.2, 5.3, 5.6, 5.8 and 5.9). Additionally, SecD of the Sec translocon (Tsukazaki et al. 2011) was also detected (Table 5.2 and 5.8). Finally, there were a number of other noteworthy proteins detected in the top 20 hits in the excised bands with less consistency such as, PflB, formate acetyltransferase 1 (Doberenz et al. 2014) (Table 5.2), Lon, ATP-dependent serine protease (Christensen et al. 2004) (Table 5.2, 5.5, 5.6 and 5.8), and YdgH, which is a currently uncharacterised putative membrane protein (Table 5.1, 5.4, 5.7). All the hits from this initial screen can be investigated further in the future to determine if they are directly involved in Oag polymerisation, thus this study paves the way for future mutagenesis and LPS analysis experiments.



**Table 5.1: Protein identification from band 1 of Figure 5.2b.**

Accession	Protein name	Description	PSMs	# unique peptides	Coverage (%)
P37784	Rfc/WzyB	O-antigen polymerase	73	9	18
I6C8N1	HflC	Modulator of FtsH protease HflC	41	21	64
Q0T240	NlpB/BamC	Outer membrane protein assembly factor BamC	21	11	43
A0A658Y1M8	GapA	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase	21	12	43
A0A2A2XR75	ManX_2	ManXYZ PTS system is involved in mannose transport.	16	13	32
A0A6D2XZS5	RpoA	DNA-directed RNA polymerase subunit alpha	13	10	36
D2ABV5	WzzE	ECA polysaccharide chain length modulation protein	13	8	32
Q0T6G6	YbhG	UPF0194 membrane protein	12	9	31
Q0SYD1	LldD	L-lactate dehydrogenase	12	11	31
D2A6V5	WzzB	Oag chain length determinant protein <i>S. flexneri</i>	11	10	37
A0A2S8DUZ3	AccA	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	11	8	29
A0A2S4MYF8	OmpC	Outer membrane protein C	11	4	15
I6D059	PyrD	Dihydroorotate dehydrogenase (quinone)	10	9	29
D2A904	RpsC	30S ribosomal protein S3	10	9	32
I6CVK9	PheS	Phenylalanine-tRNA ligase alpha subunit	9	8	28
I6CBL7	AtpA	ATP synthase subunit alpha	9	9	18
I6CHF3	AraE	MFS transporter, sugar porter family protein	9	4	9
Q0T4H3	YdgH	Uncharacterised protein	9	9	32
A0A2Y5B4B8	Mdh	Malate dehydrogenase	8	8	29
D2AHG2	YfiD	Autonomous glycy radical cofactor	8	6	43

PSMs = Peptide Spectrum Matches

**Table 5.2: Protein identification from band 2 of Figure 5.2b.**

Accession	Protein name	Description	PSMs	# unique peptides	Coverage (%)
A0A3U1DEW5	PflB	Formate C-acetyltransferase	72	40	59
A0A383JRI2	HflB/FtsH	ATP-dependent zinc metalloprotease FtsH	62	30	42
D2A6V5	WzzB	Oag chain length determinant protein <i>S. flexneri</i>	61	16	55
A0A4P7TNK7	AdhE	Aldehyde-alcohol dehydrogenase	56	36	46
A0A6N3RK72	ClpB	Chaperone protein ClpB	49	36	48
P37784	Rfc/WzyB	O-antigen polymerase	48	9	18
A0A380B9F2	PpiD	Peptidylprolyl isomerase	43	1	56
A0A380B1E8	NarG	Nitrate reductase (quinone)	41	27	26
A0A379Z1T0	FrdA	Fumarate reductase flavoprotein subunit	38	26	50
Q0SY20	TufB	Elongation factor Tu 2	37	20	62
A0A2Y4Y099	GlpD	Glycerol-3-phosphate dehydrogenase	35	19	38
A0A658XXS4	YaeT/BamA	Outer membrane protein assembly factor BamA	33	27	37
A0A4P7TR58	NdH	NAD(P)/FAD-dependent oxidoreductase	33	17	55
D2AD66	RpoB	DNA-directed RNA polymerase subunit beta	30	29	23
A0A6L6UYG8	LpdA	Dihydrolipoyl dehydrogenase	29	20	47
A0A379Z3L5	PlsB	Glycerol-3-phosphate acyltransferase	28	23	34
A0A6N3QN52	Lon	Lon protease	28	22	27
A0A6D2XMH9	AtpD	ATP synthase subunit beta	27	18	50
A0A3T2V040	FdnG	Formate dehydrogenase-N subunit alpha	23	17	24
A0A1Q8MKR1	SecD	Protein translocase subunit SecD	23	18	32

PSMs = Peptide Spectrum Matches

**Table 5.3: Protein identification from band 3 of Figure 5.2b.**

Accession	Protein name	Description	PSMs	# unique peptides	Coverage (%)
P37784	Rfc/WzyB	O-antigen polymerase	108	8	18
A0A383JRI2	HflB/FtsH	ATP-dependent zinc metalloprotease FtsH	44	24	37
A0A658XXS4	YaeT/BamA	Outer membrane protein assembly factor BamA	42	32	39
D2A6V5	WzzB	Oag chain length determinant protein <i>S. flexneri</i>	41	15	55
A0A379Z1T0	FrdA	Fumarate reductase flavoprotein subunit	37	24	49
A0A4P7TR58	NdH	NAD(P)/FAD-dependent oxidoreductase	35	18	55
A0A4P7TNK7	AdhE	Aldehyde-alcohol dehydrogenase	29	22	34
I6D635	PpiD	Peptidylprolyl isomerase	29	1	49
A0A2Y4Y099	GlpD	Glycerol-3-phosphate dehydrogenase	27	17	31
A0A2S4MYF8	OmpC	Outer membrane protein C	25	1	32
Q0T1R3	ClpB	Chaperone protein ClpB	22	20	24
D2ABV5	WzzE	ECA polysaccharide chain length modulation protein	20	11	38
A0A658Y1M8	GapA	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase	18	12	39
F5NPX4	LptD	LPS-assembly protein LptD	16	15	29
Q83SE6	HtpG	Chaperone protein HtpG	14	12	25
A0A4P7TNY5	AhpC	Alkyl hydroperoxide reductase C	14	8	47
A0A1S9KGJ3	FkpA	Peptidyl-prolyl cis-trans isomerase	14	9	47
Q0T4H5	PntB	NAD(P) transhydrogenase subunit beta	13	7	24
A0A379Z327	PurA	Adenylosuccinate synthetase	13	8	22
A0A2S8E1V7	HflK	Modulator of FtsH protease	12	9	24

PSMs = Peptide Spectrum Matches

**Table 5.4: Protein identification from band 4 of Figure 5.2b.**

Accession	Protein name	Description	PSMs	# unique peptides	Coverage (%)
P37784	Rfc/WzyB	O-antigen polymerase	128	9	18
I6C8N1	HflC	Modulator of FtsH protease HflC	40	26	68
Q0T240	NlpB/BamC	Outer membrane protein assembly factor BamC	38	12	43
P0AG02	WzzE	ECA polysaccharide chain length modulation protein	24	14	51
I6CHF3	AraE	MFS transporter, sugar porter family protein	14	6	11
A0A6N3QM36	QmcA	Multi-copy suppressor of an FtsH/HtpX protease double disruption mutant	13	13	53
Q0T6G6	YbhG	UPF0194 membrane protein	13	9	31
A0A2S4MYF8	OmpC	Outer membrane protein C	12	9	23
A0A2Y4WVQ3	DegP	Periplasmic serine endoprotease DegP-like	12	10	20
D2A6V5	WzzB	Oag chain length determinant protein <i>S. flexneri</i>	9	8	33
A0A4P7TR77	LamB	Maltoporin	9	4	21
Q0SY20	TufB	Elongation factor Tu 2	8	7	21
A0A6N3RQ35	Dps	DNA protection during starvation protein	7	6	37
A0A2A2XR75	ManX_2	ManXYZ PTS system is involved in mannose transport.	6	6	27
A0A2S8DDU2	MurG	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase	6	6	23
Q0T4H3	YdgH	Uncharacterised protein	6	6	20
A0A6D2XZS5	RpoA	DNA-directed RNA polymerase subunit alpha	6	6	19
A0A2S8DV51	DnaJ	Chaperone protein DnaJ	6	5	13
Q0T2P1	GlpT	sn-glycerol-3-phosphate permease	6	4	12
A0A6N3R6K7	DcuA	Anaerobic C4-dicarboxylate transporter	6	6	9

PSMs = Peptide Spectrum Matches

**Table 5.5: Protein identification from band 5 of Figure 5.2b.**

Accession	Protein name	Description	PSMs	# unique peptides	Coverage (%)
F5NRI1	KefA/MscK	Potassium efflux system kefA	104	41	40
A0A383JRI2	HflB/FtsH	ATP-dependent zinc metalloprotease FtsH	37	21	34
F5NPX4	LptD	LPS-assembly protein LptD	24	21	36
P37784	Rfc/WzyB	O-antigen polymerase	24	9	18
A0A2S4MYF8	OmpC	Outer membrane protein C	21	1	24
A0A658XXS4	YaeT/BamA	Outer membrane protein assembly factor BamA	18	15	21
A0A380B6Y2	AcrB	Efflux pump membrane transporter	17	16	19
A0A658YYQ4	PntA	NAD(P) transhydrogenase subunit alpha	11	8	19
D2ABV5	WzzE	ECA polysaccharide chain length modulation protein	10	8	23
A0A2S8DV74	GlpK	Glycerol kinase	10	8	19
A0A6N3QN52	Lon	Lon protease	10	10	14
F5P070	InfB	Translation initiation factor IF-2	10	10	10
Q0T4H5	PntB	NAD(P) transhydrogenase subunit beta	9	6	18
A0A2Y5I422	YfgF_2	Diguanylate cyclase	8	7	14
A0A658YRQ2	AcrD	Efflux pump membrane transporter	8	7	8
F5P2J3	DctA	C4-dicarboxylate transport protein	7	5	13
A0A6N3QUN6	ZntA	Zinc/cadmium/mercury/lead-transporting ATPase	7	7	12
A0A6N3R6K7	DcuA	Anaerobic C4-dicarboxylate transporter	7	7	9
D2AGH9	Lpp	Major outer membrane lipoprotein Lpp	6	3	40
I6CBQ2	GyrB	DNA gyrase subunit B	6	5	8

PSMs = Peptide Spectrum Matches

**Table 5.6: Protein identification from band 6 of Figure 5.2b.**

Accession	Protein name	Description	PSMs	# unique peptides	Coverage (%)
P37784	Rfc/WzyB	O-antigen polymerase	72	9	18
A0A383JRI2	HflB/FtsH	ATP-dependent zinc metalloprotease FtsH	71	31	39
A0A658XXS4	YaeT/BamA	Outer membrane protein assembly factor BamA	57	32	43
A0A2S4MYF8	OmpC	Outer membrane protein C	33	1	40
A0A380B9F2	PpiD	Peptidylprolyl isomerase	25	1	43
D2ABV5	WzzE	ECA polysaccharide chain length modulation protein	24	12	38
F5NPX4	LptD	LPS-assembly protein LptD	23	20	37
Q0SZX8	TufA/Tuf1	Elongation factor Tu 1	15	11	30
A0A658YYQ4	PntA	NAD(P) transhydrogenase subunit alpha	13	8	19
A0A2Y5I422	YfgF_2	Diguanylate cyclase	11	8	16
A0A2S8D9R8	MrcB	Penicillin-binding protein 1B	10	7	10
Q0T6Q1	LptE	LPS-assembly lipoprotein LptE	9	6	27
A0A4P7TPX8	FhuA	Ferrichrome porin FhuA	9	8	13
F5NVZ8	SFK227_2311	Outer membrane porin protein LC	8	5	19
Q0T4H5	PntB	NAD(P) transhydrogenase subunit beta	8	5	16
A0A2Y5AZK8	Lon	Lon protease	8	8	13
A0A380B6V8	CopA	Copper exporting ATPase	7	7	9
A0A6G5Z0X2	YejO	Autotransporter	7	5	6
A0A6N3R6K7	DcuA	Anaerobic C4-dicarboxylate transporter	6	6	9
A0A2X2I0N0	DamX	Cell division protein DamX	5	5	12

PSMs = Peptide Spectrum Matches

**Table 5.7: Protein identification from band 10 of Figure 5.2b.**

Accession	Protein name	Description	PSMs	# unique peptides	Coverage (%)
P37784	Rfc/WzyB	O-antigen polymerase	60	9	18
I6C8N1	HflC	Modulator of FtsH protease HflC	34	20	60
P0A9B5	GapA	Glyceraldehyde-3-phosphate dehydrogenase	28	1	50
Q0T240	NlpB/BamC	Outer membrane protein assembly factor BamC	25	11	43
A0A2A2XR75	ManX_2	ManXYZ PTS system is involved in mannose transport.	18	13	32
Q83RX6	PyrD	Dihydroorotate dehydrogenase (quinone)	16	13	44
A0A2S8DUZ3	AccA	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	12	7	22
I6CVK9	PheS	Phenylalanine--tRNA ligase alpha subunit	11	7	26
I6CBL7	AtpA	ATP synthase subunit alpha	11	11	19
A0A6D2XZS5	RpoA	DNA-directed RNA polymerase subunit alpha	10	7	25
A0A2Y4WVQ3	DegP	Periplasmic serine endoprotease DegP-like	10	9	19
Q0T4H3	YdgH	Uncharacterized protein	10	9	31
Q0SYD1	LldD	L-lactate dehydrogenase	9	9	28
A0A2S4MYF8	OmpC	Outer membrane protein C	9	7	17
I6CHF3	AraE	MFS transporter, sugar porter family protein	9	6	9
Q0SZX8	TufA/Tuf1	Elongation factor Tu 1	9	7	18
A0A2S8D5N6	PyrB	Aspartate carbamoyltransferase	8	6	20
Q0T6G6	YbhG	UPF0194 membrane protein YbhG	8	6	23
A0A2S4MYK1	AccD	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta	8	8	37
A0A2S8DCC8	HemB	Delta-aminolevulinic acid dehydratase	7	7	29

PSMs = Peptide Spectrum Matches

**Table 5.8: Protein identification from band 11 of Figure 5.2b.**

Accession	Protein name	Description	PSMs	# unique peptides	Coverage (%)
P37784	Rfc/WzyB	O-antigen polymerase	57	9	18
Q0SXC2	FrdA	Fumarate reductase flavoprotein subunit	45	24	46
A0A383JRI2	HflB/FtsH	ATP-dependent zinc metalloprotease FtsH	38	24	34
A0A380B9F2	PpiD	Peptidylprolyl isomerase	30	23	51
A0A4P7TR58	NdH	NAD(P)/FAD-dependent oxidoreductase	30	16	46
A0A658XXS4	YaeT/BamA	Outer membrane protein assembly factor BamA	30	25	31
A0A6G5YV19	TufA/Tuf1	Elongation factor Tu 1	28	2	60
A0A6D2XMH9	AtpD	ATP synthase subunit beta	26	19	54
A0A379Z3L5	PlsB	Glycerol-3-phosphate acyltransferase	25	20	27
A0A0H2UYV7	NarG	Nitrate reductase (quinone)	24	19	17
A0A2Y4Y099	GlpD	Glycerol-3-phosphate dehydrogenase	23	16	35
P0A852	Tig	Trigger factor	22	15	32
A0A2S8D6C0	SecD	Protein translocase subunit SecD	20	16	30
A0A6N3QN52	Lon	Lon protease	20	19	25
I6CMA1	Dld	Quinone-dependent D-lactate dehydrogenase	19	15	32
A0A4P7TME0	Tkt	Transketolase	18	14	22
I6D6Q1	FtsI	Peptidoglycan D,D-transpeptidase FtsI	16	14	29
Q83SE6	HtpG	Chaperone protein HtpG	16	15	29
A0A0H2VVY4	DmsA	Anaerobic dimethyl sulfoxide reductase subunit A	15	13	20
F5NRH8	ArcB	Efflux pump membrane transporter	14	12	15

PSMs = Peptide Spectrum Matches



**Table 5.9: Protein identification from band 12 of Figure 5.2b.**

Accession	Protein name	Description	PSMs	# unique peptides	Coverage (%)
P37784	RfC/WzyB	O-antigen polymerase	120	9	18
A0A658XXS4	YaeT/BamA	Outer membrane protein assembly factor BamA	49	30	39
A0A383JRI2	HflB/FtsH	ATP-dependent zinc metalloprotease FtsH	40	24	32
Q0SZX8	TufA/Tuf1	Elongation factor Tu 1	35	18	53
A0A380B9F2	PpiD	Peptidylprolyl isomerase	32	22	51
A0A4P7TR58	NhD	NAD(P)/FAD-dependent oxidoreductase	32	17	48
R4NJV1	OmpC	Outer membrane protein C	21	7	34
A0A658Y1M8	GapA	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase	18	12	39
A0A2S8DUZ3	AccA	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	16	10	30
Q83SE6	HtpG	Chaperone protein HtpG	16	15	27
F5NPX4	LptD	LPS-assembly protein LptD	15	13	23
A0A6N3RAZ3	FtsN	Cell division protein FtsN	12	9	33
A0A2S8E1V7	HflK	Protein HflK	12	8	23
A0A379Z3L5	PlsB	Glycerol-3-phosphate acyltransferase	12	12	16
Q83RX6	PryD	Dihydroorotate dehydrogenase (quinone)	11	11	37
Q2EUU0	AccD	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta	11	8	29
Q0T4H5	PntB	NAD(P) transhydrogenase subunit beta	11	6	18
A0A2Y4Y099	GlpD	Glycerol-3-phosphate dehydrogenase	11	8	16
A0A0H2VVY4	DmsA	Anaerobic dimethyl sulfoxide reductase subunit A	11	10	15
D2ABQ4	TnsC	Transposon Tn7 transposition protein TnsC	11	1	4

PSMs = Peptide Spectrum Matches

## 5.5 Discussion

---

This Chapter has attempted to identify novel WzyB protein binding partners as well as further characterise the interaction of WzyB to the Wzz proteins. Using a WzyB-FLAG purification protocol, co-purified targets were analysed via band excision, trypsin digest and mass spectrometry. As expected WzyB was detected constantly at high relative abundance levels with typically 9 unique peptides detected in the presence and absence of DSP. This is greater than previous experiments involving co-purification of WzyB using DSP which achieved detection of 5-8 unique peptides (Nath & Morona 2015). As expected WzzB and WzzE proteins were detected in both higher molecular weight bands and lower molecular weight bands from purifications in the presence and absence of DSP. Overall, these results suggest that WzyB is able to interact physically with WzzE, despite the presence of WzzB. Previous experiments highlighting a physical interaction between WzyB and WzzE were performed using an overexpression construct of WzzE and in a WzzB deficient strain (Chapter 3) (Leo et al. 2020). Therefore the data acquired in this Chapter suggests that WzyB and WzzE interact in a wild type setting; additionally WzzE was observed to be more abundant in excised bands where both WzzE and WzzB were present. Together this data suggests that WzzE and WzyB may interact in order to perform a physiological function, perhaps to aid in modal length control of Oag as previously reported (Chapter 3) (Leo et al. 2020), or more likely it may sequester WzzE from the ECA pathway leading to an impact on ECA polymerisation; experiments in Chapter 3 showed that overexpression of WzzE resulted in a reduced modal length of ECA (Chapter 3) (Leo et al. 2020). Additionally, WzzB was detected in a relative high abundance both in the presence and absence of DSP, further highlighting a previously reported interaction between these members of the Wzy-dependent pathway (Leo et al. 2020; Nath & Morona 2015). This Chapter is the first to show a direct interaction between WzyB and WzzB without the aid of a crosslinker via mass spectrometry. Therefore it can be said that these proteins need to interact to control Oag modal length.

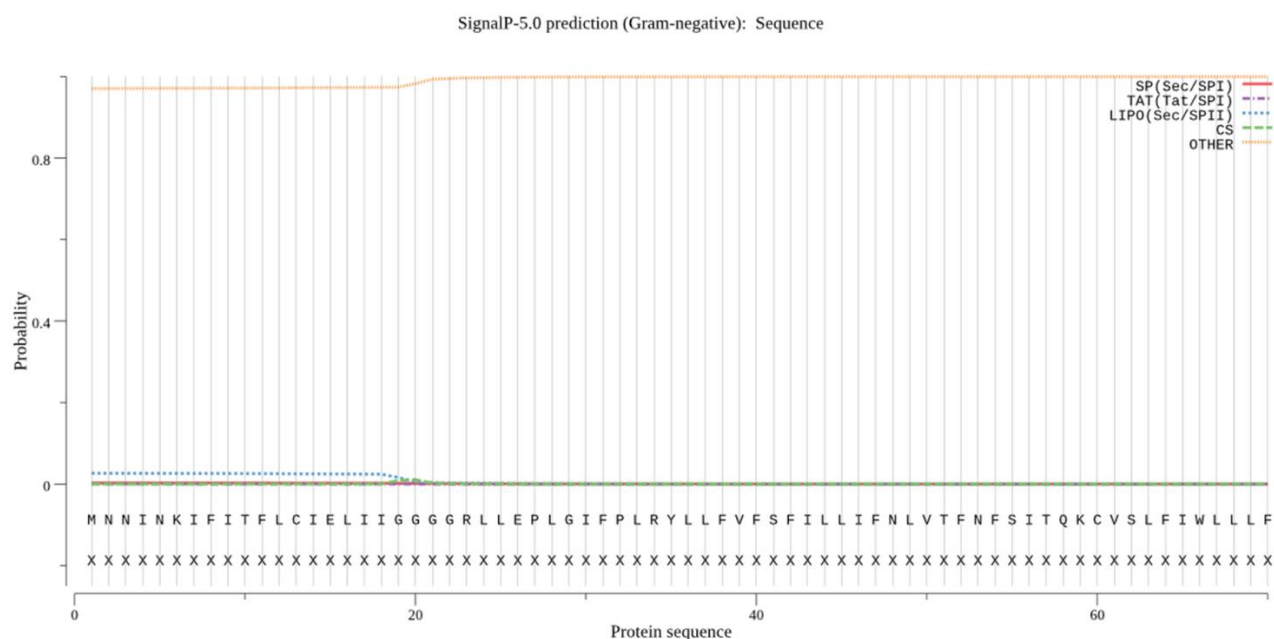
One of the most notable results from the mass spectrometry analysis was the absence of any other Wzy-dependent proteins, other than the previously shown Wzz:WzyB interactions (Leo et al. 2020; Nath & Morona 2015). WzyB has long been hypothesised to be included in a Wzy-dependent pathway complex made up of other proteins including Wzx and WaaL (Marolda et al. 2006), as depicted in Figure 1.8. However due to the results obtained in this Chapter and previously reported attempts to co-purify other members of the WzyB-dependent pathway (Carter et al. 2009; Marczak et al. 2013), the possibility of a Wzy-dependent complex including WaaL and or Wzx seems unlikely. This suggests that the steps of Oag polymerisation that involve WaaL and Wzx likely occur independent of any direct interaction with WzyB.

The protease FtsH was consistently detected at a relative high abundance, suggesting a direct interaction with WzyB. The physical interaction between WzyB and FtsH and its biological implications are yet to be investigated. FtsH is known to regulate and degrade both cytosolic and inner membrane proteins via a minimum ~ 20 aa cytoplasmic tail with no apparent sequence requirements (Langklotz et al. 2012). Additionally, FtsH has also been shown to regulate levels of LpxC and KdtA/WaaA, both of which are involved with the synthesis of LPS (Katz & Ron 2008). An *ftsH* mutation is lethal (Guest, RL et al. 2020), this is due to the increased levels of LpxC which leads to the increase level of LPS leading to abnormal membrane structures inside the periplasm. However, this lethality can be repressed if LPS synthesis is reduced (Ogura et al. 1999). Additionally, FtsH has also been implicated in colicin tolerance and CDI resistance (Narberhaus et al. 2009; Willett et al. 2015). The interaction between WzyB and FtsH could be simply explained by FtsH interacting with WzyB to degrade and regulate its cellular concentration, however WzyB has been shown to only possess a 12 aa cytoplasmic tail at the C-terminus (Daniels et al. 1998). Therefore, due to the difficulty in studying FtsH, and the fact that FtsH has been implicated in LPS previously, there may be a functional purpose for this physical interaction. Difficulties with studying WzyB may be due to its degradation by FtsH. Previous studies have reported several *wzyB* mutants have decreased protein levels in the absence of WzzB (Nath et al. 2015); this may be due to WzzB reducing the access of FtsH to WzyB for degradation.

HflC was also shown to interact with WzyB. Interestingly, it was only detected in the lower molecular weight excised bands. As HflC is known to interact and modulate FtsH, it is possible that the interaction between HflC and WzyB may be indirect. Generally, proteins detected in the lower region of the gel are likely due to indirect interactions with WzyB as they become disassociated prior to separation in the gel. Alternatively, these proteins may interact with WzyB as a homo-oligomer, obvious examples include the Wzz proteins which were detected in both higher and lower molecular weight bands. This could explain why WzzB was detected at ~40 kDa (its monomeric size) and also upwards of ~ 200 kDa.

The interaction of WzyB with proteins such as OmpC and BamA/C, was highlighted due to their previous implication with colicin and toxin resistance, suggesting a larger involvement of WzyB to colicin resistance (Cascales et al. 2007; Tran et al. 2014; Zakharov et al. 2012). Surprisingly, outer membrane members of the Lpt export system were detected. This is surprising as both LptD and LptE reside in the outer membrane, thus an interaction may be indirectly via LPS. SecD and the protein linked to the pathway, PpiD, were also detected to interact with WzyB. *In silico* analysis of the WzyB peptide sequence indicate that WzyB is not likely translocated by the Sec pathway (Figure 5.3). The analysis showed a less than 1% probability of a Sec signal

peptide at the N terminus. Therefore, the interaction of the inner membrane located SecD with WzyB was unexpected.



**Figure 5.3: *In silico* analysis of Sec dependent translocation probability of WzyB.**

Analysis of the amino acid sequence of WzyB for Sec dependent translocation via peptide identification. Probability of Sec dependent translocation is <1%. Analysis was performed by SignalP-5.0 (<http://www.cbs.dtu.dk/services/SignalP/>)

There were a number of other hits detected via mass spectrometry, some of which were inconsistent i.e. were only detected in one excised band, while others had an overall low abundance or PSMs value. These hits will need to be reproduced in repeat experiments to provide insight if these are significant WzyB binding partners. As this experiment was performed once, no statistical inference can be made with the data obtained, however, the hits presented are of proteins that were detected with a minimum of 5 fold increase in abundance compared to the negative control, and as such are highly likely to be interacting with WzyB. This Chapter paves the way for future studies of WzyB and its binding partners. Proteins such as the uncharacterised YdgH, FtsH and Lon proteases are all good candidates for future mutagenesis analysis and investigating the resulting impact on the LPS production and/or Oag modal length control.

# Chapter Six

---

## IDENTIFICATION OF NOVEL LPS GENES

---

## Chapter 6: Identification of novel LPS genes

---

### 6.1 Statement of Authorship

---

The preliminary data used and built upon was generated and submitted as part of Vincenzo Leo's Honours thesis 2016 (Leo 2016) in collaboration with Dr. Elizabeth Tran. All other data presented in this Chapter was generated during the PhD candidature for PhD thesis submission.

### 6.2 Introduction

---

LPS is the major virulence factor of many Gram negative pathogens including *S. flexneri*, however due to its complexity, aspects of its synthesis, transport and length regulation are unknown. Due to this complexity, the mass spectrometry data from Chapter 5, and the preliminary unpublished mass spectrometry data from the Morona laboratory (Nath, 2015), we hypothesise that there are uncharacterised genes involved in the biosynthesis, regulation and transport of the lipopolysaccharide molecule. A preliminary screen was performed in order to identify novel genes that affect LPS, and this was done using a site directed knock out library which was comprised of every non-lethal gene of the *E.coli* BW25113 genome being substituted with a Kanamycin resistance cassette (The Keio collection) (Baba et al. 2006). The Keio collection was screened first for any mutants that showed an increased sensitivity to colicin E2 following the addition of the *wbbL* gene; which is required for smooth LPS production. Next, a secondary screen was performed in which the LPS from colicin sensitive mutants were analysed (Leo 2016). The mutants that also showed an LPS defect via silver stained SDS-PAGE LPS analysis are summarised in Table 6.1 (Leo 2016).

**Table 6.1: Mutants with LPS defect**

Mutant	Description	LPS Profile
<i>ΔsdhC::kan<sup>R</sup></i>	Succinate dehydrogenase hydrophobic membrane anchor subunit	Core & O-antigen defect
<i>ΔargP::kan<sup>R</sup></i>	HTH-type transcriptional regulator ArgP	O-antigen complete knockout
<i>ΔyedV::kan<sup>R</sup></i>	Sensor protein – two component regulatory system	Reduced level of O-antigen
<i>ΔldhA::kan<sup>R</sup></i>	2-hydroxyacid dehydrogenase	Reduced level of O-antigen
<i>Δlhr::kan<sup>R</sup></i>	ATP-dependent helicase superfamily protein	Core & O-antigen defect
<i>ΔyegQ::kan<sup>R</sup></i>	Peptidase	O-antigen complete knockout
<i>ΔycbU::kan<sup>R</sup></i>	FimA_2 protein	Core & O-antigen defect
<i>ΔybjJ::kan<sup>R</sup></i>	DEOR-type transcriptional regulator	Reduced level of O-antigen
<i>ΔmprA::kan<sup>R</sup></i>	Regulator of RpoS	Core & O-antigen defect
<i>ΔoppF::kan<sup>R</sup></i>	Oligopeptide transport ATP-binding protein OppF	O-antigen complete knockout
<i>Δdam::kan<sup>R</sup></i>	DNA adenine methylase	Reduced level of O-antigen
<i>Δcrp::kan<sup>R</sup></i>	cAMP-activated global transcriptional regulator CRP	Reduced level of O-antigen
<i>Δwzc::kan<sup>R</sup></i>	Tyrosine-protein kinase wzc	Core & O-antigen defect

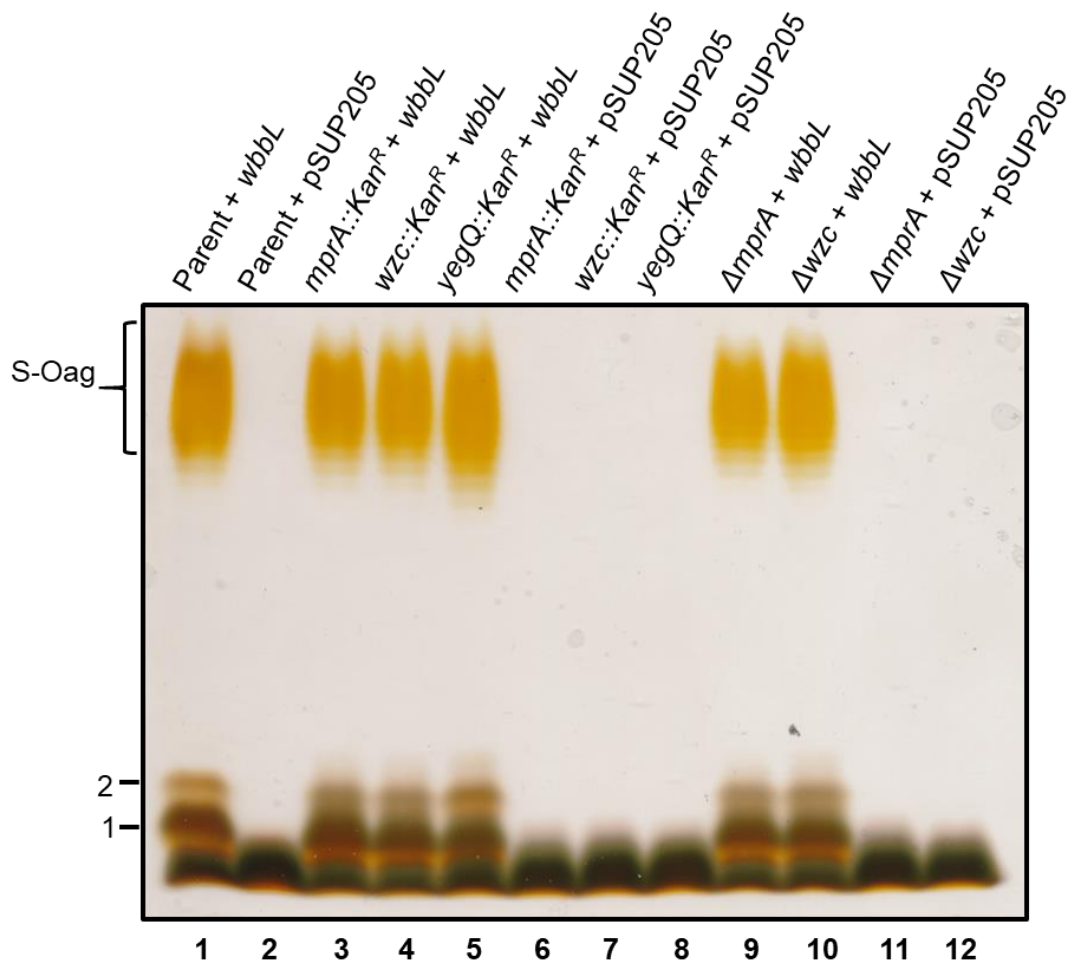
The aim of this Chapter was to confirm and characterise the phenotypes previously reported by recreating the mutants via Lambda Red mutagenesis (Section 2.3.14) and then subsequently analyse the LPS (Section 2.5.2) and colicin E2 sensitivity (Section 2.8.1) of these mutants in order to determine if the gene is connected to LPS biosynthesis, transport or modal length control.

### 6.3 Mutagenesis of select mutants in *E.coli* W3110

Initially, genes *mprA*, *wzc*, *yegQ*, which when mutated displayed obvious LPS defects, were selected for Lambda Red mutagenesis. Primers were designed (Appendix C) and the Lambda Red protocol (Section 2.3.14) was performed on *E. coli* W3110 to substitute each of the genes with a kanamycin resistance cassette (VLRM75-77) (Appendix A). The kanamycin cassette in *Δwzc::kan<sup>R</sup>* and *ΔmprA::kan<sup>R</sup>* were then subsequently removed using pCP20 (Section 2.3.14) (VLRM 78-80) (Appendix A). *E.coli* W3110 has a mutation in the gene *wbbL* which encodes a glycosyltransferase needed for the production of the smooth LPS phenotype, as such each of the mutants and the parent WT *E. coli* W3110 were transformed with pSUP205::*wbbL* or the vector control pSUP205 (VLRM 81-94) (Appendix A). The LPS profiles of these mutants were

investigated via silver-stained SDS-PAGE to determine if the original reported phenotype was reproducible (Figure 6.1). As expected when the parent *E.coli* W3110 were expressed WbbL, a smooth LPS profile was observed with an S-Oag modal length (Figure 6.1, lane 1). Furthermore, as expected, when the parent strain harboured the pSUP205 vector control the LPS profile was rough as no Oag polymerisation was detected (Figure 6.1, lane 2). However, all three mutants ( $\Delta mprA::kan^R$ ,  $\Delta wzc::kan^R$  and  $\Delta yegQ::kan^R$ ), when expressing WbbL, produced WT LPS profiles (Figure 6.1, lane 3-5), comparable to the parent strain expressing WbbL (Figure 6.1, lane 1). Therefore, the recreated mutants did not reproduce the originally reported phenotypes (Table 6.1) of the Keio collection (Baba et al. 2006). When the mutant strains were not expressing WbbL they produced rough LPS phenotypes with no Oag (Figure 6.1, lanes 6-8), comparable to the parent strain harbouring the pSUP205 vector control (Figure 6.1, lane 2). Once again, the LPS profiles did not reproduce the originally reported phenotypes, as  $\Delta mprA$  and  $\Delta wzc$  originally showed LPS core defects. Additionally, mutant strains  $\Delta mprA$  and  $\Delta wzc$ , in which the kanamycin cassette were removed, and expressing either WbbL or harbouring the pSUP205 vector, produced LPS profiles (Figure 6.1, lanes 9-12) comparable to the respective WT parent W3110 (Figure 6.1 lanes 1 and 2). Overall, these results suggest that the mutations in these genes have no effect on LPS in *E. coli* W3110.





**Figure 6.1 Analysis of LPS profiles of *E. coli* W3110 putative LPS mutants**

*E. coli* strains were grown to log-phase and  $1 \times 10^9$  collected cells were lysed in lysis buffer in the presence of proteinase K. Samples were then electrophoresed on a SDS-15% PAGE gel, and silver-stained (Section 2.5.2). Number of Oag RUs and Oag modal lengths are shown on the left-hand side. Strains are as follows: parent W3110 expressing *wbbL* (lane 1) or harbouring the pSUP205 vector control (lane 2), and Lambda Red generated mutants (2.3.15) with and without the kanamycin resistance cassette, expressing *wbbL* or harbouring the pSUP205 vector controls (as indicated).

#### 6.4 Mutagenesis of select mutants in *E. coli* BW25113

To confirm that the results obtained from the mutagenesis of *mprA*, *wzc* and *yegQ* in *E. coli* W3110 were not strain specific, Lambda Red mutagenesis was performed on *E. coli* BW25113 (the parent strain of the original Keio collection (Baba et al. 2006)) to generate two mutants,  $\Delta wzc::kan^R$  and  $\Delta mprA::kan^R$  (VLRM 95 and 96) (Appendix A). These genes were selected to be mutated as the profile originally observed affected the core sugars, therefore a mutant LPS profile could be determined without the need of the WbbL transferase, as these putative mutants were shown to produce a deep rough phenotype. The LPS profiles of these mutants along with the equivalent mutants from the Keio collection (Baba et al. 2006), were analysed via silver-stained SDS-PAGE. (Figure 6.2). The  $\Delta wzc::kan^R$  and the  $\Delta mprA::kan^R$  mutants from the Keio collection had deep rough phenotypes (Figure 6.2, lanes 2 and 4) when compared to the WT parent *E. coli*

BW25113 (Figure 6.2, lane 1). However, the regenerated mutants showed a WT rough phenotype (Figure 6.2, lane 3 and 5) comparable to the WT parent. These results suggest that the mutants identified from the Keio screen (Leo 2016) may harbour secondary mutations on the chromosome that are responsible for the LPS defects originally identified.



**Figure 6.2 Analysis of LPS profiles of *E. coli* BW25113 mutants compared to Keio mutants**

*E. coli* strains were grown to log-phase and  $1 \times 10^9$  collected cells were lysed in lysis buffer in the presence of proteinase K. Samples were then electrophoresed on a SDS-15% PAGE gel, and silver-stained (Section 2.5.2). Strains are as follows: parent BW25113 (lane 1), Lambda Red generated mutants (2.3.15) (lanes 3 and 5), mutants from the Keio collection (Baba et al. 2006) (lanes 2 and 4)

## 6.5 Whole genome sequencing of the identified mutants

As the recreated mutants did not replicate the originally identified LPS profiles, the 13 mutants (Table 6.1) from the Keio collection and the WT parent BW25113, were subjected to whole genome sequencing. The bacterial genomes were extracted (Section 2.3.2) at a concentration of  $12.5 \text{ ng } \mu\text{L}^{-1}$  and sent to BGI TECH SOLUTIONS (Hong Kong) for bacterial genome resequencing using the DNBseq™ sequencing platform (Section 2.3.18). In order to process the data, the program Geneious Prime was used, whereby each of the mutant's genome sequencing data was analysed by the “map to reference” feature using the *Escherichia coli*

BW25113 strain K-12, complete genome (NCBI reference sequence: NZ\_CP009273.1), as the reference genome. The results from the genome analysis can be seen summarised in Tables 6.2 – 6.13. The presence of the kanamycin cassette in place of a gene is listed first and highlighted in yellow, other mutations with a variant frequency of greater than 85% were considered a correct mutation and displayed with the variant frequency along with any resulting amino acid changes (Tables 6.2 – 6.13). The full details of all mutations found in each mutant sequenced along with the parent WT control can be seen in Appendix E. The kanamycin cassette was correctly located in place of the gene specified in most of the mutants analysed.

Interestingly, the  $\Delta sdhC::kan^R$  Keio mutant had no mutation affecting the *sdhC* gene. In fact the kanamycin cassette was located in place of the *rscC* gene, along with many mutations affecting the *gntX* gene (Table 6.2). These results suggest that the phenotypes observed in this mutant (Table 6.1) are likely the result of the disrupted *rscC* gene, a component of the Rcs signalling system, which controls the transcription of a number of genes involved with LPS (Clarke et al. 2002; Klein & Raina 2019), that includes *wzz* in *Salmonella Typhimurium* (Delgado et al. 2006).

Unexpectedly, the  $\Delta oppF::kan^R$  Keio mutant had no detectable kanamycin cassette in its genome and no mutations in any other gene with a variant frequency greater than 85%. The most interesting mutation identified was a substitution mutation in *wzyB*, which would explain the complete lack of Oag polymerisation in this mutant. However this mutation's variant frequency was only 25.9% - 27.7% (highlighted in red) and thus more analysis is needed to confirm this hypothesis (Table 6.10).

Keio mutants  $\Delta ldhA::kan^R$ ,  $\Delta yegQ::kan^R$ ,  $\Delta dam::kan^R$ , and  $\Delta crp::kan^R$ , possessed only the kanamycin cassette in the expected location and no other mutations affecting other genes with a variant frequency greater than 85% (Table 6.4, 6.6, 6.11 and 6.12). As such the mutation to the stated gene is likely the cause of the LPS defect, however *crp* (a cAMP-activated global transcriptional regulator (Fic et al. 2009)), *dam* (responsible for DNA methylation (Marinus & Lobner-Olesen 2014)), and *ldhA* (a lactate dehydrogenase (Kabir et al. 2005)) have global and broad impacts on the bacteria, and as such the affect to LPS is most likely an indirect one. Interestingly, the  $\Delta yegQ::kan^R$  Keio mutant possessed no other detectable changes to any coding sequence (CDS) on the genome. The cause for the originally observed phenotype of no Oag polymerisation (Table 6.1), may be due to a polar effect or an epigenetic change to this particular Keio mutant, more investigation is required to determine the cause of the LPS profile initially observed as recreation of this mutant produced a WT LPS profile (Figure 6.1).

As expected, there were several secondary mutations identified in many of the mutants analysed. The  $\Delta argP::kan^R$  Keio mutant possessed the kanamycin cassette in the correct location, however, it also harboured mutations in the *lrhA* and *wbbJ* genes (Table 6.3). The mutation to the *wbbJ* gene was a tandem repeat deletion causing early termination. Since WbbJ is a putative LPS biosynthesis O-acetyl transferase protein (Gaudet et al. 2011; Maes et al. 2020), this is likely the cause of the observed phenotype (Table 6.1). The  $\Delta lhr::kan^R$  Keio mutant possessed the kanamycin cassette in the correct location, but also, possessed a secondary mutation with a high variant frequency in the *pepP* gene (a Xaa-Pro aminopeptidase (Matos et al. 1998)). The mutation to the *pepP* gene resulted in removal the start codon (Table 6.5). The Lhr/RhlF proteins is a probable ATP-dependent helicase, which may have broad impacts on the bacterium. As there is no known link between either of these genes and LPS, further mutagenic investigation is required to determine the cause of the core and Oag defect LPS profile observed (Table 6.1). The  $\Delta ycbU::kan^R$  Keio mutant possessed the kanamycin cassette in the correct location, however it also harboured secondary mutations in *selB* and *ace* that resulted in aa substitutions (Table 6.7). The YcbU protein is an uncharacterised surface fimbrial-like protein, linked to promoting bacteria adhesion to abiotic surfaces (Korea et al. 2010). SelB is a selenocysteine-specific elongation factor (Arner et al. 1999) and AceE is a well characterised pyruvate dehydrogenase E1 component (Guest, JR & Stephens 1980; Pang et al. 2011). It is unknown which mutation is responsible for the core and Oag defect observed (Table 6.1).

The  $\Delta mprA::kan^R$  Keio mutant possessed the kanamycin cassette in the expected location, however it also possessed two other secondary mutations in *rseB* and *yraH*. The *rseB* was a missense mutation while the mutation in *yraH* was silent (Table 6.9). The RseB protein is a sigma-E factor regulatory protein, which negatively modulates the activity of sigma-E (RpoE) via interactions with RseA under non-stress conditions (De Las Penas et al. 1997). Interestingly, RseB has been shown to interact with LPS. This interaction disrupts the aforementioned interaction with RseA, as such when periplasmic LPS levels increase the sigma-E regulon is induced (Lima et al. 2013). As the *mprA* gene has been deleted in two different *E. coli* backgrounds, both of which resulted in WT LPS profile (Figure 6.1 and 6.2), the change in *rseB* is likely the cause of the LPS defect detected (Table 6.1).

Finally, as expected the  $\Delta wzc::kan^R$  Keio mutant possessed the kanamycin cassette in the correct location, however there was an additional secondary mutation causing a frame shift mutation in *gmhB*. The GmhB protein is a well characterised D-glycero-beta-D-manno-heptose-1, 7-bisphosphate 7-phosphatase. Interestingly it has been reported that cells lacking this protein form an altered LPS core causing an increase in outer membrane permeability (Kneidinger et al. 2002;

Taylor et al. 2010). Therefore, as the recreation of the *wzc* mutation in both *E. coli* backgrounds produced WT LPS, profiles it is likely that the profile observed was caused by the *gmhB* frameshift mutation detected in the strain.

**Table 6.2: Summary of the mutations found in *ΔsdhC::kan<sup>R</sup>* Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>rscC</i>	Sensor histidine kinase RcsC	N/A	N/A	N/A	Kanamycin cassette	N/A
		GCC -> CAA	130	97.1% -> 97.2%	A -> Q	Substitution
		CCG -> TAT	175	96.2% -> 96.6%	P -> Y	Substitution
		CAT -> TGA	142	94.6% -> 94.7%	N/A	Truncation
		GC -> TG	211	94.6% -> 94.7%	A -> W	Substitution
		CAAAGA -> TGGTGC	184	94.4% -> 94.8%	QR -> WC	Substitution
		CGGT -> TCTC	197	94.3% -> 94.6%	TV -> IS	Substitution
<i>gntX</i>	DNA utilization protein YhgH	AACC -> GTGG	170	93.4% -> 93.8%	KP -> SG	Substitution
		GTGGATTACC-> CACATGGGTA	119	92.1% -> 92.6%	CGLP -> SHGY	Substitution
		TGGTC -> GCAAT	191	91.9% -> 92.7%	LV -> RN	Substitution
		CCGA -> TTCC	203	91.8% -> 93.5%	AD -> VP	Substitution
		CCGCCG -> GATAAT	214	85.4% -> 88.9%	PP -> DN	Substitution
		CCTGCGGTCGCTGCCTGCAA -> TGACTACAATAATTAGTTTT	149	85.0% -> 86.6%	PCGRCLQ -> LTTIISF	Substitution
		TA -> AG	221	85.0% -> 86.4%	N/A	Truncation

**Table 6.3: Summary of the mutations found in *ΔargP::kan<sup>R</sup>* Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
-----------	-------------	-----------	--------------	-------------------	-------------------	----------------

<i>argP</i>	HTH-type transcriptional regulator ArgP	N/A	N/A	N/A	Kanamycin cassette	N/A
<i>lrhA</i>	Probable HTH-type transcriptional regulator LrhA	(-)TGCGGCAGC	85	99.6%	(-)AAA	Deletion
<i>wbbJ</i>	Lipopolysaccharide biosynthesis O-acetyl transferase WbbJ	(A)9 -> (A)8	203	99.6%	N/A	Deletion (tandem repeat)

(-) = deletion, the following nt or aa were deleted.

(A/T/G/C)<sub>x</sub>->(A/T/G/C)<sub>x</sub> = Tandem repeat mutation, addition or substitution of repeat sequence.

**Table 6.4: Summary of the mutations found in *ΔldhA::kan<sup>R</sup>* Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>ldhA</i>	2-hydroxyacid dehydrogenase	N/A	N/A	N/A	Kanamycin cassette	N/A

**Table 6.5: Summary of the mutations found in *Δlhr::kan<sup>R</sup>* Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>lhr</i>	Probable ATP-dependent helicase <i>lhr</i>	N/A	N/A	N/A	Kanamycin cassette	N/A
<i>pepP</i>	Xaa-Pro aminopeptidase	C -> A	3	99.00%	M->I	Loss of start codon

**Table 6.6: Summary of the mutations found in *ΔyegQ::kan<sup>R</sup>* Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>yegQ</i>	tRNA hydroxylation protein P	N/A	N/A	N/A	Kanamycin cassette	N/A

**Table 6.7: Summary of the mutations found in  $\Delta ycbU::kan^R$  Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>ycbU</i>	Uncharacterized fimbrial-like protein YcbU	N/A	N/A	N/A	Kanamycin cassette	N/A
<i>selB</i>	Selenocysteine-specific elongation factor	T -> G	709	95.90%	I -> L	Substitution
<i>aceE</i>	Pyruvate dehydrogenase E1 component	T -> A	1901	87.80%	L -> Q	Substitution

**Table 6.8: Summary of the mutations found in  $\Delta ybjJ::kan^R$  Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>ybjJ</i>	Inner membrane protein YbjJ	N/A	N/A	N/A	Kanamycin cassette	N/A
<i>aceE</i>	Pyruvate dehydrogenase E1 component	A -> G	2416	97.80%	K -> E	Substitution

**Table 6.9: Summary of the mutations found in  $\Delta mprA::kan^R$  Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>mprA</i>	Response regulator MprA	N/A	N/A	N/A	Kanamycin cassette	N/A
<i>rseB</i>	Sigma-E factor regulatory protein RseB	A -> C	122	98.50%	L -> R	Substitution
<i>yraH</i>	Uncharacterized fimbrial-like protein YraH	G -> A	378	98.30%	None	None

**Table 6.10: Summary of the mutations found in  $\Delta oppF::kan^R$  Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>*No kanamycin cassette located</i>						
<i>wzyB</i>	O-antigen polymerase	AAG -> TGT	1144	25.9% -> 27.7%	L -> T	Substitution

\* no kanamycin cassette was located in this strain, additionally no other mutations were located with a variant frequency greater than 85% (see Appendix E).

**Table 6.11: Summary of the mutations found in  $\Delta dam::kan^R$  Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>dam</i>	DNA adenine methylase	N/A	N/A	N/A	Kanamycin cassette	N/A

**Table 6.12: Summary of the mutations found in  $\Delta crp::kan^R$  Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>crp</i>	cAMP-activated global transcriptional regulator CRP	N/A	N/A	N/A	Kanamycin cassette	N/A

**Table 6.13: Summary of the mutations found in  $\Delta wzc::kan^R$  Keio mutant**

Gene name	Description	Nt change	CDS position	Variant frequency	Amino acid change	Protein effect
<i>wzc</i>	Inner membrane protein YbjJ	N/A	N/A	N/A	Kanamycin cassette	N/A
<i>gmhB</i>	D-glycero-beta-D-manno-heptose-1,7-bisphosphate 7-phosphatase	- C	216	99.60%	N/A	Frame shift

(-) = deletion, the following nt or aa were deleted.



## 6.6 Discussion

---

LPS is a critical component of many pathogenic Gram-negative bacteria, it is a complex glycolipid, and as such many aspects of its synthesis, transport and length regulation are unknown. A preliminary screen using the Keio collection (Baba et al. 2006) was undertaken to identify novel genes related to these aspects of LPS via firstly increased colicin E2 sensitivity, and secondly by LPS analysis via silver-stained SDS-PAGE (Leo 2016). The results from that study are summarised in Table 6.1. The aim of this Chapter was to build upon this preliminary screen by confirming the LPS defects observed. Unexpectedly when the select Keio mutants  $\Delta mprA::kan^R$ ,  $\Delta wzc::kan^R$  and  $\Delta yegQ::kan^R$  were recreated in *E. coli* W3110 using Lambda Red mutagenesis, the LPS defect initially observed were not reproduced and resulted in WT LPS profiles (Figure 6.1). Similarly when the Keio mutant's  $\Delta mprA::kan^R$  and  $\Delta wzc::kan^R$  were recreated once more in the parent strain of the Keio library *E. coli* BW25113, the mutants produced WT LPS profiles (Figure 6.2). Before further mutagenesis, it was decided that there may be a number of secondary mutations in the Keio collection causing the altered LPS profile and as such whole genome sequencing was performed on all the identified LPS mutants from the preliminary screen.

As expected upon analysing the whole genome sequencing data, there were a number of Keio mutants that contained other secondary mutations (Tables 6.2 - 6.13). The presence of secondary mutations in the Keio collection has been reported by others (Parker et al. 2019; Yamamoto et al. 2009). Only four of the thirteen mutants sequenced possessed the kanamycin substitution in the correct location of the stated gene with no other secondary mutations ( $\Delta ldhA::kan^R$ ,  $\Delta yegQ::kan^R$ ,  $\Delta dam::kan^R$ , and  $\Delta crp::kan^R$ ) (Tables 6.4, 6.6, 6.11, and 6.12, respectively). Of these genes *ldhA*, *dam*, and *crp* are involved with global metabolism and cell viability, and as such an impact, on LPS is expected and likely to be indirect. The *yegQ* gene is less characterised but is linked with tRNA hydroxylation (Sakai, Y et al. 2019), however attempts to recreate this mutant in *E. coli* W3110 resulted in a WT LPS profile. Future investigation of the *yegQ* gene should involve mutagenesis in *E. coli* BW25113 as this was the parent strain of the original Keio mutant, it is possible that the background of the strain is playing a role in the phenotype observed as their genotypes are considerably different (*E. coli* W3110 - F lambda<sup>-</sup> IN(*rrnD-rrnE*)1 *rph-1* and *E. coli* BW25113 - F<sup>-</sup> DE(*araD-araB*)567 *lacZ*4787(del)::*rrnB-3* LAM<sup>-</sup> *rph-1* DE(*rhaD-rhaB*)568 *hsdR*514).

Uniquely, the  $\Delta oppF::kan^R$  Keio mutant had no detectable kanamycin cassette along with no secondary mutations with a variant frequency above 80%. Interestingly, a mutation in *wzyB* was detected, though at a relatively low frequency, but would explain the profile observed. Further investigation and recreation of this mutant is required. Additionally the  $\Delta sdhC::kan^R$  Keio mutant

was incorrect, as no mutations were discovered in the *sdhC* gene, and the kanamycin cassette was alternatively detected in the *rscC* gene. RcsC is part of the Rcs signalling system, which controls the transcription of a number of genes involved with LPS (Clarke et al. 2002; Klein & Raina 2019), and is hence likely the cause of the LPS profile initially observed. The cause of this incorrect mutant may be due to contamination or the library being incorrectly labelled, considering both the  $\Delta rcsC::kan^R$  and  $\Delta sdhC::kan^R$  Keio mutants are on the same 96 well plate, either is a possibility.

Many of the secondary mutations found were in genes that have previously been reported to have links to LPS and as such, it is likely that these are the reasons for the LPS defects observed. For example, the  $\Delta argP::kan^R$  Keio mutant possessed a secondary mutation in the *wbbJ* gene, which encodes a putative O-acetyl transferase, WbbJ, and is involved in LPS biosynthesis (Gaudet et al. 2011; Maes et al. 2020). The  $\Delta mprA::kan^R$  Keio mutant possessed a secondary mutation in the *rseB* which is also known to be involved with LPS synthesis regulation (Lima et al. 2013). The  $\Delta wzc::kan^R$  Keio mutant possessed a secondary mutation in *gmhB*, which when knocked out has been previously reported to increase membrane permeability and alter the LPS core (Kneidinger et al. 2002). The Lambda Red protocol involves electroporation of DNA into the target cells, this inherently selects for LPS defects and increased membrane permeability, as these cells are more likely to take up the target DNA. As such it is not surprising that many mutations in known LPS affecting genes were enriched during the creation of this library. Therefore it is possible that the other secondary mutations found in this Chapter may also have direct links to LPS.

Most promising were the Keio mutants  $\Delta lhr::kan^R$ ,  $\Delta ycbU::kan^R$ , and  $\Delta ybjJ::kan^R$ , these mutants are relatively lesser characterised and have minimal secondary mutations. The  $\Delta lhr::kan^R$  Keio mutant only had one secondary mutation in *pepP*. The *pepP* gene is part of an operon, and the mutation to the *pepP* gene resulted in the removal of the start codon. As a result, the next start codon in frame is 57 nt downstream leading to 19 aa truncation if translation were to occur at this secondary site. 3-D x-ray crystallography reveals that this truncation would completely remove the first helix of the protein (6-19 aa) and as such it is likely that this protein will be non-functional (Wilce et al. 1998). The Lhr/RhlF protein is a probable ATP-dependent helicase, which may have broad impacts on the bacterium. As such further mutagenic analysis is needed to determine which of these mutation is causing the LPS defect observed (Table 6.1). Similarly, the  $\Delta ybjJ::kan^R$  Keio mutant only possessed one other secondary mutation located in the *aceE* gene. The AceE protein is a component of the pyruvate dehydrogenase (PDH) complex that catalyses the overall conversion of pyruvate to acetyl-CoA and CO<sub>2</sub>. The YbjJ protein is an uncharacterised inner membrane protein, as such it is unknown which of these mutations is the cause of the LPS defect observed. In contrast, the  $\Delta ycbU::kan^R$  Keio mutant possesses two other secondary mutations in

*selB* and *aceE*. SelB is a selenocysteine-specific elongation factor, which is required for the incorporation of selenocysteine into proteins (Forchhammer et al. 1991). A non-functional SelB protein would have broad effects, one of which may be involved with LPS. Therefore, it is not known if this mutation or the mutation to *aceE* is the cause of the LPS defect observed. The YcbU protein is an uncharacterised surface fimbrial-like protein, linked to promoting bacteria adhesion to abiotic surfaces (Korea et al. 2010). Further mutagenic analysis on *ycbU*, *aceE* and *selB* are required to investigate the cause of the LPS defect observed. Considering the  $\Delta ycbU::kan^R$  and the  $\Delta ybjJU::kan^R$  Keio mutants both have unique mutations in the *aceE* gene, it is highly likely that this is the cause of the LPS defects observed, however, these mutants produced differing LPS profiles (core and Oag defect and reduced Oag, respectively (Table 6.1)). Additionally, it is unknown whether a combination of these mutations is required to produce the LPS profiles observed, and should be considered with further investigation. Finally, to ensure the mutations identified via the whole genome sequencing are correct, direct Sanger sequencing should be performed via Sanger sequencing.

The whole genome sequencing revealed many secondary mutations, some of which affected known LPS linked genes, while others had no previously reported links with LPS. As such, this Chapter provides the foundation for future mutagenic experiments in search of novel genes that affect LPS. Future mutagenic analysis should be performed in *E. coli* BW25113 to ensure the same genetic background of the original Keio library and should consider multiple mutations if the LPS profiles are not reproduced as there is a possibility that these profiles only exist when mutations are in combination with each other.

# Chapter Seven

---

## CONCLUSIONS

---

## Chapter 7: Conclusions

---

### 7.1 WzzB interaction with WzyB via TM2

---

Complex polysaccharide chains such as the Oag element of the LPS, are synthesised by variants of the Wzy-dependent pathway (Rai & Mitchell 2020; Whitfield, Wear, et al. 2020). As such, in depth understanding of the Wzy-dependent pathway will aid in the generation of novel antimicrobials and the possibility to manipulate this system for the biotechnological, industrial, and commercial applications. In Chapter 3, our findings show that the TM2 region of WzzB and WzzE are important for function in regulating their respective polysaccharide chains, and that WzzE is able to partially act on the Oag pathway by controlling modal length. This was unexpected as it has always been thought the ECA and Oag Wzy-dependent pathways systems run alongside with one another with no cross interaction, however our data suggests otherwise. Furthermore, WzzE with only the TM2 region of WzzB was able to increase Oag modal length control significantly compared to the WT WzzE. Using this data we can infer that the TM2 region is likely responsible for the majority of the modal length control of Oag. Chapter 3 also shows a direct interaction between WzzB without the use of a crosslinker, and a novel interaction of WzzE with WzyB was detected in the absence of other PCP proteins, and this interaction was increased when the TM2 of WzzB was substituted into the protein. The interaction of WzzE and WzyB was unexpected, but possible, and was enhanced significantly only with the addition of the TM2 region of WzzB. Additionally, this data confirms a true interaction between WzyB and WzzB without the aid of any crosslinker.

We believe that this interaction occurs at the TM2 region and is required for the function of modal length control and thus this finding paves the way for future studies that focus on the TM2 region in order to fully elucidate which amino acids are involved in binding to Wzy proteins. It is also likely that considering that WzzE and WzzB are structurally similar that the interaction between WzzE and its native binding pattern, WzyE, is highly likely and thus should be investigated in the future.

### 7.2 Identification of a region in WzyB that disrupts the novel interaction with Wzz<sub>pHS2</sub>

---

As it was determined that WzzB and WzyB interact without the use of a crosslinker, the next step was to determine if WzyB and Wzz<sub>pHS2</sub> have a physical interaction to further support the hypothesis that Wzy and Wzz proteins need to interact in order to function. In Chapter 4, the data shows that WzyB and Wzz<sub>pHS2</sub> can interact using biochemical co-purification experiments without the aid of a crosslinker. This was expected as WzyB and WzzB have been shown to interact. Furthermore mutagenesis of WzyB revealed that the region 352-354 is important in WzyB

polymerisation function and is directly or indirectly involved with Wzz<sub>pHS2</sub> interaction. Mutations to this region reduced the interaction of WzyB and Wzz<sub>pHS2</sub>, however the interaction with WzzB was unchanged. This was unexpected as initially we hypothesised that Wzz proteins compete for WzyB interaction at the same location. There is still a possibility that Wzz proteins compete for Wzy but it appears to be at unique sites. It is possible that once WzyB interacts with WzzB the steric hindrance of the relatively large WzzB homooligomeric structure inhibits the ability of Wzz<sub>pHS2</sub> to interact despite the interaction sites being unique. The mutated region of WzyB appears to affect the interaction with only Wzz<sub>pHS2</sub>, however, given that the majority of the more substantial mutations that introduced a charged aa caused either complete loss or near complete loss of WzyB activity, this region is also critical for WzyB function and thus appears to be associated with the active site. Therefore it appears that this active site is not involved with interaction with WzzB and only involved with interaction with Wzz<sub>pHS2</sub>. We hypothesise that this region may be a “switch” region involved in the enzymatic activity of WzyB that is activated when interacting with Wzz<sub>pHS2</sub>. Once activated, WzyB is able to maintain the Oag chain for a longer period, potentially via increasing its affinity to Oag, leading to larger Oag chains (VL Oag-chains). Assuming WzzB and Wzz<sub>pHS2</sub> form homo-oligomeric bells of the same number of protomers, this model explains why despite being of similar sizes, WzzB and Wzz<sub>pHS2</sub> produce drastically different size Oag chains. Significant mutations to this “switch” region may result in a break of this switch leading to no polymerisation, potentially completely removing the ability to interact with Oag.

These findings aid in the understanding of WzyB polymerase activity and its interaction with partner Wzz proteins, and for the first time identifies a region that interacts with a Wzz protein. Additionally, the region identified drastically impacts the proteins ability to function and as such also paves the way for future mutagenic investigation of WzyB.

### **7.3 Identification of WzyB binding partners**

---

Unpublished preliminary data generated in 2015 revealed potentially novel and unreported binding partners of WzyB (Nath 2015; Nath & Morona 2015). Therefore, in Chapter 6 attempts were made to uncover novel binding partners of WzyB, as well as confirm the interactions of WzyB to WzzE and WzzB from Chapter 3. Using a potent expression construct pVLRM11 and the purification conditions optimised from Chapters 3 and 4, large scale purification of WzyB in the presence and absence of DSP was performed with the intention of downstream mass spectrometry analysis. Following purification, SDS-PAGE was used to separate the sample and multiple bands were excised and treated with trypsin prior to mass spectrometry analysis. Using the highly sensitive orbitrap LS-MS a large number of hits were obtained from each of the bands excised. As expected the co-polymerase proteins were detected in high abundance as well as a

number of novel protein partners including FtsH, YdgH, Lon and BamA. There were a number of other hits detected via mass spectrometry, some of which were inconsistent i.e. were only detected in one excised band, while others had an overall low abundance or PSMs value. These hits will need to be reproduced in repeat experiments to provide insight into whether they are significant WzyB binding partners. Additionally despite careful protocols to ensure pure samples, there were still a number of unavoidable hits recorded in the negative controls in which a mock purification was performed (Appendix D). These may be from equipment/buffer contaminations or nonspecific purification of these proteins from the FLAG resin. As this experiment was performed once, no statistical inference can be made with the data obtained, however, the hits presented (Chapter 5) are of proteins that were detected with a minimum of 5 fold increase in abundance compared to the negative control, and as such are highly likely to be interacting with WzyB. Additionally there was no other members of the Wzy-dependent pathway, such as, WaaL or Wzx detected. These proteins were predicted to bind with WzyB and Wzz to form a Wzy-dependent complex (Section 1.6.5) (Figure 1.8), however, this does not appear to be the case.

These results pave the way for future studies of WzyB and its binding partners. Proteins such as the uncharacterised YdgH, FtsH and Lon proteases are all good candidates for future mutagenesis analysis and the resulting impact on either LPS production or Oag modal length control. Additional purification experiments and mass spectrometry analysis using the same conditions should also be performed or direct co-purification experiments using purified FLAG-tagged WzyB and His-tagged putative binding proteins in order to validate the hits obtained in Chapter 5.

#### **7.4 Identification of novel LPS genes**

---

As mentioned numerous times LPS is a complex, but critical component of many Gram negative bacteria, however aspects of its synthesis, transport and length regulation are unknown. The goal of Chapter 6 was to build upon previous data (Leo 2016) obtained using a broad screen for colicin E2 sensitive mutants of the Keio collection (Baba et al. 2006). As previously reported LPS mutants are more susceptible to Colicin E2 (Tran et al. 2014), as such Colicin E2 was used to identify novel genes that impact the various aspects of LPS. Following the initial screen, LPS from Colicin E2 sensitive mutants were analysed via silver-stained SDS-PAGE. Following LPS analysis 13 mutants were deemed to possess notable LPS defects (Table 6.1), as such the aim of Chapter 6 was to confirm that these genes played a role in impacting LPS. More promising Keio mutants,  $\Delta mprA::kan^R$ ,  $\Delta wzc::kan^R$  and  $\Delta yegQ::kan^R$ , were recreated in *E. coli* W3110 via Lambda Red mutagenesis, to reproduce the original phenotype observed. Surprisingly each of these mutants produced WT LPS profiles and as such,  $\Delta mprA::kan^R$  and  $\Delta wzc::kan^R$ , were recreated once more

in *E. coli* BW25113. Once again these mutations produced WT LPS profiles, suggesting that there may be secondary mutations in the Keio collections that are impacting the LPS defects initially observed. The identifications of these secondary mutations may be of value as they too may be found within novel genes with no previous connection/link to LPS. As such, whole genome sequencing was performed on each of the 13 identified Keio mutations to investigate their genotype.

The whole genome sequencing revealed many secondary mutations, some of which affected known LPS linked genes and explains a number of the LPS defects initially observed. However, others have no previously reported links with LPS and will be the basis of future studies. Most promising were the Keio mutants *Alhr::kan<sup>R</sup>*, *ΔycbU::kan<sup>R</sup>*, and *ΔybjJ::kan<sup>R</sup>*, these mutants are relatively less well characterised and had minimal secondary mutations. Additionally the *ΔyegQ::kan<sup>R</sup>* mutant had no other mutations, which was unexpected as *yegQ* was recreated in *E. coli* W3110 with no LPS defect. As there is a relatively high degree of difference in genomes between *E. coli* W3110 and *E. coli* BW25113, there is a possibility that the background strain is the cause of the difference in LPS phenotype.

Chapter 6 provides the foundation for future mutagenic experiments in search of novel genes that affect LPS. Future mutagenic analysis should be performed in *E. coli* BW25113 to ensure the same genetic background of the original Keio library and should consider multiple mutations if the LPS profiles are not reproduced, as there is a possibility that these profiles only exist when mutations are in combination with each other.

## 7.5 Summary

---

The theme of this thesis was to explore LPS, in particular to investigate the most common system used to generate the Oag component, the Wzy-dependent pathway. Particular focus was placed on WzyB and its binding partners, throughout this thesis and each of the co-polymerases, WzzB, WzzE and Wzz<sub>pHS2</sub>, were all shown to physically interact with WzyB. The data suggests that the TM 2 region of the co-polymerases is likely the binding site for WzyB. Additionally the data suggests that the region of aa 352-354 of WzyB appears to be the binding site of Wzz<sub>pHS2</sub> but also likely an active site involved with Oag modal length control. The physical interaction between these members of the Wzy-dependent pathway appear to be the only members of the pathway that interact. This interaction is likely required for function and as such, furthering the understanding of this interaction is of critical importance and paves the way for the development of novel antimicrobials. This thesis greatly advances knowledge in the area of Wzy-dependent Oag polysaccharide synthesis which is the most widely used mechanism in bacteria.





# Appendices

---

STRAINS LIST

CONSTRUCT LIST

OLIGONUCLEOTIDE LIST

MASS SPEC DATA

GENOME SEQUENCING DATA

BIBLIOGRAPHY

## Appendix A - Bacterial strains generated

Strain ID	Parent (plasmid (s))	Description/Phenotype
VLRM74	W3110 (pDK46)	Amp <sup>R</sup> 30 °C Temp sensitive
VLRM75	W3110 <i>mprA::kan<sup>R</sup></i>	Kan <sup>R</sup> , Lambda Red cassette not flipped out
VLRM76	W3110 <i>wzc::kan<sup>R</sup></i>	Kan <sup>R</sup> , Lambda Red cassette not flipped out
VLRM77	W3110 <i>yegQ::kan<sup>R</sup></i>	Kan <sup>R</sup> , Lambda Red cassette not flipped out
VLRM78	W3110 $\Delta$ <i>mprA</i>	Lambda Red mutant FRT scar
VLRM79	W3110 $\Delta$ <i>wzc</i>	Lambda Red mutant FRT scar
VLRM80	W3110 $\Delta$ <i>yegQ</i>	Lambda Red mutant FRT scar
VLRM81	W3110 (pSUP205:: <i>wbbL</i> )	Tet <sup>R</sup> , WT control
VLRM82	W3110 (pSUP205)	Tet <sup>R</sup> , Vector control
VLRM83	W3110 <i>mprA::kan<sup>R</sup></i> (pSUP205:: <i>wbbL</i> )	Tet <sup>R</sup> , Kan <sup>R</sup> mutant complemented with <i>wbbL</i>
VLRM84	W3110 <i>wzc::kan<sup>R</sup></i> (pSUP205:: <i>wbbL</i> )	Tet <sup>R</sup> , Kan <sup>R</sup> mutant complemented with <i>wbbL</i>
VLRM85	W3110 <i>yegQ::kan<sup>R</sup></i> (pSUP205:: <i>wbbL</i> )	Tet <sup>R</sup> , Kan <sup>R</sup> mutant complemented with <i>wbbL</i>
VLRM86	W3110 <i>mprA::kan<sup>R</sup></i> (pSUP205)	Tet <sup>R</sup> , Kan <sup>R</sup> vector control firt scar
VLRM87	W3110 <i>wzc::kan<sup>R</sup></i> (pSUP205)	Tet <sup>R</sup> , Kan <sup>R</sup> vector control firt scar
VLRM88	W3110 <i>yegQ::kan<sup>R</sup></i> (pSUP205)	Tet <sup>R</sup> , Kan <sup>R</sup> vector control firt scar
VLRM89	W3110 $\Delta$ <i>mprA</i> (pSUP205:: <i>wbbL</i> )	Tet <sup>R</sup> , mutant complemented with <i>wbbL</i> firt scar
VLRM90	W3110 $\Delta$ <i>wzc</i> (pSUP205:: <i>wbbL</i> )	Tet <sup>R</sup> , mutant complemented with <i>wbbL</i> firt scar
VLRM91	W3110 $\Delta$ <i>yegQ</i> (pSUP205:: <i>wbbL</i> )	Tet <sup>R</sup> , mutant complemented with <i>wbbL</i> firt scar
VLRM92	W3110 $\Delta$ <i>mprA</i> (pSUP205)	Tet <sup>R</sup> , vector control
VLRM93	W3110 $\Delta$ <i>wzc</i> (pSUP205)	Tet <sup>R</sup> , vector control
VLRM94	W3110 $\Delta$ <i>yegQ</i> (pSUP205)	Tet <sup>R</sup> , vector control
VLRM95	BW25113 <i>wzc::kan<sup>R</sup></i>	Kan <sup>R</sup> , Lambda Red cassette not flipped out
VLRM96	BW25113 <i>mprA::kan<sup>R</sup></i>	Kan <sup>R</sup> , Lambda Red cassette not flipped out
VLRM97	RMA2608 (pAC/BADT7) (pWALDO)	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> PE638 <i>wzyB</i>
VLRM98	RMA2608 (pAC/BADT7) (pWALDO:: <i>wzyB</i> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> PE638 <i>wzyB</i>
VLRM99	RMA2608 (pAC/BADT7) (pWALDO:: <i>wzyB<sup>G130V</sup></i> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> PE638 <i>wzyB</i>
VLRM100	RMA2608 (pAC/BADT7) (pWALDO:: <i>wzyB<sup>L214I</sup></i> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> PE638 <i>wzyB</i>
VLRM101	RMA2608 (pAC/BADT7) (pWALDO:: <i>wzyB<sup>P352H</sup></i> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> PE638 <i>wzyB</i>

VLRM102	RMA2608 (pAC/BADT7) (pWALDO:: <i>wzyB</i> <sup>V92M</sup> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> PE638 <i>wzyB</i>
VLRM103	RMA2608 (pAC/BADT7) (pWALDO:: <i>wzyB</i> <sup>Y137H</sup> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> PE638 <i>wzyB</i>
VLRM104	RMA4337 (pAC/BADT7) (pWALDO)	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> , PE638 $\Delta$ <i>wzyB</i> <i>wzzB</i> :: <i>Tet</i> <sup>R</sup>
VLRM105	RMA4337 (pAC/BADT7) (pWALDO:: <i>wzyB</i> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta$ <i>wzyB</i> <i>wzzB</i> :: <i>Tet</i> <sup>R</sup>
VLRM106	RMA4337 (pAC/BADT7) (pWALDO:: <i>wzyB</i> <sup>G130V</sup> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta$ <i>wzyB</i> <i>wzzB</i> :: <i>Tet</i> <sup>R</sup>
VLRM107	RMA4337 (pAC/BADT7) (pWALDO:: <i>wzyB</i> <sup>L214I</sup> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta$ <i>wzyB</i> <i>wzzB</i> :: <i>Tet</i> <sup>R</sup>
VLRM108	RMA4337 (pAC/BADT7) (pWALDO:: <i>wzyB</i> <sup>P352H</sup> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta$ <i>wzyB</i> <i>wzzB</i> :: <i>Tet</i> <sup>R</sup>
VLRM109	RMA4337 (pAC/BADT7) (pWALDO:: <i>wzyB</i> <sup>V92M</sup> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta$ <i>wzyB</i> <i>wzzB</i> :: <i>Tet</i> <sup>R</sup>
VLRM110	RMA2608 (pAC/BADT7) (pWALDO:: <i>wzyB</i> <sup>Y137H</sup> )	Kan <sup>R</sup> , Cml <sup>R</sup> , Rif <sup>R</sup> PE638 $\Delta$ <i>wzyB</i>
VLRM111	DH5 $\alpha$ (pVL1 (pEXT21:: <i>wzzB-His</i> ))	Strep <sup>R</sup> WzzB expression construct
VLRM112	RMA2163 (pEXT21)	Strep <sup>R</sup> , PE860 <i>wzzB</i>
VLRM113	RMA2163 (pEXT21:: <i>wzzB-His</i> )	Strep <sup>R</sup> , PE860 <i>wzzB</i>
VLRM114	DH5 $\alpha$ (pVL2 (pBAD24:: <i>wzyB-GFP- StrepII</i> ))	Amp <sup>R</sup> , Rif <sup>R</sup> , WzyB-GFP expression construct
VLRM115	RMA2608 (pBAD24)	Amp <sup>R</sup> , Rif <sup>R</sup> , PE638 <i>wzyB</i>
VLRM116	RMA2608 (pVL2 (pBAD24:: <i>wzyB-GFP- StrepII</i> ))	Amp <sup>R</sup> , Rif <sup>R</sup> , PE638 <i>wzyB</i>
VLRM117	RMA2608 (pAC/BADT7) (pWaldo- <i>wzyB</i> <sup>G130V</sup> -TEV-GFP)	Cml <sup>R</sup> , Kan <sup>R</sup> , Rif <sup>R</sup> , PE638 <i>wzyB</i>
VLRM118	RMA2608 (pAC/BADT7) (pWaldo- <i>wzyB</i> <sup>V92M</sup> -TEV-GFP)	Cml <sup>R</sup> , Kan <sup>R</sup> , Rif <sup>R</sup> , PE638 <i>wzyB</i>
VLRM119	RMA4337 (pAC/BADT7) (pWaldo- <i>wzyB</i> <sup>G130V</sup> -TEV-GFP)	Cml <sup>R</sup> , Kan <sup>R</sup> , Tet <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta$ <i>wzyB</i> <i>wzzB</i> :: <i>Tet</i> <sup>R</sup>
VLRM120	RMA4337 (pAC/BADT7) (pWaldo- <i>wzyB</i> <sup>V92M</sup> -TEV-GFP)	Cml <sup>R</sup> , Kan <sup>R</sup> , Tet <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta$ <i>wzyB</i> <i>wzzB</i> :: <i>Tet</i> <sup>R</sup>
VLRM121	RMA4662 (pQE30:: <i>wzzE</i> )	Kan <sup>R</sup> , Amp <sup>R</sup> , PE860 $\Delta$ <i>wzzB</i> $\Delta$ <i>wzzE</i> $\Delta$ <i>wzzpHS2</i>
VLRM122	RMA46620 (pQE30:: <i>wzzB</i> )	Kan <sup>R</sup> , Amp <sup>R</sup> , PE860 $\Delta$ <i>wzzB</i> $\Delta$ <i>wzzE</i> $\Delta$ <i>wzzpHS2</i>
VLRM123	RMA4662 (pQE30)	Kan <sup>R</sup> , Amp <sup>R</sup> , PE860 $\Delta$ <i>wzzB</i> $\Delta$ <i>wzzE</i> $\Delta$ <i>wzzpHS2</i>
VLRM124	RMA4662 (pEXT21)	Kan <sup>R</sup> , Strep <sup>R</sup> , PE860 $\Delta$ <i>wzzB</i> $\Delta$ <i>wzzE</i> $\Delta$ <i>wzzpHS2</i>
VLRM125	XL10 Gold pQE30:: <i>wzzB</i> random Mutagenesis pull 1	Amp <sup>R</sup> , Random mutagenesis pull 1 ~500 colonies
VLRM126	XL10 Gold pQE30:: <i>wzzB</i> random Mutagenesis pull 2	Amp <sup>R</sup> , Random mutagenesis pull 2 ~500 colonies

VLRM127	XL10 Gold pEXT21:: <i>wzzB</i> random Mutagenesis pull 1	Strep <sup>R</sup> , Random mutagenesis pull 2 ~500 colonies
VLRM128	RMA4662 (pKD46)	Kan <sup>R</sup> , Amp <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzzpHS2$ 30 °C temp. sensitive
VLRM129	Not Used	Not Used
VLRM130	Not Used	Not Used
VLRM131	Not Used	Not Used
VLRM132	Not Used	Not Used
VLRM133	Not Used	Not Used
VLRM134	Not Used	Not Used
VLRM135	Not Used	Not Used
VLRM136	Not Used	Not Used
VLRM137	Not Used	Not Used
VLRM138	Not Used	Not Used
VLRM139	Not Used	Not Used
VLRM140	DH5 $\alpha$ (pVL5 (pBAD33:: <i>wzyB</i> -GFP-StrepII)) clone 1	Kan <sup>R</sup> , Amp <sup>R</sup> , Strep <sup>R</sup> , pBAD- <i>wzyB</i> clone 1
VLRM141	DH5 $\alpha$ (pVL5 (pBAD33:: <i>wzyB</i> -GFP-StrepII)) clone 2	Kan <sup>R</sup> , Amp <sup>R</sup> , Strep <sup>R</sup> , pBAD- <i>wzyB</i> clone 2
VLRM142	RMA2608 (pVL5 (pBAD33:: <i>wzyB</i> -GFP-StrepII)) clone 1	Kan <sup>R</sup> , Amp <sup>R</sup> , Strep <sup>R</sup> , PE638 <i>wzyB</i>
VLRM143	RMA2608 (pVL5 (pBAD33:: <i>wzyB</i> -GFP-StrepII)) clone 2	Kan <sup>R</sup> , Amp <sup>R</sup> , Strep <sup>R</sup> , PE638 <i>wzyB</i>
VLRM144	RMA4662 (pQE30:: <i>wzzE</i> ) (pCDFduet)	Kan <sup>R</sup> , Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzzpHS2$
VLRM145	RMA2608 (pBAD33)	Kan <sup>R</sup> , Amp <sup>R</sup> , Strep <sup>R</sup> , PE638 <i>wzyB</i>
VLRM146	DH5 $\alpha$ (pVL6 (pBAD33:: <i>wzyE</i> -GFP-StrepII))	Cml <sup>R</sup> , <i>wzyE</i> expression construct
VLRM147	DH5 $\alpha$ (pVL7 (pBAD33:: <i>wzyB</i> <sup>L214I</sup> -GFP-StrepII))	Cml <sup>R</sup> , pBAD- <i>wzyB</i> <sup>L214I</sup>
VLRM148	W3110 $\Delta wzyE$ NMRM6 (pVL6 (pBAD33:: <i>wzyE</i> -GFP-StrepII))	Cml <sup>R</sup> , <i>wzyE</i> expression construct in W3110 <i>wzyE</i> mutant
VLRM149	RMA 4622 (pQ30:: <i>wzzE</i> ) (min)	Kan <sup>R</sup> , Amp <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzzpHS2$
VLRM150	DH5 $\alpha$ (pVL8 ((pBAD33:: <i>wzyB</i> <sup>V92M</sup> -GFP-StrepII))	Cml <sup>R</sup> ,
VLRM151	DH5 $\alpha$ (pVL9 ((pBAD33:: <i>wzyB</i> <sup>Y137H</sup> -GFP-StrepII))	Cml <sup>R</sup> , replicate 1
VLRM152	DH5 $\alpha$ (pVL9 ((pBAD33:: <i>wzyB</i> <sup>Y137H</sup> -GFP-StrepII))	Cml <sup>R</sup> , replicate 2
VLRM153	DH5 $\alpha$ (pVL10 ((pBAD33:: <i>wzyB</i> <sup>P352H</sup> -GFP-StrepII))	Cml <sup>R</sup> , replicate 1
VLRM154	DH5 $\alpha$ (pVL10 ((pBAD33:: <i>wzyB</i> <sup>P352H</sup> -GFP-StrepII))	Cml <sup>R</sup> , replicate 2
VLRM155	DH5 $\alpha$ (pQE30:: <i>wzzE</i> <sup>*</sup> )	Amp <sup>R</sup> , Random mutagenesis mutant 1

VLRM156	DH5α (pQE30::wzzE*2)	Amp <sup>R</sup> , Random mutagenesis mutant 2
VLRM157	DH5α (pQE30::wzzE*3)	Amp <sup>R</sup> , Random mutagenesis mutant 3
VLRM158	DH5α (pQE30::wzzE*4)	Amp <sup>R</sup> , Random mutagenesis mutant 4
VLRM159	DH5α (pQE30::wzzE*5)	Amp <sup>R</sup> , Random mutagenesis mutant 5
VLRM160	DH5α (pQE30::wzzE*6)	Amp <sup>R</sup> , Random mutagenesis mutant 6
VLRM161	DH5α (pQE30::wzzE*7)	Amp <sup>R</sup> , Random mutagenesis mutant 7
VLRM162	RMA4662 (pQE30::wzzE*1)	Kan <sup>R</sup> , Amp <sup>R</sup> , mut. 1, PE860 ΔwzzB ΔwzzE ΔwzzpHS2
VLRM163	RMA4662 (pQE30::wzzE*2)	Kan <sup>R</sup> , Amp <sup>R</sup> , mut. 2, PE860 ΔwzzB ΔwzzE ΔwzzpHS2
VLRM164	RMA4662 (pQE30::wzzE*3)	Kan <sup>R</sup> , Amp <sup>R</sup> , mut. 3, PE860 ΔwzzB ΔwzzE ΔwzzpHS2
VLRM165	RMA4662 (pQE30::wzzE*4)	Kan <sup>R</sup> , Amp <sup>R</sup> , mut. 4, PE860 ΔwzzB ΔwzzE ΔwzzpHS2
VLRM166	RMA4662 (pQE30::wzzE*5)	Kan <sup>R</sup> , Amp <sup>R</sup> , mut. 5, PE860 ΔwzzB ΔwzzE ΔwzzpHS2
VLRM167	RMA4662 (pQE30::wzzE*6)	Kan <sup>R</sup> , Amp <sup>R</sup> , mut. 6, PE860 ΔwzzB ΔwzzE ΔwzzpHS2
VLRM168	RMA4662 (pQE30::wzzE*7)	Kan <sup>R</sup> , Amp <sup>R</sup> , mut. 7, PE860 ΔwzzB ΔwzzE ΔwzzpHS2
VLRM169	DH5α (pVL11 (pBAD33::wzyB-FLAGx3))	Cml <sup>R</sup> , replicate 1
VLRM170	DH5α (pVL11 (pBAD33::wzyB-FLAGx3))	Cml <sup>R</sup> , replicate 2
VLRM171	DH5α (pVL11 (pBAD33::wzyB-FLAGx3))	Cml <sup>R</sup> , replicate 3
VLRM172	RMA2608 (pVL11 (pBAD33::wzyB-FLAGx3))	Cml <sup>R</sup> , replicate 1, PE638 wzyB (Best)
VLRM173	RMA2608 (pVL11 (pBAD33::wzyB-FLAGx3))	Cml <sup>R</sup> , replicate 2, PE638 wzyB
VLRM174	RMA2608 (pVL11 (pBAD33::wzyB-FLAGx3))	Cml <sup>R</sup> , replicate 3, PE638 wzyB
VLRM175	RMA696 (pQ30::wzzE) (min)	Kan <sup>R</sup> , Amp <sup>R</sup> , 2457T VP <sup>+</sup> , ΔwzzB ΔwzzE
VLRM176	RMA696 (pQ30::wzzB)	Kan <sup>R</sup> , Amp <sup>R</sup> , 2457T VP <sup>+</sup> , ΔwzzB ΔwzzE
VLRM177	RMA696 (pQ30)	Kan <sup>R</sup> , Amp <sup>R</sup> , 2457T VP <sup>+</sup> , ΔwzzB ΔwzzE
VLRM178	Not Used	Not Used
VLRM179	RMA696 (pEXT21)	Kan <sup>R</sup> , Amp <sup>R</sup> , 2457T VP <sup>+</sup> , ΔwzzB
VLRM180	RMA696 (pVL1 (pEXT21::wzzB-His))	Kan <sup>R</sup> , Amp <sup>R</sup> , 2457T VP <sup>+</sup> , ΔwzzB
VLRM181	RMA696 (pCDFduet-1)	Kan <sup>R</sup> , Strep <sup>R</sup> , 2457T VP <sup>+</sup> , ΔwzzB
VLRM182	RMA696 (pCDFduet-1) (pEXT21)	Kan <sup>R</sup> , Strep <sup>R</sup> , Amp <sup>R</sup> , 2457T VP <sup>+</sup> , ΔwzzB
VLRM183	RMA696 (pCDFduet-1) (pQ30::wzzE)	Kan <sup>R</sup> , Strep <sup>R</sup> , Amp <sup>R</sup> , 2457T VP <sup>+</sup> , ΔwzzB
VLRM184	RMA696 (pCDFduet-1) (pQ30::wzzB)	Kan <sup>R</sup> , Strep <sup>R</sup> , Amp <sup>R</sup> , 2457T VP <sup>+</sup> , ΔwzzB
VLRM185	DH5α (pVL12 (pBAD33::wzyB <sup>V92M</sup> -FLAGx3))	Cml <sup>R</sup>
VLRM186	Not Used	Not Used
VLRM187	DH5α (pVL14 (pBAD33::wzyB <sup>p352H</sup> -FLAGx3))	Cml <sup>R</sup>

VLRM188	DH5α (pVL15 (pBAD33::wzyB <sup>p137H</sup> -FLAGx3))	Cml <sup>R</sup>
VLRM189	Not Used	Not Used
VLRM190	DH5α (pVL16 (pQ30::wzzE <sup>BTM2</sup> -His))	Cml <sup>R</sup> , TM swap
VLRM191	Not Used	Not Used
VLRM192	RMA4662 (pVL16 (pQ30::wzzE <sup>BTM2</sup> -His)) (pCDFduet-1)	Kan <sup>R</sup> , Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 ΔwzzB ΔwzzE Δwzz <sub>pHS2</sub>
VLRM193	Not Used	Not Used
VLRM194	Not Used	Not Used
VLRM195	Not Used	Not Used
VLRM196	Not Used	Not Used
VLRM197	RMA2608 (pVL12 (pBAD33::wzyB <sup>V92M</sup> -FLAGx3))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 wzyB
VLRM198	RMA4337 (pVL12 (pBAD33::wzyB <sup>V92M</sup> -FLAGx3))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM199	RMA2608 (pVL14 (pBAD33::wzyB <sup>p352H</sup> -FLAGx3))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 wzyB
VLRM200	RMA4337 (pVL14 (pBAD33::wzyB <sup>p352H</sup> -FLAGx3))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM201	RMA2608 (pVL15 (pBAD33::wzyB <sup>p137H</sup> -FLAGx3))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 wzyB
VLRM202	RMA4337 (pVL15 (pBAD33::wzyB <sup>p137H</sup> -FLAGx3))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM203	RMA4622 wzyE::cml <sup>R</sup>	Cml <sup>R</sup> , Kan <sup>R</sup> PE860 ΔwzzB ΔwzzE Δwzz <sub>pHS2</sub>
VLRM204	DH5α (pVL17 (pQ30::wzzB <sup>ETM2</sup> -His))	Amp <sup>R</sup>
VLRM205	DH5α (pVL18 (pQ30::wzzE <sup>BTM1</sup> -His))	Amp <sup>R</sup>
VLRM206	DH5α (pVL19 (pQ30::wzzB <sup>ETM1</sup> -His))	Amp <sup>R</sup>
VLRM207	RMA4622 (pVL17 (pQ30::wzzB <sup>ETM2</sup> -His)) (pCDFduet-1)	Kan <sup>R</sup> Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 ΔwzzB ΔwzzE Δwzz <sub>pHS2</sub>
VLRM208	RMA4622 (pVL18 (pQ30::wzzE <sup>BTM1</sup> -His)) (pCDFduet-1)	Kan <sup>R</sup> Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 ΔwzzB ΔwzzE Δwzz <sub>pHS2</sub>
VLRM209	RMA4622 (pVL19 (pQ30::wzzB <sup>ETM1</sup> -His)) (pCDFduet-1)	Kan <sup>R</sup> Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 ΔwzzB ΔwzzE Δwzz <sub>pHS2</sub>
VLRM210	DH5α (pVL20 (pQ30::wzzE <sup>BTM1&amp;2</sup> -His))	Amp <sup>R</sup>
VLRM211	DH5α (pVL21 (pQ30::wzzB <sup>ETM1&amp;2</sup> -His))	Amp <sup>R</sup>
VLRM212	RMA4622 (pVL20 (pQ30::wzzE <sup>BTM1&amp;2</sup> -His)) (pCDFduet-1)	Kan <sup>R</sup> Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 ΔwzzB ΔwzzE Δwzz <sub>pHS2</sub>
VLRM213	RMA4622 (pVL21 (pQ30::wzzB <sup>ETM1&amp;2</sup> -His)) (pCDFduet-1)	Kan <sup>R</sup> Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 ΔwzzB ΔwzzE Δwzz <sub>pHS2</sub>
VLRM214	RMA4622 (pVL18 (pQ30::wzzE <sup>BTM1</sup> -His))	Kan <sup>R</sup> Amp <sup>R</sup> , PE860 ΔwzzB ΔwzzE Δwzz <sub>pHS2</sub>
VLRM215	RMA4662 (pVL16 (pQ30::wzzE <sup>BTM2</sup> -His))	Kan <sup>R</sup> , Amp <sup>R</sup> , PE860 ΔwzzB ΔwzzE Δwzz <sub>pHS2</sub>
VLRM216	RMA4622 (pVL20 (pQ30::wzzE <sup>BTM1&amp;2</sup> -His))	Kan <sup>R</sup> Amp <sup>R</sup> , PE860 ΔwzzB ΔwzzE Δwzz <sub>pHS2</sub>

VLRM217	RMA4622 (pVL19 (pQ30::wzzB <sup>ETM1</sup> -His))	Kan <sup>R</sup> Amp <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM218	RMA4622 (pVL17 (pQ30::wzzB <sup>ETM2</sup> -His))	Kan <sup>R</sup> Amp <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM219	RMA4622 (pVL21 (pQ30::wzzB <sup>ETM1&amp;2</sup> -His))	Kan <sup>R</sup> Amp <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM220	DH5 $\alpha$ (pVL22 (pCactus wzyE::tet <sup>R</sup> ))	30 °C, Cml <sup>R</sup> Tet <sup>R</sup>
VLRM221	DH5 $\alpha$ (pVL22 (pCactus wzyE::tet <sup>R</sup> ))	30 °C, Cml <sup>R</sup> Tet <sup>R</sup>
VLRM222	RMA4721 (pBAD33) (pQE30::wzzB)	Kan <sup>R</sup> Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM223	RMA4721 ((pVL11 (pBAD33::wzyB-FLAGx3) (pQE30::wzzB)	Kan <sup>R</sup> Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM224	RMA4703 ((pVL11 (pBAD33::wzyB-FLAGx3) (pQE30)	Kan <sup>R</sup> Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM225	RMA4704 ((pVL11 (pBAD33::wzyB-FLAGx3) (pQE30::wzzE)	Kan <sup>R</sup> Amp <sup>R</sup> , Strep <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM226	VLRM214 ((pVL11 (pBAD33::wzyB-FLAGx3)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM227	VLRM215 ((pVL11 (pBAD33::wzyB-FLAGx3)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM228	VLRM216 ((pVL11 (pBAD33::wzyB-FLAGx3)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM229	VLRM217 ((pVL11 (pBAD33::wzyB-FLAGx3)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM230	VLRM218 ((pVL11 (pBAD33::wzyB-FLAGx3)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM231	VLRM219 ((pVL11 (pBAD33::wzyB-FLAGx3)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM232	VLRM214 (pBAD33)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM233	VLRM215 (pBAD33)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM234	VLRM216 (pBAD33)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM235	VLRM217 (pBAD33)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM236	VLRM218 (pBAD33)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM237	VLRM219 (pBAD33)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM238	RMA4703 (pQE30) (pBAD33)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM239	RMA4704 (pBAD33) (pQE30::wzzE)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$
VLRM240	RMA4703 (pQE30) (pVL14 (pBAD33::wzyB <sup>p352H</sup> -FLAGx3))	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzz_{pHS2}$



VLRM241	RMA4721 ((pVL11 (pVL14 (pBAD33::wzyB <sup>P352H</sup> -FLAGx3) (pQE30::wzzB)	Kan <sup>R</sup> Amp <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB \Delta wzzE \Delta wzzpHS2$
VLRM242	RMA4337 (pBCKS <sup>+</sup> )	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta wzyB wzzB::tet^R$
VLRM243	RMA4337 (pBCKS <sup>+</sup> ::wzyB-LacZ <sup>α</sup> -FLAG)	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta wzyB wzzB::tet^R$
VLRM244	RMA2608 (pBCKS <sup>+</sup> ::wzyB-LacZ <sup>α</sup> -FLAG)	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta wzyB$
VLRM245	RMA2608 (pBCKS <sup>+</sup> )	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta wzyB$
VLRM246	DH5α (pVL23 (pBCKS <sup>+</sup> ::wzyB <sup>T351A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM247	DH5α (pVL24 (pBCKS <sup>+</sup> ::wzyB <sup>P352A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM248	DH5α (pVL25 (pBCKS <sup>+</sup> ::wzyB <sup>M353A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM249	DH5α (pVL26 (pBCKS <sup>+</sup> ::wzyB <sup>G354A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM250	DH5α (pVL27 (pBCKS <sup>+</sup> ::wzyB <sup>I355A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM251	DH5α (pVL28 (pBCKS <sup>+</sup> ::wzyB <sup>F356A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM252	DH5α (pVL29 (pBCKS <sup>+</sup> ::wzyB <sup>I357A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM253	RMA2608 (pVL23 (pBCKS <sup>+</sup> ::wzyB <sup>T351A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta wzyB$
VLRM254	RMA4337 (pVL23 (pBCKS <sup>+</sup> ::wzyB <sup>T351A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta wzyB wzzB::tet^R$
VLRM255	RMA2608 (pVL24 (pBCKS <sup>+</sup> ::wzyB <sup>P352A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta wzyB$
VLRM256	RMA4337 (pVL24 (pBCKS <sup>+</sup> ::wzyB <sup>P352A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta wzyB wzzB::tet^R$
VLRM257	RMA2608 (pVL25 (pBCKS <sup>+</sup> ::wzyB <sup>M353A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta wzyB$
VLRM258	RMA4337 (pVL25 (pBCKS <sup>+</sup> ::wzyB <sup>M353A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta wzyB wzzB::tet^R$
VLRM259	RMA2608 (pVL26 (pBCKS <sup>+</sup> ::wzyB <sup>G354A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta wzyB$
VLRM260	RMA4337 (pVL26 (pBCKS <sup>+</sup> ::wzyB <sup>G354A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta wzyB wzzB::tet^R$
VLRM261	RMA2608 (pVL27 (pBCKS <sup>+</sup> ::wzyB <sup>I355A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta wzyB$
VLRM262	RMA4337 (pVL27 (pBCKS <sup>+</sup> ::wzyB <sup>I355A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta wzyB wzzB::tet^R$
VLRM263	RMA2608 (pVL28 (pBCKS <sup>+</sup> ::wzyB <sup>F356A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta wzyB$
VLRM264	RMA4337 (pVL28 (pBCKS <sup>+</sup> ::wzyB <sup>F356A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 $\Delta wzyB wzzB::tet^R$
VLRM265	RMA2608 (pVL29 (pBCKS <sup>+</sup> ::wzyB <sup>I357A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 $\Delta wzyB$

VLRM266	RMA4337 (pVL29 (pBCKS <sup>+</sup> ::wzyB <sup>I357A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM267	DH5α (pVL30 (pBCKS <sup>+</sup> ::wzyB <sup>p352K</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM268	DH5α (pVL31 (pBCKS <sup>+</sup> ::wzyB <sup>M353K</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM269	DH5α (pVL32 (pBCKS <sup>+</sup> ::wzyB <sup>G354K</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM270	DH5α (pVL33 (pBCKS <sup>+</sup> ::wzyB <sup>P352D</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM271	DH5α (pVL34 (pBCKS <sup>+</sup> ::wzyB <sup>M353D</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM272	DH5α (pVL35 (pBCKS <sup>+</sup> ::wzyB <sup>G354D</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM273	DH5α (pVL36 (pBCKS <sup>+</sup> ::wzyB <sup>352-354A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup>
VLRM274	RMA2608 (pVL30 (pBCKS <sup>+</sup> ::wzyB <sup>p352K</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 ΔwzyB
VLRM275	RMA4337 (pVL30 (pBCKS <sup>+</sup> ::wzyB <sup>p352K</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM276	RMA2608 (pVL31 (pBCKS <sup>+</sup> ::wzyB <sup>M353K</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 ΔwzyB
VLRM277	RMA4337 (pVL31 (pBCKS <sup>+</sup> ::wzyB <sup>M353K</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM278	RMA2608 (pVL32 (pBCKS <sup>+</sup> ::wzyB <sup>G354K</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 ΔwzyB
VLRM279	RMA4337 (pVL32 (pBCKS <sup>+</sup> ::wzyB <sup>G354K</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM280	RMA2608 (pVL33 (pBCKS <sup>+</sup> ::wzyB <sup>P352D</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 ΔwzyB
VLRM281	RMA4337 (pVL33 (pBCKS <sup>+</sup> ::wzyB <sup>P352D</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM282	RMA2608 (pVL34 (pBCKS <sup>+</sup> ::wzyB <sup>M353D</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 ΔwzyB
VLRM283	RMA4337 (pVL34 (pBCKS <sup>+</sup> ::wzyB <sup>M353D</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM284	RMA2608 (pVL35 (pBCKS <sup>+</sup> ::wzyB <sup>G354D</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 ΔwzyB
VLRM285	RMA4337 (pVL35 (pBCKS <sup>+</sup> ::wzyB <sup>G354D</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM286	RMA2608 (pVL36 (pBCKS <sup>+</sup> ::wzyB <sup>352-354A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , PE638 ΔwzyB
VLRM287	RMA4337 (pVL36 (pBCKS <sup>+</sup> ::wzyB <sup>352-354A</sup> -LacZ <sup>α</sup> -FLAG))	Cml <sup>R</sup> , Rif <sup>R</sup> , Tet <sup>R</sup> PE638 ΔwzyB wzzB::tet <sup>R</sup>
VLRM288	RMA2162 (pVL11 (pBAD33::wzyB-FLAGx3))	Cml <sup>R</sup> PE860 WT
VLRM289	RMA2162 (pBAD33)	Cml <sup>R</sup> PE860 WT

---

VLRM290	RMA4622 (pVL11 (pBAD33::wzyB-FLAGx3))	Kan <sup>R</sup> , Cml <sup>R</sup> , PE860 $\Delta wzzB$ $\Delta wzzE$ $\Delta wzz_{pHS2}$
---------	---------------------------------------	---

---

---

## Appendix B – Plasmids used

---

Construct ID	Description
pVL1	pEXT21:: <i>wzzB</i> -His6
pVL2	pBAD24 – <i>wzyB</i> -GFP-Strep
pVL3	pEXT21 <sup>i92</sup>
pVL4	pEXT21 <sup>i128</sup>
pVL5	pBAD33- <i>wzyB</i> -GFP-FLAG
pVL6	pBAD33- <i>wzyE</i> -GFP-FLAG
pVL7	pBAD33:: <i>wzyB</i> <sup>L214I</sup> -GFP-StrepII
pVL8	pBAD33:: <i>wzyB</i> <sup>V92M</sup> -GFP-StrepII
pVL9	pBAD33:: <i>wzyB</i> <sup>Y137H</sup> -GFP-StrepII
pVL10	pBAD33:: <i>wzyB</i> <sup>P352H</sup> -GFP-StrepII
pVL11	pBAD33:: <i>wzyB</i> -FLAGx3
pVL12	pBAD33:: <i>wzyB</i> <sup>V92M</sup> -FLAGx3
pVL13	Not Used
pVL14	pBAD33:: <i>wzyB</i> <sup>P352H</sup> -FLAGx3
pVL15	pBAD33:: <i>wzyB</i> <sup>Y137H</sup> -FLAGx3
pVL16	pQ30:: <i>wzzE</i> <sup>BTM2</sup> - His
pVL17	pQ30:: <i>wzzB</i> <sup>ETM2</sup> - His
pVL18	pQ30:: <i>wzzE</i> <sup>BTM1</sup> - His
pVL19	pQ30:: <i>wzzB</i> <sup>ETM1</sup> - His
pVL20	pQ30:: <i>wzzE</i> <sup>BTM1&amp;2</sup> - His
pVL21	pQ30:: <i>wzzB</i> <sup>ETM1&amp;2</sup> - His
pVL22	pCactus <i>wzyE</i> :: <i>tet</i> <sup>R</sup>
pVL23	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>T351A</sup> -LacZ <sup>α</sup> -FLAG
pVL24	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>P352A</sup> -LacZ <sup>α</sup> -FLAG
pVL25	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>M353A</sup> -LacZ <sup>α</sup> -FLAG
pVL26	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>G354A</sup> -LacZ <sup>α</sup> -FLAG
pVL27	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>I355A</sup> -LacZ <sup>α</sup> -FLAG
pVL28	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>F356A</sup> -LacZ <sup>α</sup> -FLAG
pVL29	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>I357A</sup> -LacZ <sup>α</sup> -FLAG
pVL30	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>p352K</sup> -LacZ <sup>α</sup> -FLAG
pVL31	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>M353K</sup> -LacZ <sup>α</sup> -FLAG
pVL32	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>G354K</sup> -LacZ <sup>α</sup> -FLAG
pVL33	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>P352D</sup> -LacZ <sup>α</sup> -FLAG
pVL34	pBCKS <sup>+</sup> :: <i>wzyB</i> <sup>M353D</sup> -LacZ <sup>α</sup> -FLAG

---

---

pVL35	pBCKS <sup>+</sup> ::wzyB <sup>G354D</sup> -LacZ <sup>α</sup> -FLAG
pVL36	pBCKS <sup>+</sup> ::wzyB <sup>352-354A</sup> -LacZ <sup>α</sup> -FLAG

---

## Appendix C – Oligonucleotides generated

Oligonucleotide ID	Sequence	Description
VL35 ( <i>yegQ</i> F pKD13) (70nt)	CTTCATCAGGTGAAGCGGATCTGACCTGTCATCAG AACGAGAGAATTATGATTCCGGGGATCCGTCGACC	<i>yegQ</i> , Forward Lambda Red
VL36 <i>yegQ</i> R pKD13) (70nt)	TTCCATCCGGGAAAAATAATCGAAATTAATCACTT ACCGTGGGGATTACGTGTAGGCTGGAGCTGCTTCG	<i>yegQ</i> , Reverse Lambda Red
VL37 <i>mprA</i> F pKD13) (70nt)	GTCACCTGTCGTTACTATATCGGCTGAAATTAATGA GGTCATACCCAAATGATTCCGGGGATCCGTCGACC	<i>mprA</i> , Forward Lambda Red
VL38 <i>mprA</i> R pKD13) (70nt)	AAATCTGGATTTTTGAGCGAGATGACGCGTTAGCT CATCGCTTCGAGAAGTGTAGGCTGGAGCTGCTTCG	<i>mprA</i> , Reverse Lambda Red
VL39 <i>wzc</i> F pKD13) (70nt)	TCTGCCCGCCAGTGGGCGCAGGCATTGAACGCAGA GCAGGTATAAGAATGATTCCGGGGATCCGTCGACC	<i>wzc</i> , Forward Lambda Red
VL40 <i>wzc</i> R pKD13) (70nt)	CCTACGGGGCGGTGCGAATGCAGGCGTTCGTTATTT CGCATCCGACTTATATGTAGGCTGGAGCTGCTTCG	<i>wzc</i> , Reverse Lambda Red
VL41 pKD13 p2 (20nt)	TGTAGGCTGGAGCTGCTTCG	Sequencing FRT
VL56 new <i>wzyB</i> F (64nt)	GTACACCCGGGAATAATATTAATAAAAATTTTTATTA CATTTTTATGTATTGAACTGATTATTGG	<i>wzyB</i> from pWALDO
VL57 new <i>wzyB</i> R (64nt)	TCTTCTGCATGTCAATTTTTCGAACTGCGGGTGGCTC CAGGCCGCAAGCTTTTTGTAGAG	<i>wzyB</i> from pWALDO
VL70 <i>wzyB</i> pBAD33 F (38nt)	GTAACACCCGGGTTGTTTAACTTTAAGAAGGAGAC TCG	<i>wzyB</i> +RBS pWALDO
VL71 <i>wzyB</i> FLAGx3 R (105nt)	TCTTCGCATGCTCACTTGTGCATCGTCATCCTTGTAG TCGATGTGCATGATCTTTATAATCACCGTCATGGTCT TTGTAGTCGGATCCTTTTTGCTCCAGAAGTGAGG	<i>wzyB</i> from pWALDO + FLAGx3
VL72 S1 <i>wzzB</i> TM2 F (72nt)	AGTCCGAAAAAGGCAATCACCTTGATTCTGGCAGT GCTTCTGGGCGGCATGGTTGGCGCGGGGATTGTGTT G	<i>wzzB</i> TM2 region F strand
VL73 S1 <i>wzzB</i> TM2 R (72nt)	CAACACAATCCCCGCGCCAACCATGCCGCCAGAA G CACTGCCAGAATCAAGGTGATTGCCTTTTTTCGGACT	<i>wzzB</i> TM2 region R strand
VL74 S1 check F (19nt)	CGTGGGTTGTTTCGTACCT	S1 sequencing F
VL75 S1 check R (19nt)	AGCACTGCCAGAATCAAGG	S1 sequencing R
VL76 S1 INV1 F (21nt)	ATCGCGTTTTACCGTTCTTC	Amplification, <i>wzzE</i> <sup>ΔTM2</sup> F
VL77 S1 INV2 R (15nt)	ACCCGCCGTTGCTCG	Amplification, <i>wzzE</i> <sup>ΔTM2</sup> R
VL78 S2 <i>wzzE</i> TM2 F (72nt)	AGCCCACGTCGTGCCTTCCTGATGATTATGTGGGGC ATTGTCGGGGGGCTGATCGGGGCTGGTGTTCGATTA	<i>wzzE</i> TM2 region F strand

VL79 S2 <i>wzzE</i> TM2 R (72nt)	TAATGCGACACCAGCCCCGATCAGCCCCCGACAAT GCCCCACATAATCATCAGGAAGGCACGACGTGGGC T	<i>wzzE</i> TM2 region R strand
VL80 S2 check F (22nt)	GGACGTCAACAGCAATTATCAC	S2 sequencing F
VL81 S2 check R (20nt)	TGCCCCACATAATCATCAGG	S2 sequencing R
VL82 S2 INV1 F (20nt)	ATCGCGACGAATAGGTAACG	Amplification, <i>wzzB</i> <sup>ATM2</sup> F
VL83 S2 INV2 R (16nt)	GGGCGTAACGCTCTGC	Amplification, <i>wzzB</i> <sup>ATM2</sup> R
VL84 <i>wzyB</i> Ran Mut R (22nt)	TTATTTTGCTCCAGAAGTGAGG	Random mutagenesis (use with VL70)
VL87 TM1 S1 <i>wzzB</i> F (69nt)	GGCAAGATGACAATTATCATTTCGGTCATTGTGGCT ATTGCCCTGGCTATTGGTTATTTGGCAGTAGCG	<i>wzzB</i> TM1 region F strand
VL88 TM1 S1 <i>wzzB</i> R (69nt)	CGCTACTGCCAAATAACCAATAGCCAGGGCAATAG CCACAATGACGGAAATGATAATTGTCATCTTGCC	<i>wzzB</i> TM1 region R strand
VL89 TM1 S1 INV F (19nt)	AGCCCACAAGGTACGAAAC	Amplification, <i>wzzE</i> <sup>ATM1</sup> F
VL90 TM1 S1 INV R (18nt)	CGTCAGGAGTGGAGCTCG	Amplification, <i>wzzE</i> <sup>ATM1</sup> R
VL91 TM1 S1 check F (25nt)	GCAAGATGACAATTATCATTTCGGT	S1 TM 1 sequencing F
VL92 TM1 S1 check R (17nt)	CCGACAATGCCCCACAT	S1 TM 1 sequencing R
VL93 TM1S2 <i>wzzE</i> F (69nt)	GGGAAGCTATGGATTATTGGCATGGGGCTGGCGTTT GCGTTAATCGCGCTGGCGTATACTTTTTTTTGCT	<i>wzzE</i> TM1 region F strand
VL94 TM1S2 <i>wzzE</i> R (69nt)	AGCAAAAAAAGTATACGCCAGCGGATTAACGCAA ACGCCAGCCCCATGCCAATAATCCATAGCTTCCC	<i>wzzE</i> TM1 region R strand
VL95 TM1S2 INV F (18nt)	ACGCCACAACACTGCACTAG	Amplification, <i>wzzB</i> <sup>ATM1</sup> F
VL96 TM1S2 INV R (21nt)	AAGGAGAAATGGACGTCAACA	Amplification, <i>wzzB</i> <sup>ATM1</sup> R
VL97 TM1 S2 check F (23nt)	GGAAGCTATGGATTATTGGCATG	S2 TM 1 sequencing F
VL98 TM1 S2 check R (23nt)	GTTGTAATTACGCAGAGCGTTAC	S2 TM 1 sequencing R
VL105 <i>wzyB</i> RM F (34nt)	TTTTGTTTAACTTTAAGAAGGAGATATACATATG	Mutagenesis <i>wzyB</i> pBCKS <sup>+</sup> F (Mega primer)
VL106 <i>wzyB</i> RM R (20nt)	TGGTCTTTGTAGTCGCATGC	Mutagenesis <i>wzyB</i> pBCKS <sup>+</sup> R (Mega primer)
VL107 <i>wzyB</i> RM seq F (19nt)	GGAAGAGCGCCCAATACGC	Random mutagenesis sequencing F

VL108 <i>wzyB</i> RM seq R (27nt)	GTAATACGACTCACTATAGGGCGAATT	Random mutagenesis sequencing R
VL109 ALA UNIR (53nt)	AAAAAAGGAATGGATTTCGTTATAGAAACGGTAATAC TGAAAAAAAGTATTAACG	Universal reverse primer for alanine scanning
VL110 ALA 1 F (31nt)	GCACCCATGGGAATTTTTATTATAGGCGTTG	ALA change position 1
VL111 ALA 2 F (34nt)	ACAGCAATGGGAATTTTTATTATAGGCGTTGTAG	ALA change position 2
VL112 ALA 3 F (42nt)	ACACCCGCAGGAATTTTTATTATAGGCGTTGTAGTT TTATGG	ALA change position 3
VL113 ALA 4 F (50nt)	ACACCCATGGCAATTTTTATTATAGGCGTTGTAGTTT TATGGGTATTTTC	ALA change position 4
VL114 ALA 5 F (50nt)	ACACCCATGGGAGCATTTATTATAGGCGTTGTAGTT TTATGGGTATTTTC	ALA change position 5
VL115 ALA 6 F (50nt)	ACACCCATGGGAATTGCAATTATAGGCGTTGTAGTT TTATGGGTATTTTC	ALA change position 6
VL116 ALA 7 F (50nt)	ACACCCATGGGAATTTTTGCAATAGGCGTTGTAGTT TTATGGGTATTTTC	ALA change position 7
VL117 ALA Seq F (32nt)	GGAAGAGGATTTGGTTCATTTATATTAGATCG	Alanine scanning seq
VL118 LYS 1 F (34nt)	ACAAAAATGGGAATTTTTATTATAGGCGTTGTAG	LYS change position 1
VL119 LYS 2 F (42nt)	ACACCCAAAGGAATTTTTATTATAGGCGTTGTAGTT TTATGG	LYS change position 2
VL120 LYS 3 F (50nt)	ACACCCATGAAAATTTTTATTATAGGCGTTGTAGTTT TATGGGTATTTTC	LYS change position 3
VL121 ASP 1 F (34nt)	ACAGATATGGGAATTTTTATTATAGGCGTTGTAG	ASP change position 1
VL122 ASP 2 F (34nt)	ACACCCGATGGAATTTTTATTATAGGCGTTGTAGTTT TATGG	ASP change position 2
VL123 ASP 3 F (34nt)	ACACCCATGGATATTTTTATTATAGGCGTTGTAGTTT TATGGGTATTTTC	ASP change position 3
VL 124 3xALA 1 F (50nt)	ACAGCAGCAGCAATTTTTATTATAGGCGTTGTAGTT TTATGGGTATTTTC	Triple alanine change



## Appendix D – Mass spectrometry raw data

### Appx. D.1: Full protein identification from band 1 of Figure 5.2b.

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score	Sequest HT
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=r/c PE=3 SV=1	34.872	18	9	73	9	382	43.7	147.31	
I6C8N1	Modulator of FtsH protease HflC OS=Shigella flexneri K-315 OX=766150 GN=hflC PE=3 SV=1	88.628	64	21	41	21	334	37.6	123.84	
Q0T240	Outer membrane protein assembly factor BamC OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nlpB PE=3 SV=1	71.957	43	11	21	11	344	36.8	79.21	
A0A658Y1M8	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=gapA PE=3 SV=1	58.418	43	12	21	12	331	35.5	70.34	
A0A2A2XR75	EIIAB-Man OS=Shigella flexneri OX=623 GN=manX_2 PE=4 SV=1	55.591	32	13	16	13	323	35	50.9	
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	32.966	36	10	13	10	329	36.5	42.01	
Q0T6G6	UPF0194 membrane protein YbhG OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ybhG PE=3 SV=1	29.633	31	9	12	9	331	36.3	35.34	
D2ABV5	ECA polysaccharide chain length modulation protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=wzzE PE=3 SV=1	32.979	32	8	13	8	349	39.6	34.4	
Q0SYD1	L-lactate dehydrogenase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lldD PE=3 SV=1	28.258	31	11	12	11	396	42.7	30.84	
D2A6V5	Chain length determinant protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_2324 PE=4 SV=1	25.128	37	10	11	10	337	37.9	29.71	
I6D059	Dihydroorotate dehydrogenase (quinone) OS=Shigella flexneri K-315 OX=766150 GN=pyrD PE=3 SV=1	22.26	29	9	10	9	336	36.8	28.44	
A0A2S8DUZ3	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=Shigella boydii OX=621 GN=accA PE=3 SV=1	27.478	29	8	11	8	319	35.2	28	
A0A2S4MYF8	Outer membrane protein C OS=Shigella flexneri OX=623 GN=ompC PE=1 SV=1	24.679	15	7	11	4	373	41.4	27.11	
I6CVK9	Phenylalanine-tRNA ligase alpha subunit OS=Shigella flexneri K-315 OX=766150 GN=pheS PE=3 SV=1	23.905	28	8	9	8	327	36.8	24.85	
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	18.68	18	9	9	9	513	55.2	24.81	
A0A2Y5B4B8	Malate dehydrogenase OS=Shigella flexneri OX=623 GN=mdh PE=3 SV=1	23.605	29	8	8	8	312	32.3	23.94	
I6CHF3	MFS transporter, sugar porter family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3768 PE=3 SV=1	15.606	9	4	9	4	472	51.7	23.63	
A0A379ZSJ2	Alcohol dehydrogenase OS=Shigella flexneri OX=623 GN=yqhD PE=4 SV=1	19.327	17	6	7	6	387	42.1	20.96	
Q0T4H3	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1619 PE=4 SV=1	15.847	32	9	9	9	314	33.9	19.26	
I6D072	Outer membrane protein A OS=Shigella flexneri K-315 OX=766150 GN=ompA PE=3 SV=1	14.738	19	7	7	7	344	37	19.19	
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsC PE=3 SV=1	18.396	32	9	10	9	224	24.8	18.73	
D2AHG2	Autonomous glycy radical cofactor OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfiD PE=3 SV=1	23.014	43	6	8	6	127	14.2	18.65	
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	19.538	18	7	7	7	376	41	18.59	
A0A2S8DDU2	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OS=Shigella dysenteriae OX=622 GN=murG PE=3 SV=1	17.876	22	6	6	6	355	37.8	18.29	
A0A6N3R6K7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4998 PE=3 SV=1	15.026	9	6	6	6	402	42.7	18.09	
A0A4P7TR77	Maltoporin OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=lamb PE=2 SV=1	16.785	21	4	7	4	446	50	17.6	
Q0SZX8	Elongation factor Tu 1 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tuf1 PE=3 SV=1	13.869	19	6	6	6	394	43.3	16.57	
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	16.714	11	5	5	5	474	49.3	15.99	
Q2EUU0	AccD (Fragment) OS=Shigella flexneri OX=623 GN=accD PE=3 SV=1	14.69	24	6	8	6	296	32.6	15.31	
A0A6N3QLJ7	Magnesium transport protein CorA OS=Shigella flexneri CDC 796-83 OX=945360 GN=corA PE=3 SV=1	15.097	16	4	5	4	316	36.6	15.11	
Q7AK95	Chloramphenicol acetyltransferase OS=Shigella flexneri 2b OX=1288825 GN=cat PE=3 SV=1	10.635	7	2	6	2	219	25.6	14.95	
I6CBL5	ATP synthase F0, B subunit OS=Shigella flexneri K-315 OX=766150 GN=atpF PE=3 SV=1	14.452	43	5	5	5	127	14	14.93	
I6D6H9	Transaldolase OS=Shigella flexneri K-315 OX=766150 GN=tal PE=3 SV=1	12.933	25	7	7	7	294	32.8	14.92	
A0A6L6V6S8	Mobilization protein OS=Shigella flexneri OX=623 GN=GLO03_22460 PE=4 SV=1	13.564	27	4	6	4	176	19.9	14.65	
A0A3T2UTX6	Biotin_lipoyl_2 domain-containing protein OS=Shigella flexneri OX=623 GN=DK174_07550 PE=4 SV=1	13.614	19	6	6	6	355	38.8	14.13	
A0A6N3RB60	50S ribosomal protein L2 OS=Shigella flexneri 1485-80 OX=766155 GN=rpIB PE=3 SV=1	10.645	21	5	5	5	273	29.8	13.91	
A0A6A9TRT7	ATP synthase subunit beta OS=Shigella flexneri OX=623 GN=atpD PE=3 SV=1	10.522	12	5	5	5	460	50.3	13.1	
A0A2S8DCK4	Outer membrane porin protein C OS=Shigella dysenteriae OX=622 GN=ompC PE=3 SV=1	11.767	9	4	5	1	376	41.4	12.66	
F5NT24	Lipoprotein releasing system, transmembrane protein LolE OS=Shigella flexneri K-227 OX=766147 GN=lolE PE=3 SV=1	12.143	8	3	4	3	414	45.3	12.25	
A0A2S4MYJ5	Glycerophosphodiester phosphodiesterase OS=Shigella flexneri OX=623 GN=glpQ PE=4 SV=1	10.822	19	5	5	5	358	40.9	12.18	

D2A917	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	12.765	20	5	5	5	124	13.7	11.82
A0A1Q8M994	ADP-L-glycero-D-manno-heptose-6-epimerase OS=Shigella boydii OX=621 GN=rfaD PE=3 SV=1	11.889	20	6	6	6	310	34.9	11.6
A0A1S9JMU3	Cell shape-determining protein MreB OS=Shigella boydii OX=621 GN=mreB PE=3 SV=1	7.143	16	4	4	4	347	36.9	11.4
A0A3T2UWB2	ATP synthase gamma chain OS=Shigella flexneri OX=623 GN=atpG PE=3 SV=1	11.474	19	5	5	5	287	31.6	11.13
A0A658Z505	Transporter, small conductance mechanosensitive ion channel (MscS) family OS=Shigella flexneri OX=623 GN=mscM PE=3 SV=1	8.047	9	4	5	4	415	46.6	11.1
Q0T6B3	Aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=aspC PE=3 SV=1	10.392	15	6	6	6	396	43.6	10.93
I6D3P2	Tol-Pal system protein TolR OS=Shigella flexneri K-315 OX=766150 GN=tolR PE=3 SV=1	8.957	23	3	6	3	142	15.4	10.19
D2AA83	Outer membrane protein induced after carbon starvation OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=slp PE=4 SV=1	11.864	16	3	3	3	243	27.4	9.89
A0A2S8DKM8	Lipoprotein-releasing ABC transporter permease subunit LolC OS=Shigella boydii OX=621 GN=lolC PE=3 SV=1	9.929	10	3	3	3	399	43.3	9.58
A0A6N3QME5	50S ribosomal protein L1 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rplA PE=3 SV=1	12.087	15	3	3	3	234	24.7	9.45
A0A6N3R4Q2	50S ribosomal protein L6 OS=Shigella flexneri 1485-80 OX=766155 GN=rplF PE=3 SV=1	7.689	21	3	4	3	177	18.9	9.36
P69785	PTS system glucose-specific EIIA component OS=Shigella flexneri OX=623 GN=crr PE=3 SV=2	5.961	20	2	3	2	169	18.2	8.93
I6CAV2	Cell division protein FtsX OS=Shigella flexneri K-315 OX=766150 GN=ftsX PE=3 SV=1	6.985	16	4	5	4	352	38.5	8.44
Q0SYE6	2-amino-3-ketobutyrate coenzyme A ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=kbl PE=3 SV=1	11.297	9	3	4	3	398	43.2	8.4
Q0SZA6	ATP-dependent 6-phosphofructokinase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pfkA PE=3 SV=1	3.462	5	2	3	2	340	37.1	7.61
A0A0H2VYI3	Nucleoside permease OS=Shigella flexneri OX=623 GN=nupC PE=3 SV=1	8.295	9	4	4	4	400	43.5	7.6
A0A1Q8M8J0	50S ribosomal protein L7/L12 OS=Shigella boydii OX=621 GN=rplL PE=3 SV=1	6.521	21	2	3	2	121	12.3	7.57
A0A6N3RAY3	50S ribosomal protein L15 OS=Shigella flexneri 1485-80 OX=766155 GN=rplO PE=3 SV=1	7.259	28	3	3	3	126	13.1	7.4
A0A4P7TQF0	50S ribosomal protein L9 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rplI PE=3 SV=1	3.505	15	2	3	2	149	15.8	7.35
A0A3T2UY84	Cyclopropane-fatty-acyl-phospholipid synthase OS=Shigella flexneri OX=623 GN=DK174_15880 PE=3 SV=1	5.973	7	2	3	2	382	43.9	7.34
A0A2S8DCC8	Delta-aminolevulinic acid dehydratase OS=Shigella dysenteriae OX=622 GN=C5K18_12345 PE=3 SV=1	6.473	9	3	3	3	324	35.6	7.31
A0A4P7TQT3	50S ribosomal protein L10 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rplJ PE=3 SV=1	7.092	19	4	4	4	165	17.7	7.24
F5P3L0	Fumarate reductase iron-sulfur subunit OS=Shigella flexneri K-227 OX=766147 GN=SFK227_5105 PE=3 SV=1	10.894	14	2	2	2	244	27	7.19
Q0T5C6	Putative protease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=sohB PE=3 SV=1	6.045	8	3	3	3	296	33.4	7.01
A0A658Z1V1	UPF0283 membrane protein YcjF OS=Shigella flexneri OX=623 GN=ycjF PE=3 SV=1	5.072	7	2	3	2	353	39.4	6.97
A0A3T2UX48	Outer membrane protein F OS=Shigella flexneri OX=623 GN=ompF PE=3 SV=1	8.637	9	3	3	3	362	39.3	6.73
A0A2Y4XVQ2	Outer membrane protein assembly complex subunit YfgL OS=Shigella flexneri OX=623 GN=yfgL PE=4 SV=1	11.367	19	3	3	3	184	20.1	6.7
A0A6N3RKA3	Succinate dehydrogenase iron-sulfur subunit OS=Shigella flexneri 1485-80 OX=766155 GN=sdhB PE=3 SV=1	8.528	17	4	4	4	238	26.8	6.5
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	6.654	24	2	2	2	129	13.8	6.47
A0A6H1JH06	30S ribosomal protein S5 OS=Shigella flexneri OX=623 GN=rpsE PE=3 SV=1	7.625	22	3	3	3	167	17.6	6.46
F5NZ29	Serine transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3429 PE=4 SV=1	3.099	3	1	2	1	429	46.9	6.42
A0A379Z1S8	Protein HflK OS=Shigella flexneri OX=623 GN=hflK PE=3 SV=1	7.051	8	3	3	3	419	45.6	6.37
I6C9W2	30S ribosomal protein S10 OS=Shigella flexneri K-315 OX=766150 GN=rpsJ PE=3 SV=1	6.982	24	2	3	2	103	11.7	6.33
F5NQQ0	UDP-glucose 4-epimerase OS=Shigella flexneri K-227 OX=766147 GN=galE PE=3 SV=1	4.632	10	2	2	2	338	37.3	6.09
A0A383JXY5	NAD dependent epimerase/dehydratase family OS=Shigella flexneri OX=623 GN=DOD76_09530 PE=4 SV=1	5.149	7	2	2	2	337	38	5.84
A0A4P7TTH9	30S ribosomal protein S2 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsB PE=3 SV=1	16.372	20	4	5	4	241	26.8	5.7
A0A2S8D3U0	Catabolite activator protein OS=Shigella dysenteriae OX=622 GN=crp PE=4 SV=1	5.184	10	2	2	2	210	23.7	5.69
A0A2S8DCH8	Cysteine synthase OS=Shigella dysenteriae OX=622 GN=cysK PE=3 SV=1	3.147	8	2	2	2	323	34.5	5.66
F5NZV9	Mechanosensitive ion channel family protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3712 PE=3 SV=1	9.039	9	2	2	2	286	30.9	5.65
Q83PC3	50S ribosomal protein L11 OS=Shigella flexneri OX=623 GN=rplK PE=3 SV=3	5.13	17	2	2	2	142	14.9	5.64
A0A658Y5Z7	L-1,2-propanediol oxidoreductase OS=Shigella flexneri OX=623 GN=fucO PE=4 SV=1	3.776	7	2	2	2	382	40.4	5.45
I6CB41	Tryptophan-tRNA ligase OS=Shigella flexneri K-315 OX=766150 GN=trpS PE=3 SV=1	3.372	7	2	2	2	319	35.9	5.34
D2AGV7	TPR_21 domain-containing protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfgM PE=3 SV=1	3.961	13	1	1	1	206	22.1	5.17
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	2.926	9	2	2	2	206	23.4	5.07
A0A6N3QPX8	Prophage P4 integrase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03306 PE=4 SV=1	1.487	2	1	2	1	422	48.2	5.07
A0A4P7TNY5	Alkyl hydroperoxide reductase C OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=ahpC PE=3 SV=1	3.055	11	2	2	2	187	20.7	5.04
F5NQ21	Cell division protein FtsQ OS=Shigella flexneri K-227 OX=766147 GN=ftsQ PE=3 SV=1	4.051	9	2	2	2	276	31.4	4.81
A0A2S8DV96	50S ribosomal protein L5 OS=Shigella boydii OX=621 GN=rplE PE=3 SV=1	7.315	12	1	1	1	179	20.3	4.75
A0A6N3QM36	Protein QmcA OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01963 PE=3 SV=1	2.622	7	2	2	2	305	33.7	4.7
Q83PS4	C4-dicarboxylate transport protein OS=Shigella flexneri OX=623 GN=dctA PE=3 SV=1	2.663	2	2	2	2	428	45.4	4.7

A0A3U1DEW5	Formate C-acetyltransferase OS=Shigella flexneri OX=623 GN=pfIB PE=3 SV=1	3.11	3	2	2	2	760	85.3	4.68
A0A6S8YW90	Fructose-bisphosphate aldolase OS=Shigella flexneri OX=623 GN=fbaB PE=4 SV=1	3.68	6	2	2	2	350	38.1	4.64
I6CBY6	Lipopolysaccharide heptosyltransferase II OS=Shigella flexneri K-315 OX=766150 GN=rfaF PE=4 SV=1	4.092	5	2	2	2	348	39	4.59
A0A6N3QLC2	Universal stress protein G OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02439 PE=3 SV=1	2.516	10	1	2	1	142	15.9	4.4
A0A2S8D5N6	Aspartate carbamoyltransferase OS=Shigella dysenteriae OX=622 GN=pyrB PE=3 SV=1	3.822	11	3	3	3	311	34.4	4.37
F5NX93	EIII-Fru OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2769 PE=4 SV=1	6.656	10	3	3	3	376	39.7	4.24
F5P2C5	L-threonine 3-dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=tdh PE=3 SV=1	3.835	6	2	2	2	341	37.3	4.24
A0A2S8DD79	Threonine/serine transporter Tdc OS=Shigella dysenteriae OX=622 GN=tdcC PE=3 SV=1	4.126	9	3	3	3	443	48.9	4.23
I6CFU5	Peptidase Do OS=Shigella flexneri K-315 OX=766150 GN=degS PE=3 SV=1	3.861	5	2	2	2	355	37.6	4.2
P0A6P4	Elongation factor Ts OS=Shigella flexneri OX=623 GN=tsf PE=3 SV=2	3.332	6	2	2	2	283	30.4	4.06
I6CJU1	Acetate kinase OS=Shigella flexneri K-315 OX=766150 GN=ackA PE=3 SV=1	6.46	8	2	2	2	400	43.3	4.02
A0A0H2V319	Putative amino acid/amine transport protein OS=Shigella flexneri OX=623 GN=yiFK PE=4 SV=1	5.42	6	3	3	3	417	45.3	4.01
A0A6N3RB16	Protein translocase subunit SecY OS=Shigella flexneri 1485-80 OX=766155 GN=prlA PE=3 SV=1	4.493	7	3	3	3	443	48.5	3.94
A0A1Q8M4D3	30S ribosomal protein S18 OS=Shigella boydii OX=621 GN=rpsR PE=3 SV=1	7.102	24	1	1	1	75	9	3.88
A0A2Y4XZJ9	Ferrocyclase OS=Shigella flexneri OX=623 GN=hemH PE=3 SV=1	3.521	7	1	1	1	320	35.9	3.85
I6C9W8	50S ribosomal protein L22 OS=Shigella flexneri K-315 OX=766150 GN=rplV PE=3 SV=1	5.209	19	2	2	2	110	12.2	3.82
A0A6D2W4F9	Chaperone protein Skp OS=Shigella flexneri OX=623 GN=skp PE=3 SV=1	3.838	17	3	3	3	161	17.7	3.78
F5NVZ8	Outer membrane porin protein LC OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2311 PE=3 SV=1	3.363	6	2	3	2	360	39.7	3.76
Q83MF6	Cell division protein FtsZ OS=Shigella flexneri OX=623 GN=ftsZ PE=3 SV=2	3.61	3	1	1	1	383	40.1	3.6
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=Igk PE=1 SV=1	2.81	6	1	1	1	219	24.2	3.58
D2AE64	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4630 PE=3 SV=1	3.641	9	1	1	1	141	15.2	3.32
Q7UCQ9	Lipopolysaccharide assembly protein B OS=Shigella flexneri OX=623 GN=yCIM PE=3 SV=1	3.744	3	1	1	1	389	44.4	3.23
F5P2D1	Rhodanese-like domain protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4672 PE=4 SV=1	6.865	16	2	2	2	143	15.6	3.22
A0A1Q8NKE7	Phosphoheptose isomerase OS=Shigella dysenteriae OX=622 GN=gmhA PE=3 SV=1	2.074	5	1	1	1	192	20.8	3.12
A0A3T2UTJ3	N-acetylglucosamine-6-phosphate deacetylase OS=Shigella flexneri OX=623 GN=DK174_06800 PE=3 SV=1	1.099	3	1	1	1	382	41	3.09
I6CPN2	Ferritin OS=Shigella flexneri K-315 OX=766150 GN=ftnA PE=3 SV=1	3.13	7	1	1	1	165	19.4	2.99
A0A4P7TR58	NAD(P)/FAD-dependent oxidoreductase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_010675 PE=4 SV=1	3.445	3	1	1	1	434	47.3	2.99
I6C9X6	30S ribosomal protein S14 OS=Shigella flexneri K-315 OX=766150 GN=rpsN PE=3 SV=1	4.605	19	2	2	2	96	11	2.98
A0A6N3R6J7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4987 PE=3 SV=1	2.449	2	1	1	1	446	47.8	2.96
A0A2S8DL49	NADH-quinone oxidoreductase subunit N OS=Shigella boydii OX=621 GN=nuoN PE=3 SV=1	2.804	2	1	1	1	485	52	2.95
A0A2S8D3C6	S-ribosylhomocysteine lyase OS=Shigella dysenteriae OX=622 GN=luxS PE=3 SV=1	4.201	14	2	2	2	171	19.4	2.94
I6CAR9	Universal stress protein OS=Shigella flexneri K-315 OX=766150 GN=uspA PE=3 SV=1	3.004	14	1	1	1	144	16.1	2.94
A0A2Y4XMF0	Sec-independent protein translocase protein TatA OS=Shigella flexneri OX=623 GN=tatA PE=3 SV=1	3.777	15	1	1	1	89	9.7	2.93
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	3.163	15	1	1	1	78	8.3	2.92
A0A383JR12	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=ftsH PE=3 SV=1	1.455	2	1	1	1	647	71	2.91
A0A6N3QQ51	Maltose regulon modulator MalY OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01515 PE=4 SV=1	3.395	3	1	1	1	390	43.6	2.86
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	2.517	3	1	1	1	477	50.7	2.85
A0A2Y4WU12	Pyrimidine-specific ribonucleoside hydrolase RihA OS=Shigella flexneri OX=623 GN=rhA PE=3 SV=1	5.903	9	2	2	2	311	33.8	2.83
A0A0H2V3T2	L-lactate permease OS=Shigella flexneri OX=623 GN=SF4369 PE=3 SV=1	1.197	4	1	1	1	300	32.5	2.8
I6CMC4	Lysine-specific permease OS=Shigella flexneri K-315 OX=766150 GN=lysP PE=4 SV=1	1.404	2	1	1	1	489	53.5	2.76
F5P2M7	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4769 PE=4 SV=1	2.665	6	1	1	1	236	25.6	2.73
Q0SY57	ATP-dependent protease subunit HslV OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hslV PE=3 SV=1	3.686	6	1	1	1	176	19.1	2.66
I6CG83	Phosphoglycerate kinase OS=Shigella flexneri K-315 OX=766150 GN=pgk PE=3 SV=1	4.978	4	1	2	1	382	40.5	2.58
P84077	ADP-ribosylation factor 1 OS=Homo sapiens OX=9606 GN=ARF1 PE=1 SV=2	2.221	6	1	1	1	181	20.7	2.56
A0A6N3RQ35	DNA protection during starvation protein OS=Shigella flexneri 1485-80 OX=766155 GN=dps PE=3 SV=1	1.25	11	1	1	1	167	18.7	2.55
I6CBX6	Lipopolysaccharide heptosyltransferase III, putative OS=Shigella flexneri K-315 OX=766150 GN=SFK315_4420 PE=4 SV=1	1.867	4	1	1	1	254	28	2.45
A0A2Y5A6X9	30S ribosomal protein S9 OS=Shigella flexneri OX=623 GN=rpsI PE=3 SV=1	2.54	14	1	1	1	85	9.7	2.44
A0A6N3RR48	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0546 PE=3 SV=1	1.332	4	1	1	1	259	28.2	2.39
A0A6N3RL91	L-arabinose isomerase OS=Shigella flexneri 1485-80 OX=766155 GN=araA PE=3 SV=1	1.9	2	1	1	1	500	56.1	2.38
A0A6L6UVT0	Lipid A biosynthesis lauroyltransferase OS=Shigella flexneri OX=623 GN=lpXL PE=3 SV=1	1.665	4	1	1	1	306	35.4	2.36

D2A9E7	Cytochrome d ubiquinol oxidase subunit I OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=cydA PE=3 SV=1	2.64	2	1	1	1	1	523	58.3	2.35
A0A2S8DX13	Aromatic amino acid transporter AroP OS=Shigella boydii OX=621 GN=C5K23_02025 PE=4 SV=1	1.604	2	1	1	1	1	457	49.7	2.31
A0A4P7TME5	Osmotic shock tolerance protein YggT OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=yggT PE=3 SV=1	1.203	6	1	1	1	1	188	21.1	2.26
A0A0H2VXW8	Putative reductase OS=Shigella flexneri OX=623 GN=SF3047 PE=4 SV=1	3.661	3	1	1	1	1	346	38.8	2.24
A0A4P7TM74	30S ribosomal protein S21 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsU PE=3 SV=1	1.005	11	1	1	1	1	71	8.5	2.22
A0A2S8E636	Acetyltransferase component of pyruvate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=aceF PE=3 SV=1	2.414	3	2	2	2	2	630	66.1	2.18
F5P004	L-asparaginase 2 OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3757 PE=3 SV=1	2.261	5	1	1	1	1	235	25.2	2.14
A0A383JU60	Chorismate synthase OS=Shigella flexneri OX=623 GN=aroC PE=3 SV=1	3.43	6	2	2	2	2	361	39.1	2.13
A0A658YW89	Riboflavin biosynthesis protein RibD OS=Shigella flexneri OX=623 GN=ribD PE=3 SV=1	1.455	2	1	1	1	1	401	44.2	2.13
F5P3C9	30S ribosomal protein S13 OS=Shigella flexneri K-227 OX=766147 GN=rpsM PE=3 SV=1	1.365	8	1	1	1	1	118	13.1	2.12
D2A8T7	Cytochrome d ubiquinol oxidase subunit III OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yhcB PE=4 SV=1	1.229	8	1	1	1	1	134	15.2	2.11
A0A6N3QQN1	Trehalosemaltose-specific PTS system components IIBC OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02485 PE=4 SV=1	1.629	2	1	1	1	1	473	51.2	2.11
I6D3R0	Succinate dehydrogenase flavoprotein subunit OS=Shigella flexneri K-315 OX=766150 GN=sdhA PE=3 SV=1	2.241	2	1	1	1	1	588	64.4	2.08
A0A0H2V1E3	SpoU_sub_bind domain-containing protein OS=Shigella flexneri OX=623 GN=yfif PE=4 SV=1	2.934	3	1	1	1	1	345	37.8	2.01
A0A2S8DA55	SH3 domain-containing protein OS=Shigella dysenteriae OX=622 GN=ygiM PE=4 SV=1	2.071	4	1	1	1	1	206	23.1	2
F5P3D3	50S ribosomal protein L17 OS=Shigella flexneri K-227 OX=766147 GN=rplQ PE=3 SV=1	1.096	6	1	1	1	1	127	14.4	1.92
A0A6N3RIF3	Enoyl-acyl-carrier-protein reductase [NADH] OS=Shigella flexneri 1485-80 OX=766155 GN=fabI PE=3 SV=1	1.51	4	1	1	1	1	262	27.8	1.92
I6C8L3	Fumarate reductase subunit C OS=Shigella flexneri K-315 OX=766150 GN=frdC PE=3 SV=1	2.242	7	1	1	1	1	120	13.6	1.91
A0A2S8DI33	Sec translocon accessory complex subunit YajC OS=Shigella boydii OX=621 GN=yajC PE=3 SV=1	2.797	18	2	2	2	2	110	11.9	1.88
A0A1S9KIA0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	2.181	2	1	1	1	1	419	47	1.83
Q0T0X5	Fructose-bisphosphate aldolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fba PE=3 SV=1	2.466	2	1	1	1	1	387	42.7	1.82
A0A6N3QNZ8	Inorganic pyrophosphatase OS=Shigella flexneri CDC 796-83 OX=945360 GN=ppa PE=3 SV=1	3.419	10	2	2	2	2	176	19.7	1.81
Q2EUF8	Glycine cleavage system T protein (Fragment) OS=Shigella flexneri OX=623 GN=gcvT PE=4 SV=1	2.198	8	1	1	1	1	176	19.2	1.81
D2AHT6	Co-chaperone protein DjIA OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=djIA PE=3 SV=1	1.822	3	1	1	1	1	271	30.5	1.8
A0A379Z9W6	Heme biosynthesis protein HemY OS=Shigella flexneri OX=623 GN=hemY PE=4 SV=1	0.931	3	1	1	1	1	398	45.2	1.7
A0A2S8DHH6	30S ribosomal protein S15 OS=Shigella dysenteriae OX=622 GN=rpsO PE=3 SV=1	1.6	8	1	1	1	1	89	10.3	1.69
F5NVB6	Glutamate/gamma-aminobutyrate antiporter OS=Shigella flexneri K-227 OX=766147 GN=gadC PE=3 SV=1	1.449	2	1	1	1	1	511	55.1	1.68
QOSX09	Phosphoserine aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=serC PE=3 SV=1	1.99	3	1	1	1	1	350	38.6	1.67
A0A6D2X1F2	30S ribosomal protein S19 OS=Shigella flexneri OX=623 GN=rpsS PE=3 SV=1	1.113	12	1	1	1	1	92	10.4	0
I6C9W3	50S ribosomal protein L3 OS=Shigella flexneri K-315 OX=766150 GN=rplC PE=3 SV=1	2.672	9	2	2	2	2	209	22.2	0
A0A6N3QGJ9	UPF0301 protein YqgE OS=Shigella flexneri CDC 796-83 OX=945360 GN=yqgE PE=3 SV=1	1.194	6	1	1	1	1	187	20.7	0
I6CGG2	Biopolymer transport protein ExbB OS=Shigella flexneri K-315 OX=766150 GN=exbB PE=3 SV=1	1.82	4	1	1	1	1	230	24.8	0
A0A6N3RLA6	Dihydroorotase OS=Shigella flexneri 1485-80 OX=766155 GN=pyrC PE=3 SV=1	0.929	4	1	1	1	1	324	35.9	0
P59330	D-cysteine desulfhydrase OS=Shigella flexneri OX=623 GN=dcyD PE=3 SV=2	0.931	4	1	1	1	1	328	35.1	0
A0A6N3RAZ3	Cell division protein FtsN OS=Shigella flexneri 1485-80 OX=766155 GN=ftsN PE=3 SV=1	1.138	4	1	1	1	1	284	31.8	0
A0A1Q8MQJ7	Protein RecA OS=Shigella boydii OX=621 GN=recA PE=3 SV=1	1.142	3	1	1	1	1	353	38	0
A0A2Y4Y1X7	D-alanine/D-serine/glycine permease OS=Shigella flexneri OX=623 GN=cycA PE=4 SV=1	1.638	3	1	1	1	1	470	51.7	0
F5P0W4	Uroporphyrinogen decarboxylase OS=Shigella flexneri K-227 OX=766147 GN=hemE PE=3 SV=1	1.086	3	1	1	1	1	354	39.2	0
A0A6N3QWE0	33 kDa chaperonin OS=Shigella flexneri CDC 796-83 OX=945360 GN=hsIO PE=3 SV=1	1.744	3	1	1	1	1	294	32.7	0
A0A6G5YYG3	Lipoprotein-releasing system ATP-binding protein LoLD OS=Shigella flexneri OX=623 GN=loLD PE=3 SV=1	0.933	3	1	1	1	1	233	25.5	0
A0A2S8D6C3	Proline-specific permease ProY OS=Shigella dysenteriae OX=622 GN=C5K18_23740 PE=4 SV=1	2.883	3	1	1	1	1	457	50.1	0
A0A2S8DET8	Galactose-1-phosphate uridylyltransferase OS=Shigella dysenteriae OX=622 GN=C5K18_07390 PE=3 SV=1	1.252	3	1	1	1	1	348	39.6	0
A0A3T2UZP3	Glyoxylate/hydroxypyruvate reductase B OS=Shigella flexneri OX=623 GN=ghrB PE=3 SV=1	1.169	2	1	1	1	1	324	35.4	0
Q0T2P1	sn-glycerol-3-phosphate permease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=glpT PE=3 SV=1	1.866	2	1	1	1	1	452	50.4	0
A0A3U1D6H5	Erythronate-4-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=pxdB PE=3 SV=1	1.885	2	1	1	1	1	378	41.3	0
A0A2Y4X2P5	Proline/glycine betaine transporter OS=Shigella flexneri OX=623 GN=proP_1 PE=4 SV=1	0.972	2	1	1	1	1	366	40.2	0
F5NXM7	NADH-quinone oxidoreductase subunit L OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2904 PE=3 SV=1	1.344	2	1	1	1	1	611	66.1	0
A0A3U1D7K9	Sucrose phosphorylase (Fragment) OS=Shigella flexneri OX=623 GN=DOD76_04610 PE=4 SV=1	1.033	2	1	1	1	1	366	41.9	0

## Appx. D.2: Full protein identification from band 2 of Figure 5.2b.

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score Sequest HT
A0A4P7TP61	Mechanosensitive channel MscK OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=mscK PE=3 SV=1	289.804	48	46	95	46	1120	127.2	286.17
A0A3U1DEW5	Formate C-acetyltransferase OS=Shigella flexneri OX=623 GN=pfIB PE=3 SV=1	216.761	59	40	72	40	760	85.3	199.51
A0A383JRI2	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=hfIB PE=3 SV=1	139.128	42	30	62	30	647	71	138.21
D2A6V5	Chain length determinant protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_2324 PE=4 SV=1	135.201	55	16	61	16	337	37.9	189.25
A0A4P7TNK7	Aldehyde-alcohol dehydrogenase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=adhE PE=3 SV=1	204.616	46	36	56	36	891	96.1	162.92
A0A6N3RK72	Chaperone protein ClpB OS=Shigella flexneri 1485-80 OX=766155 GN=clpB PE=3 SV=1	164.39	48	36	49	36	798	89.3	136.37
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=rfc PE=3 SV=1	41.725	18	9	48	9	382	43.7	83.38
A0A4P7TRN0	Pyruvate dehydrogenase E1 component OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=aceE PE=4 SV=1	134.607	39	31	44	31	887	99.6	98.63
A0A1Q8MQG3	Chaperone protein DnaK OS=Shigella boydii OX=621 GN=dnaK PE=2 SV=1	164.711	50	31	43	31	638	69.1	138.95
A0A380B9F2	Peptidylprolyl isomerase OS=Shigella flexneri OX=623 GN=ppiD PE=4 SV=1	169.875	56	29	43	1	623	68.1	131.38
A0A6N3RL18	Peptidylprolyl isomerase OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0488 PE=4 SV=1	150.686	54	29	41	1	623	68.1	120.95
A0A380B1E8	Nitrate reductase (quinone) OS=Shigella flexneri OX=623 GN=narG PE=3 SV=1	139.566	26	27	41	27	1247	140.4	116.78
A0A379Z1T0	Fumarate reductase flavoprotein subunit OS=Shigella flexneri OX=623 GN=frdA PE=3 SV=1	144.593	50	26	38	26	602	66	107.36
Q0SY20	Elongation factor Tu 2 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tuf2 PE=3 SV=1	93.703	62	20	37	20	394	43.3	82.38
A0A2Y4Y099	Glycerol-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=glpD PE=3 SV=1	97.887	38	19	35	19	501	56.7	73.94
A0A658XXS4	Outer membrane protein assembly factor YaeT OS=Shigella flexneri OX=623 GN=yaeT PE=4 SV=1	134.013	37	27	33	27	810	90.5	96.92
A0A4P7TR58	NAD(P)/FAD-dependent oxidoreductase OS=Shigella flexneri serotype 5a (OX=1086030 GN=EKN05_010675 PE=4 SV=1	106.547	55	17	33	17	434	47.3	87.39
F5P3A0	Elongation factor G OS=Shigella flexneri K-227 OX=766147 GN=fusA PE=3 SV=1	115.141	37	25	33	25	704	77.6	82.01
D2AD66	DNA-directed RNA polymerase subunit beta OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpoB PE=3 SV=1	92.745	23	29	30	29	1342	150.5	67.84
A0A658YVY8	L-tryptophan indole-lyase OS=Shigella flexneri OX=623 GN=tnaA PE=3 SV=1	104.274	44	20	29	20	471	52.8	76.27
A0A6L6UYG8	Dihydropyridyl dehydrogenase OS=Shigella flexneri OX=623 GN=lpdA PE=3 SV=1	90.921	47	20	29	20	474	50.7	64.87
A0A379Z3L5	Glycerol-3-phosphate acyltransferase OS=Shigella flexneri OX=623 GN=plsB PE=3 SV=1	92.821	34	23	28	23	807	91.4	74.92
A0A6N3QN52	Lon protease OS=Shigella flexneri CDC 796-83 OX=945360 GN=lon PE=2 SV=1	80.796	27	22	28	22	799	89	67.59
A0A6D2XMH9	ATP synthase subunit beta OS=Shigella flexneri OX=623 GN=atpD PE=3 SV=1	112.499	50	18	27	18	460	50.3	87.95
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	82.405	51	20	27	20	513	55.2	78.83
A0A379Z256	60 kDa chaperonin OS=Shigella flexneri OX=623 GN=groEL PE=3 SV=1	93.319	46	20	27	20	551	57.5	75.29
A0A3T2V040	Formate dehydrogenase-N subunit alpha OS=Shigella flexneri OX=623 GN=fdnG PE=3 SV=1	99.692	24	18	23	17	1016	112.5	63.57
A0A1Q8MKR1	Protein translocase subunit SecD OS=Shigella boydii OX=621 GN=secD PE=3 SV=1	70.013	32	18	23	18	615	66.6	50.83
I6D6Q1	Peptidoglycan D,D-transpeptidase FtsI OS=Shigella flexneri K-315 OX=766150 GN=ftsI PE=3 SV=1	83.127	30	15	21	15	588	63.9	66.59
P0A852	Trigger factor OS=Shigella flexneri OX=623 GN=tig PE=3 SV=1	66.671	39	18	21	18	432	48.2	48.97
A0A6G5YXG7	Formate dehydrogenase-N subunit alpha OS=Shigella flexneri OX=623 GN=fdnG PE=3 SV=1	75.855	23	19	21	18	1015	112.9	48.46
A0A658Y003	DNA-directed RNA polymerase subunit OS=Shigella flexneri OX=623 GN=rpoC PE=3 SV=1	56.504	13	17	21	17	1407	155.1	45.52
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	74.66	31	14	20	14	474	49.3	63.88
A0A2S8D9R8	Penicillin-binding protein 1B OS=Shigella dysenteriae OX=622 GN=mrCB PE=3 SV=1	75.415	22	15	20	15	840	93.8	55.87
Q83SE6	Chaperone protein HtpG OS=Shigella flexneri OX=623 GN=htpG PE=3 SV=1	75.799	32	17	20	17	624	71.3	54.09
A0A0H2VY4	Anaerobic dimethyl sulfoxide reductase subunit A OS=Shigella flexneri OX=623 GN=dmsA PE=3 SV=1	65.631	23	17	20	17	785	87.4	49.45
A0A6N3RM75	Oxoglutarate dehydrogenase (succinyl-transferring) OS=Shigella flexneri 1485-80 OX=766155 GN=sucA PE=3 SV=1	47.833	18	15	20	15	933	105	44.87
I6CMA1	Quinone-dependent D-lactate dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=dld PE=3 SV=1	63.444	30	16	20	16	571	64.6	41.77
Q83SN2	Protein translocase subunit SecA OS=Shigella flexneri OX=623 GN=secA PE=3 SV=1	69.255	18	17	19	17	901	102	54.08
A0A4P7TME0	Transketolase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tkt PE=3 SV=1	66.374	23	15	19	15	663	72.1	42.26
A0A6N3QD48	Succinate dehydrogenase flavoprotein subunit OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04889 PE=3 SV=1	53.413	21	12	18	12	588	64.4	51.27
R4NJV1	Outer membrane protein C (Fragment) OS=Shigella flexneri 3a OX=424717 GN=ompC PE=3 SV=1	51.315	25	11	18	6	351	39.3	46.16
A0A2S8E1V7	Protein HflK OS=Shigella dysenteriae OX=622 GN=hflK PE=3 SV=1	55.949	27	12	18	12	419	45.5	43.02
D2ABG4	30S ribosomal protein S1 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsA PE=3 SV=1	64.188	26	13	17	13	557	61.1	49.72
A0A0H2VWY5	Carboxy-terminal protease for penicillin-binding protein 3 OS=Shigella flexneri OX=623 GN=prc PE=3 SV=1	60.917	25	15	17	15	680	76.4	45.34
A0A2S8DJM4	Translation initiation factor IF-2 OS=Shigella boydii OX=621 GN=infB PE=3 SV=1	49.755	15	13	17	13	890	97.3	38.21

A0A1S9KGJ3	Peptidyl-prolyl cis-trans isomerase OS=Shigella dysenteriae OX=622 GN=fkpa PE=3 SV=1	40.58	50	11	17	11	270	28.9	33.15
A0A2S8DTV4	Phosphoenolpyruvate synthase OS=Shigella boydii OX=621 GN=ppsA PE=3 SV=1	33.381	15	13	17	13	792	87.4	30.97
A0A6N3R3T7	Aspartate ammonia-lyase OS=Shigella flexneri 1485-80 OX=766155 GN=aspA PE=3 SV=1	50.601	30	13	16	13	478	52.3	45.53
D2ABV5	ECA polysaccharide chain length modulation protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=wzzE PE=3 SV=1	50.626	38	10	16	10	349	39.6	43.48
F5NPX4	LPS-assembly protein LptD OS=Shigella flexneri K-227 OX=766147 GN=lptD PE=3 SV=1	67.046	30	16	16	16	753	86.3	42.76
F5NRH8	Efflux pump membrane transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0715 PE=3 SV=1	63.949	18	15	16	15	1049	113.6	41.99
A0A658YVC1	Aconitate hydratase B OS=Shigella flexneri OX=623 GN=acnB PE=3 SV=1	60.737	17	13	16	13	874	94.5	35.47
A0A2S8DJU5	Polyribonucleotide nucleotidyltransferase OS=Shigella boydii OX=621 GN=pnp PE=3 SV=1	38.6	18	12	16	12	711	77.1	30.8
A0A6N3RM01	Dihydropyridoxyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex OS=Shigella flexneri 1485-80 OX=766155 GN=sucB PE=3 SV=1	57.832	29	13	15	13	405	44	39.32
A0A6N3QUN6	Zinc/cadmium/mercury/lead-transporting ATPase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00074 PE=3 SV=1	54.105	21	13	15	13	732	76.7	35.3
F5NRX2	Serine-type D-Ala-D-Ala carboxypeptidase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0860 PE=3 SV=1	43.619	27	10	15	10	403	44.4	33.93
Q7UB84	Glycerol kinase OS=Shigella flexneri OX=623 GN=glpK PE=3 SV=2	37.979	25	12	14	12	502	56.2	35.51
A0A3T2UWB2	ATP synthase gamma chain OS=Shigella flexneri OX=623 GN=atpG PE=3 SV=1	41.718	34	12	14	12	287	31.6	28.46
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rrsC PE=3 SV=1	35.652	34	10	14	10	224	24.8	23.33
A0A6N3RCV1	Alanine--tRNA ligase OS=Shigella flexneri 1485-80 OX=766155 GN=alaS PE=3 SV=1	60.244	17	13	13	13	876	96	37.58
A0A2S8E636	Acetyltransferase component of pyruvate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=aceF PE=3 SV=1	44.428	22	12	13	12	630	66.1	32.42
Q0T8D5	L-arabinose isomerase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=araA PE=3 SV=1	37.252	25	10	13	10	500	56	30.87
Q0SYD1	L-lactate dehydrogenase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lldD PE=3 SV=1	43.62	36	12	13	12	396	42.7	30.21
Q0T1P7	Enolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=eno PE=3 SV=1	35.113	19	8	13	8	431	45.7	28.58
I6CJU2	Phosphate acetyltransferase OS=Shigella flexneri K-315 OX=766150 GN=pta PE=3 SV=1	45.856	20	12	13	12	709	76.5	27.68
Q7AK95	Chloramphenicol acetyltransferase OS=Shigella flexneri 2b OX=1288825 GN=cat PE=3 SV=1	32.562	17	3	12	3	219	25.6	41.92
Q0T4H5	NAD(P) transhydrogenase subunit beta OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pntB PE=3 SV=1	64.624	27	8	12	8	462	48.7	40.14
A0A658Y1M8	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=gapA PE=3 SV=1	45.916	31	10	12	10	331	35.5	38.15
A0A2S8DKT5	NADH-quinone oxidoreductase OS=Shigella boydii OX=621 GN=nuoG PE=3 SV=1	44.376	15	10	12	10	908	100.3	34.17
A0A3T2URE0	Catalase-peroxidase OS=Shigella flexneri OX=623 GN=katG PE=3 SV=1	52.037	22	12	12	12	726	80	28.02
A0A6N3RB60	50S ribosomal protein L2 OS=Shigella flexneri 1485-80 OX=766155 GN=rlpB PE=3 SV=1	33.848	36	8	11	2	273	29.8	32.18
F5NUR0	Threonine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=thrS PE=3 SV=1	29.712	13	10	11	10	642	74	27.34
A0A2Y5C3Q2	Ribonuclease E OS=Shigella flexneri OX=623 GN=me PE=3 SV=1	35.394	12	11	11	11	1063	118.4	26.52
I6CI36	Cytoskeleton protein RodZ OS=Shigella flexneri K-315 OX=766150 GN=rodZ PE=3 SV=1	41.427	27	9	11	9	337	36.2	26.21
I6CDA5	ATP-dependent protease ATPase subunit HslU OS=Shigella flexneri K-315 OX=766150 GN=hslU PE=2 SV=1	27.537	19	9	11	9	443	49.6	25.02
A0A658YV63	Bifunctional acyl-[acyl carrier protein] synthetase/2-acylglycerophosphoethanolamine acyltransferase OS=Shigella flexneri OX=623 GN=aas PE=4 SV=1	26.723	13	10	11	10	719	80.6	23.34
A0A658YYQ4	NAD(P) transhydrogenase subunit alpha OS=Shigella flexneri OX=623 GN=pntA PE=3 SV=1	33.506	20	9	11	9	510	54.5	22.95
Q7BCK4	Outer membrane protein IcsA autotransporter OS=Shigella flexneri OX=623 GN=icsA PE=1 SV=1	25.567	7	8	11	8	1102	116.2	21.99
A0A2S4MYJ5	Glycerophosphodiester phosphodiesterase OS=Shigella flexneri OX=623 GN=glpQ PE=4 SV=1	23.944	24	9	11	9	358	40.9	17.56
A0A2Y5C0F7	Putative uroporphyrinogen III C-methyltransferase OS=Shigella flexneri OX=623 GN=hemX PE=4 SV=1	49.685	28	10	10	10	401	43.6	33.51
A0A1S9KIA0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	41.881	26	9	10	9	419	47	28.38
A0A2Y5B4B8	Malate dehydrogenase OS=Shigella flexneri OX=623 GN=mdh PE=3 SV=1	32.102	29	9	10	9	312	32.3	27.2
D2AFY9	NADH-quinone oxidoreductase subunit C/D OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=nuoC PE=3 SV=1	31.375	16	9	10	9	600	68.7	24.19
I6CG83	Phosphoglycerate kinase OS=Shigella flexneri K-315 OX=766150 GN=pgk PE=3 SV=1	27.264	28	9	10	9	382	40.5	23.7
A0A2Y4JWQ5	MbeB-like, N-term conserved region OS=Shigella flexneri OX=623 GN=SAMEA3710514_05138 PE=4 SV=1	32.015	36	7	10	7	176	19.9	23.58
I6C8N1	Modulator of FtsH protease HflC OS=Shigella flexneri K-315 OX=766150 GN=hflC PE=3 SV=1	31.64	30	7	10	7	334	37.6	23.07
A0A380B6P9	PTS N-acetylglucosamine EIICBA component OS=Shigella flexneri OX=623 GN=nagE PE=4 SV=1	31.774	12	8	10	8	648	68.3	21.89
A0A379Z9W6	Heme biosynthesis protein HemY OS=Shigella flexneri OX=623 GN=hemY PE=4 SV=1	23.777	17	9	10	9	398	45.2	18.26
Q0T8G8	Isoleucine--tRNA ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ileS PE=3 SV=1	23.483	9	9	10	9	922	102.5	18.05
A0A383JWV6	Membrane protein insertase YidC OS=Shigella flexneri OX=623 GN=yidC PE=3 SV=1	19.854	11	5	10	5	548	61.5	15.01
A0A6N3R842	Adenylosuccinate synthetase OS=Shigella flexneri 1485-80 OX=766155 GN=purA PE=3 SV=1	15.034	17	7	10	7	432	47.3	8.65
A0A2A2XR75	EIIAB-Man OS=Shigella flexneri OX=623 GN=manX_2 PE=4 SV=1	48.306	22	8	9	8	323	35	28.46
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	35.918	18	7	9	7	477	50.7	26.46

I6C9W6	50S ribosomal protein L2 OS=Shigella flexneri K-315 OX=766150 GN=rpIB PE=3 SV=1	26.204	33	7	9	1	255	27.9	25.69
A0A658Y285	Protein ydgA OS=Shigella flexneri OX=623 GN=ydgA PE=4 SV=1	35.396	18	8	9	8	502	54.6	23.85
I6CBL5	ATP synthase F0, B subunit OS=Shigella flexneri K-315 OX=766150 GN=atpF PE=3 SV=1	33.315	59	9	9	9	127	14	23.08
A0A4P7TQY3	Bifunctional purine biosynthesis protein PurH OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=purH PE=3 SV=1	28.169	19	8	9	8	529	57.3	22.46
I6D072	Outer membrane protein A OS=Shigella flexneri K-315 OX=766150 GN=ompA PE=3 SV=1	24.471	27	8	9	8	344	37	22.13
A0A4P7TRQ9	Ribulokinase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=araB PE=3 SV=1	31.423	19	8	9	8	566	61.1	21.08
A0A2S8DCK4	Outer membrane porin protein C OS=Shigella dysenteriae OX=622 GN=ompC PE=3 SV=1	24.264	13	6	9	1	376	41.4	21.08
A0A4V1CUD1	Histidine kinase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=cpxA PE=4 SV=1	25.324	17	8	9	8	457	51.6	20.27
A0A4P7TJ80	Glycine dehydrogenase (decarboxylating) OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=gcvP PE=3 SV=1	28.337	10	8	9	8	957	104.3	19.86
A0A2Y4Y9J3	Pyruvate kinase OS=Shigella flexneri OX=623 GN=pykF PE=3 SV=1	25.499	15	9	9	9	542	58.7	19.77
A0A3T2UYA0	Carbamoyl-phosphate synthase large chain OS=Shigella flexneri OX=623 GN=carB PE=3 SV=1	30.296	10	9	9	9	1073	117.8	19.56
A0A380AZK5	D-amino acid dehydrogenase OS=Shigella flexneri OX=623 GN=dadA PE=3 SV=1	26.355	20	8	9	8	432	47.6	18.87
D2ADL5	Respiratory nitrate reductase I beta chain OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=narH PE=4 SV=1	20.92	13	7	9	7	512	58	18.66
A0A2S8DEF0	Chaperone SurA OS=Shigella dysenteriae OX=622 GN=surA PE=3 SV=1	25.866	16	6	9	6	428	47.3	15.46
A0A6N3RKA3	Succinate dehydrogenase iron-sulfur subunit OS=Shigella flexneri 1485-80 OX=766155 GN=sdhB PE=3 SV=1	20.185	35	9	9	9	238	26.8	13.15
A0A379ZH24	Glycine--tRNA ligase beta subunit OS=Shigella flexneri OX=623 GN=glyS PE=3 SV=1	15.396	9	6	9	6	689	76.7	8.78
A0A6N3QJ73	Fructose-specific PTS system IIBC component OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03096 PE=4 SV=1	34.495	13	6	8	6	563	57.5	27.58
A0A6N3R6K7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4998 PE=3 SV=1	29.477	9	7	8	7	402	42.7	25.37
A0A2S8DUZ3	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=Shigella boydii OX=621 GN=accA PE=3 SV=1	29.296	24	7	8	7	319	35.2	25.08
A0A2X2I0N0	Cell division protein DamX OS=Shigella flexneri OX=623 GN=damX PE=3 SV=1	46.408	32	8	8	8	428	46.1	24.82
A0A6N3RAZ3	Cell division protein FtsN OS=Shigella flexneri 1485-80 OX=766155 GN=ftsN PE=3 SV=1	29.69	30	8	8	8	284	31.8	22.92
D2AHG2	Autonomous glycol radical cofactor OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfiD PE=3 SV=1	33.349	51	6	8	6	127	14.2	21.67
A0A658Y983	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degQ PE=3 SV=1	26.692	20	8	8	8	455	47.2	20.27
A0A2S8DKK2	Ribonucleoside-diphosphate reductase OS=Shigella boydii OX=621 GN=nrdA PE=3 SV=1	18.911	10	8	8	8	761	85.7	15.19
Q0T4H3	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1619 PE=4 SV=1	19.273	22	7	8	7	314	33.9	13.47
A0A658XZL3	Cytochrome d terminal oxidase, polypeptide subunit I OS=Shigella flexneri OX=623 GN=cydA PE=3 SV=1	19.836	13	8	8	8	522	58.2	12.26
Q0T240	Outer membrane protein assembly factor BamC OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nlpB PE=3 SV=1	30.24	28	7	7	7	344	36.8	23.41
F5POF1	Diguanylate cyclase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3908 PE=4 SV=1	28.115	11	6	7	6	602	68.4	21.3
A0A380B6V8	Copper exporting ATPase OS=Shigella flexneri OX=623 GN=copA PE=3 SV=1	26.422	9	7	7	7	834	87.9	20.49
A0A2S8DI03	Signal recognition particle-docking protein FtsY (Fragment) OS=Shigella boydii OX=621 GN=C5K23_31365 PE=3 SV=1	27.769	15	5	7	5	486	53.2	19.84
A0A6H1JG84	Phosphoenolpyruvate carboxylase OS=Shigella flexneri OX=623 GN=ppc PE=3 SV=1	24.434	9	7	7	7	883	99	19.82
F5NY87	Phosphoenolpyruvate-protein phosphotransferase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3048 PE=3 SV=1	28.436	14	7	7	7	585	64.5	19.6
A0A4V1CTY5	ATP-dependent Clp protease ATP-binding subunit ClpA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=clpA PE=3 SV=1	28.498	11	7	7	7	758	84.2	19.57
D2A8B5	Cytoplasmic membrane protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yrbD PE=4 SV=1	24.751	43	6	7	6	183	19.6	18.06
F5NX93	EIII-Fru OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2769 PE=4 SV=1	24.794	23	7	7	7	376	39.7	17.34
A0A4P7TNY5	Alkyl hydroperoxide reductase C OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=ahpC PE=3 SV=1	24.478	39	6	7	6	187	20.7	16.51
A0A3T2UUK6	Energy-dependent translational throttle protein EttA OS=Shigella flexneri OX=623 GN=yjjk PE=3 SV=1	22.307	13	6	7	6	555	62.4	16.14
I6CBQ2	DNA gyrase subunit B OS=Shigella flexneri K-315 OX=766150 GN=gyrB PE=3 SV=1	20.175	10	7	7	7	804	89.9	15.85
I6CVL0	Phenylalanine--tRNA ligase beta subunit OS=Shigella flexneri K-315 OX=766150 GN=pheT PE=3 SV=1	21.681	8	6	7	6	795	87.3	14.93
P0A6P4	Elongation factor Ts OS=Shigella flexneri OX=623 GN=tsf PE=3 SV=2	16.906	25	7	7	7	283	30.4	13.72
A0A6N3REQ7	Nickel-dependent hydrogenase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3604 PE=3 SV=1	16.345	10	6	7	6	567	62.5	13.56
A0A2S8D3U0	Catabolite activator protein OS=Shigella dysenteriae OX=622 GN=crp PE=4 SV=1	15.445	27	6	7	6	210	23.7	10.16
A0A3T2UZZ4	Glucans biosynthesis glucosyltransferase H OS=Shigella flexneri OX=623 GN=mdoH PE=3 SV=1	19.364	7	6	7	6	847	96.9	10.15
I6CDH9	DNA polymerase I OS=Shigella flexneri K-315 OX=766150 GN=polA PE=3 SV=1	16.648	11	5	7	5	605	68.1	7.64
I6CE82	Fumarate hydratase class I OS=Shigella flexneri K-315 OX=766150 GN=fumB PE=3 SV=1	13.716	9	5	7	3	548	60.1	6.24
A0A658YX10	Acriflavine resistance protein A OS=Shigella flexneri OX=623 GN=acrA PE=3 SV=1	29.964	20	6	6	6	397	42.1	22.06
Q0T239	4-hydroxy-tetrahydrodipicolinate synthase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dapA PE=3 SV=1	23.128	23	5	6	5	292	31.3	18.26
A0A6N3QN50	Cysteine synthase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02637 PE=3 SV=1	28.989	29	6	6	6	323	34.5	18.2
I6D632	ATP-dependent Clp protease ATP-binding subunit ClpX OS=Shigella flexneri K-315 OX=766150 GN=clpX PE=3 SV=1	22.973	13	5	6	5	424	46.4	16.58
A0A6N3RBT1	GTP-binding protein TypA/BipA OS=Shigella flexneri 1485-80 OX=766155 GN=typA PE=4 SV=1	26.32	11	6	6	6	607	67.4	16.08

Q0T8M9	Serine--tRNA ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=serS PE=3 SV=1	23.503	17	6	6	6	430	48.4	16.04
A0A4P7TSC3	3-octaprenyl-4-hydroxybenzoate carboxy-lyase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=ubiD PE=3 SV=1	20.568	11	5	6	5	497	55.6	14.64
A0A2Y4ZTN4	Xaa-Pro dipeptidase OS=Shigella flexneri OX=623 GN=pepQ PE=3 SV=1	18.225	14	6	6	6	443	50.2	14.56
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	22.235	22	6	6	6	329	36.5	14.2
A0A658ZZJ6	Aminopeptidase N OS=Shigella flexneri OX=623 GN=pepN PE=3 SV=1	16.021	7	6	6	6	870	98.9	14.13
I6D6H9	Transaldolase OS=Shigella flexneri K-315 OX=766150 GN=tal PE=3 SV=1	20.17	21	6	6	6	294	32.8	14.11
A0A658XY46	Glucose dehydrogenase OS=Shigella flexneri OX=623 GN=gcd PE=3 SV=1	21.268	9	6	6	6	796	86.7	13.69
A0A658YX45	L-arabinose-binding periplasmic protein OS=Shigella flexneri OX=623 GN=araF PE=3 SV=1	15.907	21	6	6	6	329	35.5	13.68
A0A2S8DA81	Succinate--CoA ligase [ADP-forming] subunit beta OS=Shigella dysenteriae OX=622 GN=sucC PE=3 SV=1	17.158	15	6	6	6	388	41.4	13.6
A0A6L6V006	Ribose-phosphate pyrophosphokinase OS=Shigella flexneri OX=623 GN=prs PE=3 SV=1	14.043	16	5	6	5	315	34.2	13.55
A0A6N3RAY3	50S ribosomal protein L15 OS=Shigella flexneri 1485-80 OX=766155 GN=rlpO PE=3 SV=1	13.656	39	4	6	4	126	13.1	13.36
D2AAJ1	2,3-bisphosphoglycerate-independent phosphoglycerate mutase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=gpmM PE=3 SV=1	14.915	8	4	6	4	548	60.1	12.72
A0A1S9JMU3	Cell shape-determining protein MreB OS=Shigella boydii OX=621 GN=mreB PE=3 SV=1	19.327	15	4	6	4	347	36.9	12.39
A0A658YT81	Penicillin-binding protein 2 OS=Shigella flexneri OX=623 GN=mrdA PE=4 SV=1	13.917	9	6	6	6	633	70.8	12.39
A0A3U1D5H5	Alpha-D-glucose phosphate-specific phosphoglucomutase OS=Shigella flexneri OX=623 GN=pgm PE=3 SV=1	17.778	8	4	6	4	546	58.3	12.25
Q0T2Q0	DNA gyrase subunit A OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=gyrA PE=3 SV=1	20.641	7	6	6	6	875	96.9	11.7
F5NRY2	Leucine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=leuS PE=3 SV=1	15.745	8	6	6	6	860	97.2	10.22
A0A0H2VYI3	Nucleoside permease OS=Shigella flexneri OX=623 GN=nupC PE=3 SV=1	13.707	11	5	6	5	400	43.5	9.54
I6CI73	Elongation factor 4 OS=Shigella flexneri K-315 OX=766150 GN=lepA PE=3 SV=1	13.397	8	5	6	5	599	66.6	9.37
A0A3T2UZJ5	Protease 4 OS=Shigella flexneri OX=623 GN=DK174_18290 PE=3 SV=1	15.011	8	6	6	6	618	67.2	8.09
A0A2Y4XRM3	CTP synthase OS=Shigella flexneri OX=623 GN=pyrG PE=3 SV=1	12.586	10	6	6	6	545	60.4	7.03
A0A4P7TTH9	30S ribosomal protein S2 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsB PE=3 SV=1	32.213	20	4	5	4	241	26.8	20.15
A0A658Y016	Glucan biosynthesis protein G OS=Shigella flexneri OX=623 GN=mdoG PE=3 SV=1	22.607	14	5	5	5	500	56.6	15.86
I6CHF3	MFS transporter, sugar porter family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3768 PE=3 SV=1	11.904	7	3	5	3	472	51.7	14.57
A0A2S8DV96	50S ribosomal protein L5 OS=Shigella boydii OX=621 GN=rlpE PE=3 SV=1	23.568	29	5	5	5	179	20.3	14.33
Q2EUU0	AccD (Fragment) OS=Shigella flexneri OX=623 GN=accD PE=3 SV=1	19.361	20	5	5	5	296	32.6	14.31
A0A3T2UY26	Beta-glucosidase BglX OS=Shigella flexneri OX=623 GN=DK174_15745 PE=3 SV=1	22.01	7	5	5	5	765	83.5	14.3
Q83MF6	Cell division protein FtsZ OS=Shigella flexneri OX=623 GN=ftsZ PE=3 SV=2	21.636	15	5	5	5	383	40.1	14.06
A0A4P7TP07	Tol-Pal system protein TolB OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tolB PE=3 SV=1	17.285	11	4	5	4	430	45.9	13.49
A0A380A6R4	3-oxoacyl-ACP synthase OS=Shigella flexneri OX=623 GN=fabB PE=3 SV=1	16.232	10	5	5	5	406	42.7	12.71
D2AGX5	Cysteine desulfurase IscS OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=iscS PE=3 SV=1	13.615	11	5	5	5	412	46	12.33
A0A383K220	Valine--tRNA ligase OS=Shigella flexneri OX=623 GN=valS PE=3 SV=1	16.916	5	4	5	4	951	108.1	12.05
A0A2S8DNR9	Methionine--tRNA ligase OS=Shigella boydii OX=621 GN=metG PE=3 SV=1	16.661	8	5	5	5	677	76.2	11.08
F5P2R0	Oligopeptidase A OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4802 PE=3 SV=1	12.27	6	4	5	4	651	73.9	10.68
A0A379ZSJ2	Alcohol dehydrogenase OS=Shigella flexneri OX=623 GN=yqhD PE=4 SV=1	15.184	7	3	5	3	387	42.1	10.67
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	13.273	12	4	5	4	376	41	10.23
A0A2Y4XMF0	Sec-independent protein translocase protein TatA OS=Shigella flexneri OX=623 GN=tatA PE=3 SV=1	18.058	49	4	5	4	89	9.7	10.16
A0A6N3RJH6	Sulfatase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_1839 PE=3 SV=1	9.288	7	4	5	4	539	60.7	10.11
A0A6N3R4Q2	50S ribosomal protein L6 OS=Shigella flexneri 1485-80 OX=766155 GN=rlpF PE=3 SV=1	13.76	28	4	5	4	177	18.9	10.04
I6CBM2	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS=Shigella flexneri K-315 OX=766150 GN=glmS PE=3 SV=1	8.152	7	3	5	3	609	66.9	9.54
D2A9I7	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	15.83	27	5	5	5	124	13.7	9.47
D2A6V6	6-phosphogluconate dehydrogenase, decarboxylating OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=gnd PE=3 SV=1	12.124	11	5	5	5	468	51.3	9.45
I6CX73	Cell division inhibitor MinD OS=Shigella flexneri K-315 OX=766150 GN=minD PE=3 SV=1	9.98	17	4	5	4	235	26.3	9.44
I6D3P2	Tol-Pal system protein TolR OS=Shigella flexneri K-315 OX=766150 GN=tolR PE=3 SV=1	11.773	17	2	5	2	142	15.4	8.99
Q0T0X8	Putative actin OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yggE PE=4 SV=1	12.031	26	5	5	5	235	25.4	8.94
Q0T6B3	Aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=aspC PE=3 SV=1	9.761	10	4	5	4	396	43.6	8.29
I6C9W2	30S ribosomal protein S10 OS=Shigella flexneri K-315 OX=766150 GN=rpsJ PE=3 SV=1	11.273	39	4	5	4	103	11.7	7.06
A0A0H2VZP5	RNA polymerase sigma factor RpoD OS=Shigella flexneri OX=623 GN=rpoD PE=3 SV=1	7.427	6	4	5	4	608	69.6	4.12
A0A6N3RGK2	FAD linked oxidase domain protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_1959 PE=4 SV=1	9.67	4	5	5	5	1018	113.2	3.99



A0A2S8DKX6	Insulinase family protein OS=Shigella boydii OX=621 GN=yhjJ PE=3 SV=1	23.098	12	4	4	4	4	498	55.4	15.33
F5P3L0	Fumarate reductase iron-sulfur subunit OS=Shigella flexneri K-227 OX=766147 GN=SFK227_5105 PE=3 SV=1	17.882	15	3	4	3	3	244	27	13.11
F5P2D1	Rhodanese-like domain protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4672 PE=4 SV=1	19.48	37	4	4	4	4	143	15.6	12.75
A0A2S8DA55	SH3 domain-containing protein OS=Shigella dysenteriae OX=622 GN=ygim PE=4 SV=1	15.547	20	4	4	4	4	206	23.1	12.73
I6CAV2	Cell division protein FtsX OS=Shigella flexneri K-315 OX=766150 GN=ftsX PE=3 SV=1	18.576	12	3	4	3	3	352	38.5	12.51
A0A2S8DDU2	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OS=Shigella dysenteriae OX=622 GN=murG PE=3 SV=1	16.747	14	4	4	4	4	355	37.8	12.33
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	16.164	25	3	4	3	3	129	13.8	12.17
A0A6N3QME5	50S ribosomal protein L1 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rplA PE=3 SV=1	15.544	21	4	4	4	4	234	24.7	11.45
A0A2S8DUL5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase OS=Shigella boydii OX=621 GN=dapD PE=3 SV=1	16.895	16	4	4	4	4	274	29.9	11.19
Q0T2K3	NADH-quinone oxidoreductase subunit F OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nuoF PE=3 SV=1	9.385	8	3	4	3	3	445	49.4	9.9
A0A2Y4XZK1	Citrate synthase OS=Shigella flexneri OX=623 GN=glta PE=3 SV=1	10.105	9	4	4	4	4	427	48	9.84
D2ACQ6	Sulfatase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yijP PE=4 SV=1	14.124	8	4	4	4	4	577	66.5	9.28
A0A6H1JH06	30S ribosomal protein S5 OS=Shigella flexneri OX=623 GN=rpsE PE=3 SV=1	10.332	22	3	4	3	3	167	17.6	9.08
A0A658YV21	Auxiliary transport protein, membrane fusion protein (MFP) family OS=Shigella flexneri OX=623 GN=macA_2 PE=4 SV=1	8.246	10	4	4	4	4	355	38.7	9.08
A0A380B3G3	Asparagine--tRNA ligase OS=Shigella flexneri OX=623 GN=asnC_2 PE=3 SV=1	10.162	9	4	4	4	4	466	52.6	9.08
Q83IY3	Bifunctional protein GlmU OS=Shigella flexneri OX=623 GN=glmU PE=3 SV=1	10.271	9	4	4	4	4	456	49.2	8.94
A0A2Y5I422	Diguanylate cyclase OS=Shigella flexneri OX=623 GN=yfgF_2 PE=4 SV=1	11.615	8	4	4	4	4	505	57.7	8.8
F5NST7	Paraquat-inducible protein B OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1183 PE=4 SV=1	10.675	9	4	4	4	4	527	58.3	8.58
A0A2S8DCL9	Adenylosuccinate lyase OS=Shigella dysenteriae OX=622 GN=purB PE=3 SV=1	11.719	9	4	4	4	4	456	51.6	8.47
A0A2S4N348	Low-affinity transport protein proline permease II OS=Shigella flexneri OX=623 GN=proP PE=3 SV=1	7.95	6	4	4	4	4	500	54.9	7.61
A0A6N3QLC2	Universal stress protein G OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02439 PE=3 SV=1	5.399	14	2	4	2	2	142	15.9	7.53
A0A3T2UX48	Outer membrane protein F OS=Shigella flexneri OX=623 GN=ompF PE=3 SV=1	11.949	9	3	4	3	3	362	39.3	7.42
F5NRS1	Oxygen-insensitive NAD(P)H nitroreductase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0809 PE=3 SV=1	12.221	10	3	4	3	3	217	23.8	7.3
A0A4P7TKC9	NADP-dependent oxaloacetate-decarboxylating malate dehydrogenase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_003280 PE=3 SV=1	9.445	4	4	4	4	4	759	82.3	7.18
A0A379ZPY0	Aerobic respiration control sensor protein OS=Shigella flexneri OX=623 GN=arcB PE=4 SV=1	15.754	6	4	4	4	4	778	87.9	7.09
A0A6G5Z3B5	Biotin carboxylase OS=Shigella flexneri OX=623 GN=accC PE=4 SV=1	9.103	8	4	4	4	4	449	49.3	6.14
A0A2Y4YFW7	Adenylate kinase OS=Shigella flexneri OX=623 GN=adk PE=3 SV=1	8.225	16	4	4	4	4	234	25.8	6.05
I6CXI2	Putative protease sohB OS=Shigella flexneri K-315 OX=766150 GN=sohB PE=3 SV=1	10.571	11	4	4	4	4	349	39.4	6.04
Q0T6G6	UPF0194 membrane protein YbhG OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ybhG PE=3 SV=1	11.253	12	4	4	4	4	331	36.3	6.03
F5NZF4	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3556 PE=3 SV=1	7.829	6	4	4	4	4	493	53.7	5.75
F5POM6	10 kDa chaperonin OS=Shigella flexneri K-227 OX=766147 GN=groS PE=3 SV=1	9.893	48	4	4	4	4	97	10.4	5.7
A0A6H1JLX0	AsmA2 domain-containing protein OS=Shigella flexneri OX=623 GN=FOC24_08420 PE=4 SV=1	12.452	4	4	4	4	4	1266	139	5.33
A0A6N3QQ54	Histidine kinase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01033 PE=4 SV=1	11.51	7	3	4	3	3	450	50.3	5.25
P59107	Exoribonuclease 2 OS=Shigella flexneri OX=623 GN=mb PE=3 SV=1	10.277	6	4	4	4	4	644	72.4	4.63
Q0T5P3	Sensor histidine protein kinase/phosphatase PhoQ OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=phoQ PE=4 SV=1	8.267	5	3	4	3	3	486	55.3	4.38
A0A2Y4Y1C7	Fumarate hydratase class I OS=Shigella flexneri OX=623 GN=fumA PE=3 SV=1	7.96	6	3	4	1	1	548	60.3	4.23
A0A3T6J5Y5	Involved in lipopolysaccharide biosynthesis OS=Shigella flexneri OX=623 GN=htrL PE=4 SV=1	6.249	12	4	4	4	4	285	33.5	3.69
D2ADW1	Putative transport protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yegH PE=3 SV=1	7.705	6	4	4	4	4	549	62.1	3.64
A0A3T2UTU6	Sensor protein OS=Shigella flexneri OX=623 GN=DK174_07305 PE=4 SV=1	6.504	5	3	4	3	3	598	67.1	3.51
A0A2A2XF62	Cell division ATP-binding protein FtsE OS=Shigella flexneri OX=623 GN=ftsE PE=3 SV=1	7.407	17	4	4	4	4	222	24.4	3.43
F5P0R0	Maltodextrin-binding protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4033 PE=3 SV=1	9.707	13	4	4	4	4	396	43.4	2.73
A0A380B7T8	UvrABC system protein B OS=Shigella flexneri OX=623 GN=uvrB PE=3 SV=1	6.073	6	4	4	4	4	673	76.2	1.73
A0A2S8DD79	Threonine/serine transporter TdcC OS=Shigella dysenteriae OX=622 GN=tdcC PE=3 SV=1	13.067	9	3	3	3	3	443	48.9	12.32
P69785	PTS system glucose-specific EIIA component OS=Shigella flexneri OX=623 GN=crr PE=3 SV=2	12.602	20	2	3	2	2	169	18.2	11.29
I6D602	Protein-export membrane protein SecF OS=Shigella flexneri K-315 OX=766150 GN=secF PE=3 SV=1	16.893	17	3	3	3	3	304	33.2	10.85
F5P2W0	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4852 PE=4 SV=1	16.212	23	3	3	3	3	185	19.8	10.42
F5P004	L-asparaginase 2 OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3757 PE=3 SV=1	18.943	16	3	3	3	3	235	25.2	9.86
A0A1Q8MQJ7	Protein RecA OS=Shigella boydii OX=621 GN=recA PE=3 SV=1	10.543	10	3	3	3	3	353	38	9.45
F5NZV9	Mechanosensitive ion channel family protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3712 PE=3 SV=1	13.358	13	3	3	3	3	286	30.9	9.09

F5P316	Fe/S biogenesis protein NfuA OS=Shigella flexneri K-227 OX=766147 GN=nfuA PE=3 SV=1	8.309	12	2	3	2	191	21	8.74
A0A1Q8M8J0	50S ribosomal protein L7/L12 OS=Shigella boydii OX=621 GN=rpL PE=3 SV=1	8.181	11	2	3	2	121	12.3	8.64
A0A6N3RAZ0	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4958 PE=3 SV=1	10.578	7	3	3	3	428	47.7	8.63
A0A2Y4XSH5	Periplasmic protein of mal regulon OS=Shigella flexneri OX=623 GN=malM PE=4 SV=1	8.11	10	3	3	3	306	31.9	8.57
A0A6N3RBI6	Protein translocase subunit SecY OS=Shigella flexneri 1485-80 OX=766155 GN=prlA PE=3 SV=1	7.299	7	3	3	3	443	48.5	8.51
F5NPS2	Lipoprotein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0205 PE=3 SV=1	10.314	13	3	3	3	271	29.4	8.24
F5NZ29	Serine transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3429 PE=4 SV=1	10.595	7	3	3	3	429	46.9	8.24
F5NPR9	Proline--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=proS PE=3 SV=1	12.437	6	3	3	3	572	63.6	8.15
A0A6N3QJK5	AsmA protein OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04099 PE=4 SV=1	10.552	6	3	3	3	617	68.9	7.95
Q0T2P1	sn-glycerol-3-phosphate permease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=glpT PE=3 SV=1	8.53	6	3	3	3	452	50.4	7.79
A0A658Z2B7	GDP/GTP pyrophosphokinase OS=Shigella flexneri OX=623 GN=relA PE=3 SV=1	8.957	4	3	3	3	744	83.8	7.75
A0A658Z233	Inner membrane protein yjgQ OS=Shigella flexneri OX=623 GN=yjgQ PE=3 SV=1	5.94	6	2	3	2	360	39.6	7.73
A0A2S8DCH3	Lipid ABC transporter permease/ATP-binding protein (Fragment) OS=Shigella dysenteriae OX=622 GN=C5K18_12120 PE=4 SV=1	7.186	5	3	3	3	573	63.4	7.72
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	7.764	14	3	3	3	206	23.4	7.69
A0A4P7TVF5	Cyclic di-GMP-binding protein OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=bcsB PE=3 SV=1	8.76	4	3	3	3	779	86.1	7.58
A0A383JTR9	Cardiolipin synthase A OS=Shigella flexneri OX=623 GN=cls PE=3 SV=1	10.877	7	3	3	3	486	54.8	7.51
A0A2S8DRB8	Glucose-6-phosphate 1-dehydrogenase OS=Shigella boydii OX=621 GN=zwf PE=3 SV=1	7.984	6	3	3	3	491	55.7	7.28
A0A383JTM9	Periplasmic glutamine-binding protein permease OS=Shigella flexneri OX=623 GN=fliY PE=3 SV=1	9.239	10	3	3	3	285	31	7.25
Q83PS4	C4-dicarboxylate transport protein OS=Shigella flexneri OX=623 GN=dctA PE=3 SV=1	9.52	6	3	3	3	428	45.4	7.21
A0A6N3R6J7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4987 PE=3 SV=1	8.64	4	2	3	2	446	47.8	7.06
I6CGI0	Bifunctional glutathionylspermidine synthetase/amidase OS=Shigella flexneri K-315 OX=766150 GN=gsp PE=3 SV=1	9.19	5	3	3	3	619	70.5	6.81
A0A3T2V110	Aspartate--tRNA ligase OS=Shigella flexneri OX=623 GN=aspS PE=3 SV=1	7.562	6	3	3	3	590	65.8	6.75
Q0T0X5	Fructose-bisphosphate aldolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fba PE=3 SV=1	6.407	4	2	3	2	387	42.7	6.64
A0A4V1CUG6	Transcription termination/antitermination protein NusA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=nusA PE=3 SV=1	4.146	5	2	3	2	495	54.8	6.55
A0A3T2UQA1	Glutamate-1-semialdehyde 2,1-aminomutase OS=Shigella flexneri OX=623 GN=hemL PE=3 SV=1	8.725	9	3	3	3	426	45.3	6.36
A0A2S8DI33	Sec translocon accessory complex subunit YajC OS=Shigella boydii OX=621 GN=yajC PE=3 SV=1	7.425	26	3	3	3	110	11.9	6.3
A0A3T2UY84	Cyclopropane-fatty-acyl-phospholipid synthase OS=Shigella flexneri OX=623 GN=DK174_15880 PE=3 SV=1	8.821	8	3	3	3	382	43.9	6.28
A0A658Z505	Transporter, small conductance mechanosensitive ion channel (MscS) family OS=Shigella flexneri OX=623 GN=mscM PE=3 SV=1	8.48	7	3	3	3	415	46.6	6.26
A0A3T3S7B2	ParB/RepB/Spo0J family partition protein OS=Shigella flexneri OX=623 GN=sopB_2 PE=3 SV=1	10.936	10	3	3	3	326	36.8	6.08
I6CXR3	Universal stress protein E OS=Shigella flexneri K-315 OX=766150 GN=uspE PE=4 SV=1	6.097	7	3	3	3	314	35.5	6.05
I6D405	PhoH-like protein OS=Shigella flexneri K-315 OX=766150 GN=ybeZ PE=3 SV=1	7.814	10	3	3	3	346	39	5.93
A0A658YRM6	Peptidase, M48 family OS=Shigella flexneri OX=623 GN=SAMEA3710514_00010 PE=4 SV=1	9.556	6	3	3	3	487	53.9	5.67
I6CVA3	L-serine dehydratase OS=Shigella flexneri K-315 OX=766150 GN=SFK315_1603 PE=3 SV=1	6.996	6	3	3	3	432	46.6	5.66
A0A6N3RBM8	Diguanylate cyclase OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4553 PE=4 SV=1	7.946	5	3	3	3	633	71.2	5.58
Q0SZA6	ATP-dependent 6-phosphofructokinase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pfkA PE=3 SV=1	4.723	5	2	3	2	340	37.1	5.49
F5P1H3	Uridine phosphorylase OS=Shigella flexneri K-227 OX=766147 GN=udp PE=3 SV=1	6.99	11	3	3	3	253	27.2	5.37
A0A1S9KGS8	Phosphatidylserine decarboxylase proenzym OS=Shigella dysenteriae OX=622 GN=psd PE=3 SV=1	10.572	9	3	3	3	322	35.9	5.06
A0A4P7TQT3	50S ribosomal protein L10 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpLJ PE=3 SV=1	8.831	19	3	3	3	165	17.7	5.04
A0A658Z1V1	UPF0283 membrane protein YcjF OS=Shigella flexneri OX=623 GN=yjF PE=3 SV=1	8.651	9	3	3	3	353	39.4	5.02
I6CXD2	UTP--glucose-1-phosphate uridylyltransferase OS=Shigella flexneri K-315 OX=766150 GN=galU PE=3 SV=1	6.759	8	2	3	2	302	33	4.96
I6D059	Dihydroorotate dehydrogenase (quinone) OS=Shigella flexneri K-315 OX=766150 GN=pyrD PE=3 SV=1	9.395	8	3	3	3	336	36.8	4.86
Q0T5J2	Ribosome-binding ATPase YchF OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ychF PE=3 SV=1	7.919	8	3	3	3	363	39.6	4.86
F5P300	Glucose-1-phosphate adenyltransferase OS=Shigella flexneri K-227 OX=766147 GN=glgC PE=3 SV=1	6.871	7	3	3	3	431	48.7	4.81
I6CBY6	Lipopolysaccharide heptosyltransferase II OS=Shigella flexneri K-315 OX=766150 GN=rfaF PE=4 SV=1	3.468	5	2	3	2	348	39	4.77
I6CH97	AMP nucleosidase OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3709 PE=4 SV=1	7.071	7	3	3	3	444	49.9	4.63
A0A383JXY5	NAD dependent epimerase/dehydratase family OS=Shigella flexneri OX=623 GN=DOD76_09530 PE=4 SV=1	8.12	9	3	3	3	337	38	4.57
A0A6N3R8Q1	Mechanosensitive ion channel family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_5021 PE=3 SV=1	5.105	2	3	3	3	1107	123.9	4.53
A0A2S8DRX8	Sulfate ABC transporter substrate-binding protein OS=Shigella boydii OX=621 GN=cysP PE=3 SV=1	6.299	8	3	3	3	338	37.7	4.26
A0A3T2UTS0	Arginine ABC transporter substrate-binding protein OS=Shigella flexneri OX=623 GN=DK174_07240 PE=3 SV=1	7.303	10	3	3	3	243	26.9	4.22
F5P3D3	50S ribosomal protein L17 OS=Shigella flexneri K-227 OX=766147 GN=rpLQ PE=3 SV=1	4.323	13	2	3	2	127	14.4	4.12

A0A2S8DBS7	Glycosyltransferase family 1 protein OS=Shigella dysenteriae OX=622 GN=mshA PE=4 SV=1	5.969	7	3	3	3	362	40.6	4.06
D2AHT6	Co-chaperone protein DjlA OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=djlA PE=3 SV=1	4.149	8	3	3	3	271	30.5	2.28
A0A2Y5AKI5	Ribonuclease R OS=Shigella flexneri OX=623 GN=rnr PE=3 SV=1	5.1	3	3	3	3	813	92	2.18
A0A383JQI3	3-oxoacyl-[acyl-carrier-protein] reductase OS=Shigella flexneri OX=623 GN=fabG_1 PE=3 SV=1	3.734	7	2	3	2	244	25.5	2.04
Q0T3A5	Histidine biosynthesis bifunctional protein HisB OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hisB PE=3 SV=1	3.715	4	2	3	2	355	40.1	2.03
A0A2S8E9Y4	Acid-resistance membrane protein OS=Shigella dysenteriae OX=622 GN=hdeD_2 PE=4 SV=1	4.649	7	3	3	3	190	20.9	0
Q0SZA9	Triosephosphate isomerase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tpiA PE=3 SV=1	14.373	16	2	2	2	255	27	9.1
D2AGV7	TPR_21 domain-containing protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfgM PE=3 SV=1	13.542	16	2	2	2	206	22.1	8.03
A0A1Q8N7Q2	50S ribosomal protein L11 OS=Shigella dysenteriae OX=622 GN=rplK PE=3 SV=1	9.05	17	2	2	2	142	14.9	6.52
A0A6D2W4F9	Chaperone protein Skp OS=Shigella flexneri OX=623 GN=skp PE=3 SV=1	8.209	14	2	2	2	161	17.7	6.43
Q7UCQ9	Lipopolysaccharide assembly protein B OS=Shigella flexneri OX=623 GN=yciM PE=3 SV=1	7.178	5	2	2	2	389	44.4	6.06
A0A2Y4YUV3	Peptidyl-prolyl cis-trans isomerase OS=Shigella flexneri OX=623 GN=fklB PE=3 SV=1	8.737	12	2	2	2	206	22.2	6.05
A0A2S8DN4	RNA-binding transcriptional accessory protein (Fragment) OS=Shigella boydii OX=621 GN=C5K23_19395 PE=4 SV=1	9.829	8	2	2	2	304	34.4	5.81
A0A3Y2RM52	Phosphoglucosamine mutase OS=Shigella flexneri OX=623 GN=glmM PE=3 SV=1	9.013	6	2	2	2	445	47.5	5.6
A0A2S8DD51	Cell division protein ZipA OS=Shigella dysenteriae OX=622 GN=zipA PE=3 SV=1	5.044	3	1	2	1	328	36.5	5.57
A0A6N3QM36	Protein QmcA OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01963 PE=3 SV=1	6.952	7	2	2	2	305	33.7	5.43
F5NV08	Tyrosine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=tyrS PE=3 SV=1	4.18	3	1	2	1	424	47.6	5.42
A0A0H2VZX0	Thiol:disulfide interchange protein DsbD OS=Shigella flexneri OX=623 GN=dsbD PE=3 SV=1	8.118	4	2	2	2	565	61.8	5.37
A0A6N3RIF3	Enoyl-[acyl-carrier-protein] reductase [NADH] OS=Shigella flexneri 1485-80 OX=766155 GN=fabI PE=3 SV=1	6.628	8	2	2	2	262	27.8	5.33
A0A658XZH7	Oxidoreductase OS=Shigella flexneri OX=623 GN=NCTC8524_01188 PE=4 SV=1	8.034	3	2	2	2	804	89.3	5.3
F5NVZ8	Outer membrane porin protein LC OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2311 PE=3 SV=1	7.858	6	2	2	2	360	39.7	5.29
Q0SYE6	2-amino-3-ketobutyrate coenzyme A ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=kbl PE=3 SV=1	7.656	6	2	2	2	398	43.2	5.29
F5P2M7	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4769 PE=4 SV=1	7.455	13	2	2	2	236	25.6	5.27
Q83J96	Phosphoenolpyruvate carboxykinase (ATP) OS=Shigella flexneri OX=623 GN=pckA PE=3 SV=4	5.612	5	2	2	2	540	59.6	5.24
A0A380AZ11	DNA topoisomerase 1 OS=Shigella flexneri OX=623 GN=topA PE=3 SV=1	6.773	2	2	2	2	865	97.3	5.24
I6CB18	Transcriptional regulatory protein ompR OS=Shigella flexneri K-315 OX=766150 GN=ompR PE=4 SV=1	5.649	8	2	2	2	239	27.4	5.15
A0A3T3S2E7	Pyruvate kinase OS=Shigella flexneri OX=623 GN=pyk PE=3 SV=1	7.477	4	2	2	2	480	51.3	5.05
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	5.939	24	2	2	2	78	8.3	5.01
A0A6N3QSN2	DNA helicase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02367 PE=3 SV=1	3.859	2	1	2	1	720	82	4.9
I6CI93	Serine hydroxymethyltransferase OS=Shigella flexneri K-315 OX=766150 GN=glyA PE=3 SV=1	4.717	2	2	2	2	417	45.3	4.87
A0A6N3RQG4	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydD OS=Shigella flexneri 1485-80 OX=766155 GN=cydD PE=4 SV=1	3.649	3	2	2	2	588	65	4.82
A0A6N3QN40	Glutamate--tRNA ligase OS=Shigella flexneri CDC 796-83 OX=945360 GN=glT PE=3 SV=1	7.033	4	2	2	2	471	53.8	4.79
Q2EW05	Galactokinase OS=Shigella flexneri OX=623 GN=galK PE=3 SV=1	7.411	7	2	2	2	382	41.4	4.78
A0A6G5Z350	Serine-type D-Ala-D-Ala carboxypeptidase OS=Shigella flexneri OX=623 GN=dacB PE=3 SV=1	4.79	5	2	2	2	477	51.8	4.77
A0A6N3R9B1	PTS system, trehalose-specific IIBC component OS=Shigella flexneri 1485-80 OX=766155 GN=treP PE=4 SV=1	7.253	5	2	2	2	472	51.1	4.74
A0A6N3R6Z9	Lipopolysaccharide export system permease protein LptF OS=Shigella flexneri 1485-80 OX=766155 GN=yjgP PE=3 SV=1	7.876	7	2	2	2	335	36.6	4.73
F5NQ21	Cell division protein FtsQ OS=Shigella flexneri K-227 OX=766147 GN=ftsQ PE=3 SV=1	5.405	9	2	2	2	276	31.4	4.63
A0A3U1DEI9	Electron transport complex subunit RxC (Fragment) OS=Shigella flexneri OX=623 GN=DOD76_18490 PE=3 SV=1	5.636	4	2	2	2	565	61.3	4.56
Q0SZ45	Thiol:disulfide interchange protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dsbA PE=3 SV=1	3.979	8	2	2	2	208	23.1	4.52
F5P3C9	30S ribosomal protein S13 OS=Shigella flexneri K-227 OX=766147 GN=rpsM PE=3 SV=1	4.465	16	2	2	2	118	13.1	4.48
A0A2S8DFJ4	Chaperone protein HscA OS=Shigella dysenteriae OX=622 GN=hscA PE=3 SV=1	4.811	3	2	2	2	616	65.7	4.47
A0A6N3RKR4	Iron-sulfur cluster binding protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0392 PE=4 SV=1	4.564	3	2	2	2	475	53	4.44
A0A2S8DXT0	High frequency lysogenization protein HflD homolog OS=Shigella boydii OX=621 GN=hflD PE=3 SV=1	4.793	9	2	2	2	213	22.9	4.37
F5NQK6	Glutamine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=glN PE=3 SV=1	4.908	4	2	2	2	554	63.4	4.36
A0A2Y4XSL4	Peptidyl-prolyl cis-trans isomerase OS=Shigella flexneri OX=623 GN=ppiB PE=3 SV=1	4.884	7	1	2	1	164	18.2	4.34
A0A380B6A5	BAX inhibitor (BI)-1/YccA family protein OS=Shigella flexneri OX=623 GN=ybhL_2 PE=3 SV=1	3.477	6	2	2	2	234	25.9	4.29
A0A2S8DWY7	Lysine--tRNA ligase OS=Shigella boydii OX=621 GN=lysS PE=3 SV=1	5.063	4	2	2	2	505	57.6	4.27
A0A1Q8NZW8	DNA-binding transcriptional regulator PhoP OS=Shigella dysenteriae OX=622 GN=phoP PE=4 SV=1	2.437	5	1	2	1	223	25.5	4.26
A0A658Y5D6	Arginyl-tRNA synthetase OS=Shigella flexneri OX=623 GN=argS PE=3 SV=1	4.898	4	2	2	2	577	64.7	4.26
A0A4P7TQF0	50S ribosomal protein L9 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rplI PE=3 SV=1	2.829	7	1	2	1	149	15.8	4.22

A0A6N3RE59	S-adenosylmethionine synthase OS=Shigella flexneri 1485-80 OX=766155 GN=metK PE=3 SV=1	4.503	5	2	2	2	384	41.9	4.18
Q6R2C3	Isocitrate dehydrogenase [NADP] (Fragment) OS=Shigella flexneri OX=623 GN=icd PE=3 SV=1	5.546	5	2	2	2	389	42.8	4.14
Q83Q93	Biosynthetic arginine decarboxylase OS=Shigella flexneri OX=623 GN=speA PE=3 SV=2	4.466	2	2	2	2	662	74.3	4.14
F5P090	50S ribosomal protein L21 OS=Shigella flexneri K-227 OX=766147 GN=rplU PE=3 SV=1	5.969	19	2	2	2	103	11.5	4.13
A0A6A9TSA3	Bifunctional protein HldE OS=Shigella flexneri OX=623 GN=hldE PE=3 SV=1	5.801	5	2	2	2	477	51	4.1
Q5IPI8	Heat shock protein (Fragment) OS=Shigella flexneri OX=623 GN=grpE PE=3 SV=1	8.481	13	2	2	2	158	17.6	4.06
I6D448	Alkyl hydroperoxide reductase subunit F OS=Shigella flexneri K-315 OX=766150 GN=ahpF PE=3 SV=1	4.436	3	2	2	2	521	56.1	4
A0A2S8DFP5	Cell division protein DedD OS=Shigella dysenteriae OX=622 GN=dedD PE=3 SV=1	5.928	10	2	2	2	220	22.9	3.98
A0A6N3RLZ1	Cytochrome d ubiquinol oxidase, subunit II OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0759 PE=3 SV=1	4.299	4	2	2	2	379	42.4	3.88
A0A2Y4XP64	Oligopeptide ABC transporter periplasmic oligopeptide-binding protein OS=Shigella flexneri OX=623 GN=oppA PE=4 SV=1	6.824	6	2	2	2	442	50.4	3.86
A0A4P7TRM2	Lipopolysaccharide N-acetylglucosaminyltransferase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_019395 PE=4 SV=1	4.583	5	2	2	2	380	43.5	3.71
I6C9X6	30S ribosomal protein S14 OS=Shigella flexneri K-315 OX=766150 GN=rpsN PE=3 SV=1	4.983	19	2	2	2	96	11	3.36
I6C9W8	50S ribosomal protein L22 OS=Shigella flexneri K-315 OX=766150 GN=rplV PE=3 SV=1	7.454	19	2	2	2	110	12.2	3.12
A0A2S8D3C6	S-ribosylhomocysteine lyase OS=Shigella dysenteriae OX=622 GN=luxS PE=3 SV=1	7.283	14	2	2	2	171	19.4	2.94
D2ACS5	Putative lipoprotein yceB OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yceB PE=4 SV=1	6.589	11	2	2	2	205	22.6	2.87
Q83Q07	30S ribosomal protein S9 OS=Shigella flexneri OX=623 GN=rpsI PE=3 SV=3	5.974	15	2	2	2	130	14.9	2.65
A0A2S8DU80	Agmatinase OS=Shigella boydii OX=621 GN=speB PE=3 SV=1	4.63	6	2	2	2	306	33.5	2.63
A0A6G5Z373	Sensor protein QseC OS=Shigella flexneri OX=623 GN=qseC PE=4 SV=1	5.03	4	2	2	2	449	50.3	2.6
A0A4P7TNZ8	Cell envelope integrity protein TolA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tolA PE=4 SV=1	3.55	5	2	2	2	392	40.3	2.49
A0A6N3QP90	Glucans biosynthesis protein D OS=Shigella flexneri CDC 796-83 OX=945360 GN=mdoD PE=3 SV=1	5.183	4	2	2	2	539	61.3	2.45
A0A4P7TP15	Cu(+)/Ag(+) efflux RND transporter permease subunit CusA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=cusA PE=3 SV=1	4.553	2	2	2	2	1059	115.8	2.43
A0A1W2MD82	Protease HtpX OS=Shigella flexneri OX=623 GN=htpX PE=3 SV=1	4.122	4	1	2	1	293	31.9	2.35
A0A4V1CUE4	Alpha-1,4 glucan phosphorylase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_021860 PE=3 SV=1	4.183	2	2	2	2	815	93.1	2.3
F5P2C5	L-threonine 3-dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=tdh PE=3 SV=1	3.592	6	2	2	2	341	37.3	2.25
I6C9X3	50S ribosomal protein L14 OS=Shigella flexneri K-315 OX=766150 GN=rplN PE=3 SV=1	1.941	11	2	2	2	117	12.8	2.13
Q0T7Q8	Aminoacyl-histidine dipeptidase (Peptidase D) OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pepD PE=4 SV=1	3.634	4	2	2	2	485	52.9	2.02
Q0SZ40	Protoporphyrin oxidase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hemG PE=3 SV=1	1.896	4	1	2	1	181	21.2	1.95
P0A6A2	Probable protein kinase UbiB OS=Shigella flexneri OX=623 GN=ubiB PE=3 SV=1	3.26	3	2	2	2	546	63.2	1.85
I6CG52	tRNA-modifying protein YgfZ OS=Shigella flexneri K-315 OX=766150 GN=ygfZ PE=3 SV=1	3.351	6	2	2	2	326	36.1	1.81
I6D6W3	Poly(A) polymerase I OS=Shigella flexneri K-315 OX=766150 GN=pcnB PE=3 SV=1	2.41	3	2	2	2	454	52.5	1.75
A0A6G5YYG3	Lipoprotein-releasing system ATP-binding protein LolD OS=Shigella flexneri OX=623 GN=lolD PE=3 SV=1	2.771	6	2	2	2	233	25.5	0
A0A6S8Z2S5	GMP synthase OS=Shigella flexneri OX=623 GN=guaA PE=4 SV=1	2.016	3	2	2	2	525	58.7	0
I6CJU1	Acetate kinase OS=Shigella flexneri K-315 OX=766150 GN=ackA PE=3 SV=1	11.512	6	1	1	1	400	43.3	5.39
I6CAR9	Universal stress protein OS=Shigella flexneri K-315 OX=766150 GN=uspA PE=3 SV=1	4.999	14	1	1	1	144	16.1	4.43
F5NXM7	NADH-quinone oxidoreductase subunit L OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2904 PE=3 SV=1	5.684	2	1	1	1	611	66.1	4.03
A0A6L6UVT0	Lipid A biosynthesis lauroyltransferase OS=Shigella flexneri OX=623 GN=lpXL PE=3 SV=1	5.439	4	1	1	1	306	35.4	3.89
D2A8T7	Cytochrome d ubiquinol oxidase subunit III OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yhcB PE=4 SV=1	3.286	8	1	1	1	134	15.2	3.69
A0A1Q8M4D3	30S ribosomal protein S18 OS=Shigella boydii OX=621 GN=rpsR PE=3 SV=1	6.603	24	1	1	1	75	9	3.62
A0A2S8DCN0	Lipoprotein-releasing ABC transporter permease subunit LolE OS=Shigella dysenteriae OX=622 GN=C5K18_10460 PE=3 SV=1	4.12	2	1	1	1	414	45.3	3.54
A0A2Y4XVQ2	Outer membrane protein assembly complex subunit YfgL OS=Shigella flexneri OX=623 GN=yfgL PE=4 SV=1	4.677	7	1	1	1	184	20.1	3.43
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=Igkc PE=1 SV=1	4.778	6	1	1	1	219	24.2	3.41
Q83J75	Putative ATP-binding component of a transport system OS=Shigella flexneri OX=623 GN=yhiH PE=4 SV=1	2.937	1	1	1	1	911	100.6	3.32
A0A2S8DKM1	Putrescine-binding periplasmic protein OS=Shigella boydii OX=621 GN=potD PE=3 SV=1	3.895	3	1	1	1	348	38.9	3.27
A0A379ZAE3	Dihydroxy-acid dehydratase OS=Shigella flexneri OX=623 GN=ilvD PE=3 SV=1	4.262	2	1	1	1	616	65.5	3.26
I6CGU4	Signal recognition particle protein OS=Shigella flexneri K-315 OX=766150 GN=fth PE=3 SV=1	6.795	3	1	1	1	453	49.8	3.25
I6CFV1	Putative stringent starvation protein A OS=Shigella flexneri K-315 OX=766150 GN=sspA PE=3 SV=1	4.267	7	1	1	1	212	24.3	3.22
D2AE64	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4630 PE=3 SV=1	4.552	9	1	1	1	141	15.2	3.2
Q0SZX6	30S ribosomal protein S7 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsG PE=3 SV=1	3.24	9	1	1	1	179	20	3.18
D2AG45	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfcZ PE=3 SV=1	5.224	13	1	1	1	102	11.3	3.1

A0A6N3RFX8	N-acetylmuramoyl-L-alanine amidase OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3202 PE=4 SV=1	4.078	2	1	1	1	1	417	45.6	2.97
Q0T4A8	Possible enzyme OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1686 PE=4 SV=1	3.476	3	1	1	1	1	418	42.9	2.91
A0A2S4MTA8	Lactate utilization protein C OS=Shigella flexneri OX=623 GN=ykgG PE=4 SV=1	2.811	4	1	1	1	1	231	25.2	2.87
I6CFU5	Peptidase Do OS=Shigella flexneri K-315 OX=766150 GN=degS PE=3 SV=1	3.618	7	1	1	1	1	355	37.6	2.84
A0A2S8DHN0	Ubiquinone/menaquinone biosynthesis C-methyltransferase UbiE OS=Shigella dysenteriae OX=622 GN=ubiE PE=3 SV=1	5.057	5	1	1	1	1	251	28.1	2.84
Q0SZX1	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yheO PE=4 SV=1	4.603	5	1	1	1	1	240	26.8	2.84
A0A383JXW9	UPF0145 protein YbjQ OS=Shigella flexneri OX=623 GN=ybjQ PE=3 SV=1	3.228	11	1	1	1	1	107	11.4	2.81
I6CIP8	Oxidoreductase ucpA OS=Shigella flexneri K-315 OX=766150 GN=ucpA PE=4 SV=1	2.439	5	1	1	1	1	263	27.8	2.77
I6D070	Endopeptidase La OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0989 PE=3 SV=1	4.258	2	1	1	1	1	586	65.9	2.72
A0A379ZX42	Glutamate-cysteine ligase OS=Shigella flexneri OX=623 GN=gshA PE=3 SV=1	3.576	2	1	1	1	1	518	58.3	2.69
D2ACJ8	Formate dehydrogenase iron-sulfur subunit OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=fdoH PE=4 SV=1	4.291	4	1	1	1	1	300	33.1	2.67
A0A6N3R9K0	Phosphotransferase system, EIIC family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_2851 PE=4 SV=1	3.335	3	1	1	1	1	345	36.5	2.67
A0A1Q8NKE7	Phosphoheptose isomerase OS=Shigella dysenteriae OX=622 GN=gmhA PE=3 SV=1	3.106	5	1	1	1	1	192	20.8	2.63
Q0T3Y0	Fumarate and nitrate reduction Regulatory protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fnr PE=4 SV=1	4.357	4	1	1	1	1	250	27.9	2.59
A0A658YSH2	Dehydrogenase OS=Shigella flexneri OX=623 GN=yrrF PE=3 SV=1	2.081	1	1	1	1	1	711	79.5	2.59
A0A658Z0B8	AsmA family protein OS=Shigella flexneri OX=623 GN=SAMEA3710514_03157 PE=4 SV=1	2.149	2	1	1	1	1	569	62.3	2.55
I6CMC4	Lysine-specific permease OS=Shigella flexneri K-315 OX=766150 GN=lysP PE=4 SV=1	3.46	2	1	1	1	1	489	53.5	2.54
A0A1Q8M6D1	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase OS=Shigella boydii OX=621 GN=gpmA PE=3 SV=1	4.38	5	1	1	1	1	250	28.5	2.53
A0A379ZL4	DNA mismatch repair protein MutS OS=Shigella flexneri OX=623 GN=mutS PE=3 SV=1	3.591	1	1	1	1	1	853	95.2	2.53
A0A2S8D7U4	Phosphoribosylaminoimidazole-succinocarboxamide synthase OS=Shigella dysenteriae OX=622 GN=purC PE=3 SV=1	2.338	5	1	1	1	1	237	27	2.47
F5P339	DD-transpeptidase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4931 PE=3 SV=1	2.65	1	1	1	1	1	843	92.6	2.42
A0A379Z3I1	(Fe-S)-binding protein OS=Shigella flexneri OX=623 GN=ykgE PE=4 SV=1	3.593	5	1	1	1	1	239	26.1	2.41
Q32SZ3	Acyl_transf_3 domain-containing protein OS=Shigella flexneri OX=623 PE=4 SV=1	2.797	3	1	1	1	1	349	40.1	2.4
A0A6N3QPX8	Prophage P4 integrase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03306 PE=4 SV=1	1.508	2	1	1	1	1	422	48.2	2.39
D2ABV0	ATP-dependent RNA helicase RhlB OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rhlB PE=3 SV=1	3.5	3	1	1	1	1	421	47.1	2.37
I6CXN4	Phage shock protein A OS=Shigella flexneri K-315 OX=766150 GN=pspA PE=3 SV=1	3.286	4	1	1	1	1	222	25.5	2.36
A0A2Y4XVQ3	Carbon starvation family protein OS=Shigella flexneri OX=623 GN=yjiY PE=4 SV=1	2.392	3	1	1	1	1	322	35	2.36
D2ADZ6	Glucose-6-phosphate isomerase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=pgi PE=3 SV=1	6.579	3	1	1	1	1	549	61.5	2.36
A0A2Y4MDE1	Bifunctional aspartokinase/homoserine dehydrogenase OS=Shigella flexneri OX=623 GN=thrA PE=3 SV=1	3.905	1	1	1	1	1	820	89	2.36
D2AH71	Universal stress protein F OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=uspF PE=3 SV=1	4.141	6	1	1	1	1	168	18.4	2.35
F5NT19	Transcription-repair-coupling factor OS=Shigella flexneri K-227 OX=766147 GN=mfd PE=3 SV=1	1.464	1	1	1	1	1	1148	129.9	2.35
A0A4P7TM74	30S ribosomal protein S21 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsU PE=3 SV=1	2.239	11	1	1	1	1	71	8.5	2.34
A0A237FIF1	UPF0234 protein YajQ OS=Shigella boydii OX=621 GN=yajQ PE=3 SV=1	1.65	11	1	1	1	1	163	18.3	2.34
F5P3W0	UDP-N-acetylmuramate-L-alanyl-gamma-D-glutamyl-meso-2,6-diaminoheptandioate ligase OS=Shigella flexneri K-227 OX=766147 GN=mpl PE=3 SV=1	2.562	2	1	1	1	1	457	49.9	2.34
A0A2Y5A0I3	Repressor protein for FtsI OS=Shigella flexneri OX=623 GN=sufI PE=4 SV=1	3.73	4	1	1	1	1	370	40.8	2.33
I6CAS1	Phosphate transporter OS=Shigella flexneri K-315 OX=766150 GN=pitA PE=3 SV=1	4.038	2	1	1	1	1	499	53.4	2.32
F5NS7	Thiol:disulfide interchange protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3680 PE=3 SV=1	2.243	6	1	1	1	1	236	25.6	2.3
I6CI71	Sigma-E factor regulatory protein rseB OS=Shigella flexneri K-315 OX=766150 GN=rseB PE=3 SV=1	2.37	4	1	1	1	1	318	35.7	2.29
A0A383K2I0	Lipopolysaccharide heptosyltransferase RfaC OS=Shigella flexneri OX=623 GN=rfaC PE=4 SV=1	2.716	3	1	1	1	1	330	36.7	2.29
A0A2S8DJM8	Na+/H+ antiporter OS=Shigella boydii OX=621 GN=C5K23_27965 PE=3 SV=1	4.342	4	1	1	1	1	517	56.9	2.28
A0A6N3QGJ9	UPF0301 protein YggE OS=Shigella flexneri CDC 796-83 OX=945360 GN=yggE PE=3 SV=1	4.434	6	1	1	1	1	187	20.7	2.27
A0A380A2S8	Histidine kinase OS=Shigella flexneri OX=623 GN=yfhK PE=4 SV=1	3.536	2	1	1	1	1	460	51.4	2.27
P59341	Sensor histidine kinase DcuS OS=Shigella flexneri OX=623 GN=dcuS PE=3 SV=1	4.181	2	1	1	1	1	543	60.5	2.27
F5P3E9	3'(2'),5'-biphosphate nucleotidase CysQ OS=Shigella flexneri K-227 OX=766147 GN=cysQ PE=3 SV=1	2.468	4	1	1	1	1	230	25.4	2.26
A0A6N3RN87	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ OS=Shigella flexneri 1485-80 OX=766155 GN=fabZ PE=3 SV=1	2.108	5	1	1	1	1	151	17	2.25
A0A6N3QNZ8	Inorganic pyrophosphatase OS=Shigella flexneri CDC 796-83 OX=945360 GN=ppa PE=3 SV=1	2.941	5	1	1	1	1	176	19.7	2.24
A0A4P7TJQ1	Dual-specificity RNA methyltransferase RlmN OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rlmN PE=3 SV=1	2.102	2	1	1	1	1	384	43.1	2.24
Q0SY57	ATP-dependent protease subunit HslV OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hslV PE=3 SV=1	3.557	6	1	1	1	1	176	19.1	2.23
A0A6D2X1F2	30S ribosomal protein S19 OS=Shigella flexneri OX=623 GN=rpsS PE=3 SV=1	2.337	12	1	1	1	1	92	10.4	2.22
A0A4P7TTY8	Autotransporter assembly complex protein TamB OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tamB PE=4 SV=1	3.636	1	1	1	1	1	1259	136.8	2.22

A0A658YTG8	Putative F0F1-type ATP synthase, subunit b OS=Shigella flexneri OX=623 GN=SAMEA3710514_00636 PE=4 SV=1	1.694	6	1	1	1	1	160	18.9	2.18
F5P3V8	Fructose-1,6-bisphosphatase class 1 OS=Shigella flexneri K-227 OX=766147 GN=fbp PE=3 SV=1	3.651	3	1	1	1	1	332	36.8	2.17
A0A6G5Z0C8	Inosine-5'-monophosphate dehydrogenase OS=Shigella flexneri OX=623 GN=guaB PE=3 SV=1	3.359	2	1	1	1	1	488	52	2.13
I6C8L3	Fumarate reductase subunit C OS=Shigella flexneri K-315 OX=766150 GN=frdC PE=3 SV=1	2.11	7	1	1	1	1	120	13.6	2.11
F5NTG0	2-dehydro-3-deoxyphosphooctonate aldolase OS=Shigella flexneri K-227 OX=766147 GN=kdsA PE=3 SV=1	3.391	4	1	1	1	1	284	30.8	2.1
A0A6N3QSE0	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00807 PE=3 SV=1	3.186	2	1	1	1	1	401	41.7	2.1
A0A2S8DHD8	AI-2E family transporter OS=Shigella dysenteriae OX=622 GN=C5K18_00760 PE=3 SV=1	2.331	2	1	1	1	1	370	39.9	2.1
D2ABT9	UPF0438 protein YifE OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4187 PE=3 SV=1	1.803	10	1	1	1	1	112	13.1	2.06
I6CFZ0	50S ribosomal protein L27 OS=Shigella flexneri K-315 OX=766150 GN=rpmA PE=3 SV=1	1.264	8	1	1	1	1	85	9.1	2.06
I6CFX7	Lipopolysaccharide export system protein LptC OS=Shigella flexneri K-315 OX=766150 GN=lptC PE=3 SV=1	2.652	4	1	1	1	1	191	21.7	2.05
A0A2S8DSZ9	Putative glucose-6-phosphate 1-epimerase OS=Shigella boydii OX=621 GN=yeaD PE=3 SV=1	1.827	3	1	1	1	1	294	32.6	2.05
I6CVK9	Phenylalanine--tRNA ligase alpha subunit OS=Shigella flexneri K-315 OX=766150 GN=pheS PE=3 SV=1	1.771	2	1	1	1	1	327	36.8	2.05
D2AD60	Protein translocase subunit SecE OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=secE PE=3 SV=1	2.348	7	1	1	1	1	127	13.6	2.04
A0A6N3REG1	NADH-quinone oxidoreductase subunit H OS=Shigella flexneri 1485-80 OX=766155 GN=nuoH PE=3 SV=1	1.929	2	1	1	1	1	325	36.2	2.04
A0A2S8D986	SelB translation factor OS=Shigella dysenteriae OX=622 GN=C5K18_18765 PE=4 SV=1	2.367	2	1	1	1	1	614	68.8	2.04
D2AIS5	RNA polymerase-binding transcription factor DksA OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=dksA PE=3 SV=1	2.421	6	1	1	1	1	151	17.5	2.01
A0A6N3RJ42	Glutaredoxin OS=Shigella flexneri 1485-80 OX=766155 GN=ydhD PE=3 SV=1	1.808	7	1	1	1	1	115	12.9	2
D2A8B1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=murA PE=3 SV=1	2.687	3	1	1	1	1	419	44.8	1.98
Q0SXI3	Magnesium-transporting ATPase, P-type 1 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=mgtA PE=3 SV=1	2.497	1	1	1	1	1	898	99.4	1.98
A0A4P7TKA6	Glycerol-3-phosphate dehydrogenase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=glpA PE=3 SV=1	2.332	3	1	1	1	1	542	58.9	1.97
I6D6Z3	Ribosome-recycling factor OS=Shigella flexneri K-315 OX=766150 GN=frf PE=3 SV=1	1.163	4	1	1	1	1	185	20.6	1.96
A0A383JSX6	Chaperedoxin OS=Shigella flexneri OX=623 GN=ybbN PE=4 SV=1	2.216	4	1	1	1	1	284	31.8	1.96
A0A6N3R946	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin) OS=Shigella flexneri 1485-80 OX=766155 GN=ispG PE=3 SV=1	1.654	3	1	1	1	1	372	40.7	1.96
A0A2S8DCI2	Na(+)/H(+) antiporter NhaB OS=Shigella dysenteriae OX=622 GN=nhaB PE=3 SV=1	1.414	1	1	1	1	1	513	56.7	1.95
I6CGI6	1-acyl-sn-glycerol-3-phosphate acyltransferase OS=Shigella flexneri K-315 OX=766150 GN=plsC PE=3 SV=1	2.072	3	1	1	1	1	211	23.4	1.92
I6CG61	Aminomethyltransferase OS=Shigella flexneri K-315 OX=766150 GN=gcvT PE=3 SV=1	1.456	3	1	1	1	1	347	38.2	1.91
D2AEV8	Putative NADPH-dependent glutamate synthase beta chain-like oxidoreductase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yeiT PE=4 SV=1	1.428	3	1	1	1	1	412	44.4	1.9
A0A6N3RM33	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase OS=Shigella flexneri 1485-80 OX=766155 GN=murE PE=3 SV=1	2.647	2	1	1	1	1	495	53.3	1.89
A0A4P7TUP9	Efflux pump membrane transporter OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_018995 PE=3 SV=1	3.126	1	1	1	1	1	1037	111.5	1.89
I6CI31	GTPase Der OS=Shigella flexneri K-315 OX=766150 GN=der PE=3 SV=1	1.358	2	1	1	1	1	490	55	1.88
A0A2S8DH97	Pyridoxine/pyridoxamine 5'-phosphate oxidase OS=Shigella dysenteriae OX=622 GN=pxdH PE=3 SV=1	2.14	4	1	1	1	1	218	25.6	1.87
D2AH92	Glutathione-binding protein GsiB OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=mppA PE=3 SV=1	2.35	2	1	1	1	1	544	60.7	1.85
A0A6N3QEL1	50S ribosomal protein L19 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rplS PE=3 SV=1	2.086	7	1	1	1	1	115	13.1	1.82
I6D6Z7	Zinc metalloprotease OS=Shigella flexneri K-315 OX=766150 GN=rseP PE=3 SV=1	1.758	2	1	1	1	1	450	49.1	1.82
A0A2S8DE90	Long-chain-fatty-acid--CoA ligase FadD OS=Shigella dysenteriae OX=622 GN=C5K18_08425 PE=4 SV=1	2.243	2	1	1	1	1	561	62.2	1.82
I6CBX0	50S ribosomal protein L28 OS=Shigella flexneri K-315 OX=766150 GN=rpmB PE=3 SV=1	2.657	15	1	1	1	1	66	7.7	1.81
A0A6L6UWY8	Flavodoxin family protein OS=Shigella flexneri OX=623 GN=mdaB PE=4 SV=1	1.837	4	1	1	1	1	193	22	1.79
A0A2S8DDN5	Glutamine synthetase OS=Shigella dysenteriae OX=622 GN=glnA PE=3 SV=1	2.576	2	1	1	1	1	461	51	1.78
A0A658YW43	Phosphotransfer intermediate protein in two-component regulatory system with RcsBC OS=Shigella flexneri OX=623 GN=yojN PE=4 SV=1	1.91	2	1	1	1	1	890	100.3	1.77
I6CI44	Peptidase B OS=Shigella flexneri K-315 OX=766150 GN=pepB PE=3 SV=1	1.701	3	1	1	1	1	427	46.3	1.76
F5P096	Toluene tolerance, Ttg2 family protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3853 PE=4 SV=1	1.508	4	1	1	1	1	211	24	1.75
Q0T2J7	Putative aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_2357 PE=4 SV=1	1.829	3	1	1	1	1	405	45.5	1.71
A0A2Y4XYI3	Esterase FrsA OS=Shigella flexneri OX=623 GN=frsA PE=3 SV=1	1.364	2	1	1	1	1	414	47	1.71
A0A658YBA8	Alpha-1,4 glucan phosphorylase OS=Shigella flexneri OX=623 GN=malP PE=3 SV=1	3.677	2	1	1	1	1	797	90.4	1.71
A0A4P7TS84	Probable cytosol aminopeptidase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=pepA PE=3 SV=1	2.285	2	1	1	1	1	503	54.8	1.71
Q83PW3	Fe(2+) transporter FeoB OS=Shigella flexneri OX=623 GN=feoB PE=3 SV=4	1.648	2	1	1	1	1	773	84.4	1.7

F5NY55	Phosphoribosylformylglycinamide synthase OS=Shigella flexneri K-227 OX=766147 GN=purL PE=3 SV=1	1.801	1	1	1	1	1294	141.2	1.69
A0A6N3RIF1	Intermembrane phospholipid transport system permease protein MlaE OS=Shigella flexneri 1485-80 OX=766155 GN=yrbE PE=3 SV=1	2.04	5	1	1	1	260	27.8	1.66
A0A658Y5Y4	Putative CBS domain-containing protein OS=Shigella flexneri OX=623 GN=yfjD PE=3 SV=1	2.014	2	1	1	1	413	46.3	1.66
C9EAD7	Mannitol-1-phosphate dehydrogenase (Fragment) OS=Shigella flexneri OX=623 GN=mtlD PE=4 SV=1	1.147	4	1	1	1	180	19.2	1.64
A0A2S8D6C3	Proline-specific permease ProY OS=Shigella dysenteriae OX=622 GN=C5K18_23740 PE=4 SV=1	3.791	3	1	1	1	457	50.1	1.64
A0A6N3RFW5	Penicillin-binding protein 7 OS=Shigella flexneri 1485-80 OX=766155 GN=pbpG PE=3 SV=1	2.616	3	1	1	1	293	32.5	1.61
A0A3T2UXZ7	DctR protein OS=Shigella flexneri OX=623 GN=DK174_15225 PE=4 SV=1	1.381	43	1	1	1	60	7	0
A0A2S8DUX8	IS66 family transposase (Fragment) OS=Shigella boydii OX=621 GN=C5K23_05970 PE=4 SV=1	1.457	18	1	1	1	231	26.3	0
I6D3N6	Tetratricopeptide repeat family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0613 PE=4 SV=1	2.332	16	1	1	1	57	6.2	0
A0A1Q8M2L6	50S ribosomal protein L30 OS=Shigella boydii OX=621 GN=rpM PE=3 SV=1	1.676	15	1	1	1	59	6.5	0
A0A0H2V114	Glutamate decarboxylase OS=Shigella flexneri OX=623 GN=SF2498 PE=3 SV=1	1.209	10	1	1	1	70	7.8	0
A0A658Z1R1	Dipeptidyl carboxypeptidase II OS=Shigella flexneri OX=623 GN=dcp_1 PE=3 SV=1	1.472	9	1	1	1	96	11.2	0
A0A658Y206	Putative mannitol-1-phosphate/altronate dehydrogenase OS=Shigella flexneri OX=623 GN=uxaB PE=4 SV=1	0.944	8	1	1	1	483	54.8	0
A0A6N3RC82	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3360 PE=4 SV=1	0.902	8	1	1	1	117	13.8	0
A0A2Y4GI23	Iron-dicitrate transporter substrate-binding subunit OS=Shigella flexneri OX=623 GN=fecB PE=4 SV=1	1.018	7	1	1	1	300	33.2	0
F5NQG4	Transcription antitermination protein NusB OS=Shigella flexneri K-227 OX=766147 GN=nusB PE=3 SV=1	0.933	7	1	1	1	139	15.7	0
A0A2Y4XZJ9	Ferrocyclase OS=Shigella flexneri OX=623 GN=hemH PE=3 SV=1	1.899	7	1	1	1	320	35.9	0
A0A2S8DK85	Single-stranded DNA-binding protein (Fragment) OS=Shigella boydii OX=621 GN=C5K23_27980 PE=3 SV=1	2.016	6	1	1	1	156	16.5	0
A0A2Y5B179	DNA-binding transcriptional repressor PurR OS=Shigella flexneri OX=623 GN=purR_2 PE=4 SV=1	0.916	6	1	1	1	174	19.5	0
Q0SY18	Transcription termination/antitermination protein NusG OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nusG PE=3 SV=1	3.421	6	1	1	1	181	20.5	0
F5NTJ9	Putative potassium channel protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1447 PE=4 SV=1	0.908	5	1	1	1	402	44.3	0
A0A658YZ45	Glutamine ABC transporter ATP-binding protein OS=Shigella flexneri OX=623 GN=glnQ PE=3 SV=1	0.937	5	1	1	1	240	26.6	0
A0A3T2US69	Uncharacterized protein OS=Shigella flexneri OX=623 GN=DK174_04225 PE=4 SV=1	1.062	5	1	1	1	124	13.5	0
I6D3R9	Uncharacterized protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0646 PE=4 SV=1	1.042	5	1	1	1	124	14.1	0
I6CQ07	Zinc import ATP-binding protein ZnuC OS=Shigella flexneri K-315 OX=766150 GN=znuC PE=3 SV=1	1.1	5	1	1	1	251	27.9	0
A0A2Y5AY14	DNA topoisomerase IV subunit A OS=Shigella flexneri OX=623 GN=parC PE=4 SV=1	0.914	5	1	1	1	273	29.6	0
A0A3T2UX13	Probable transcriptional regulatory protein YeeN OS=Shigella flexneri OX=623 GN=yeeN PE=3 SV=1	2.428	5	1	1	1	238	25.8	0
A0A6N3QV54	YciO family OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00295 PE=4 SV=1	1.109	5	1	1	1	218	24.5	0
P0A8A1	Probable transcriptional regulatory protein YebC OS=Shigella flexneri OX=623 GN=yebC PE=3 SV=1	1.037	4	1	1	1	246	26.4	0
P0AE94	Protein CreA OS=Shigella flexneri OX=623 GN=creA PE=3 SV=1	0.917	4	1	1	1	157	17.1	0
A0A6N3QRI2	ATP synthase epsilon chain OS=Shigella flexneri CDC 796-83 OX=945360 GN=atpC PE=3 SV=1	0.946	4	1	1	1	139	15	0
F5P1X7	Phosphate-specific transport system accessory protein PhoU OS=Shigella flexneri K-227 OX=766147 GN=phoU PE=3 SV=1	1.474	4	1	1	1	241	27.4	0
F5P327	Transcription elongation factor GreB OS=Shigella flexneri K-227 OX=766147 GN=greB PE=3 SV=1	0.936	4	1	1	1	158	18.5	0
I6D2B3	Amino ABC transporter, permease, 3-TM region, His/Glu/Gln/Arg/opine family domain protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0742 PE=3 SV=1	1.087	4	1	1	1	219	24.3	0
Q0T3B2	Putative enzyme of sugar metabolism OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_2075 PE=4 SV=1	1.252	4	1	1	1	274	29.7	0
A0A2S8DWD4	Oligopeptide ABC transporter ATP-binding protein OppF OS=Shigella boydii OX=621 GN=oppF PE=4 SV=1	1.666	4	1	1	1	334	37.1	0
F5NV18	Lipid A biosynthesis myristoyltransferase OS=Shigella flexneri K-227 OX=766147 GN=lpzM PE=3 SV=1	2.018	3	1	1	1	331	38.3	0
Q5IPA5	Aerobic respiratory control protein (Fragment) OS=Shigella flexneri OX=623 GN=arcA PE=4 SV=1	1.054	3	1	1	1	182	21	0
A0A2S8D890	Gluconate transporter OS=Shigella dysenteriae OX=622 GN=C5K18_19750 PE=4 SV=1	1.163	3	1	1	1	438	45.9	0
A0A3T2US15	Glucokinase OS=Shigella flexneri OX=623 GN=glk PE=3 SV=1	0.95	3	1	1	1	321	34.7	0
A0A2Y4ELK4	Phosphate import ATP-binding protein PstB OS=Shigella flexneri OX=623 GN=pstB PE=3 SV=1	2.318	3	1	1	1	257	29	0
I6D6M8	L-ribulose-5-phosphate 4-epimerase OS=Shigella flexneri K-315 OX=766150 GN=araD PE=3 SV=1	0.991	3	1	1	1	231	25.5	0
I6CDZ8	Short chain dehydrogenase family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3944 PE=3 SV=1	0.96	3	1	1	1	268	29.1	0
Q0SZY4	50S ribosomal protein L4 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpL4 PE=3 SV=1	0.922	3	1	1	1	201	22.1	0
A0A2Y4XHG0	Alkyl hydroperoxide reductase subunit F OS=Shigella flexneri OX=623 GN=ahpF PE=4 SV=1	1.019	3	1	1	1	202	21.8	0
A0A658YX65	Phosphopentomutase OS=Shigella flexneri OX=623 GN=deoB PE=3 SV=1	2.032	3	1	1	1	407	44.4	0
A0A4P7TR77	Maltoporin OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=lamb PE=2 SV=1	1.355	3	1	1	1	446	50	0
D2A949	Bifunctional protein FolD OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=folD PE=3 SV=1	1.219	3	1	1	1	288	30.9	0
A0A3T2UU59	Metalloprotease PmbA OS=Shigella flexneri OX=623 GN=DK174_07880 PE=3 SV=1	1.895	3	1	1	1	450	48.4	0

I6CXG7	Tryptophan synthase alpha chain OS=Shigella flexneri K-315 OX=766150 GN=trpA PE=3 SV=1	0.974	3	1	1	1	268	28.7	0
S5FUZ3	CI repressor OS=Shigella phage SIII OX=66284 GN=cI PE=4 SV=1	1.338	3	1	1	1	230	25.6	0
A0A1Q8M994	ADP-L-glycero-D-manno-heptose-6-epimerase OS=Shigella boydii OX=621 GN=rfaD PE=3 SV=1	1.566	3	1	1	1	310	34.9	0
A0A6N3QFD2	VirK OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04903 PE=4 SV=1	1.426	3	1	1	1	316	36.8	0
A0A6N3RN78	Uridylate kinase OS=Shigella flexneri 1485-80 OX=766155 GN=pyrH PE=3 SV=1	0.946	2	1	1	1	241	26	0
A0A658Y1F9	Mannitol dehydrogenase family protein OS=Shigella flexneri OX=623 GN=por PE=4 SV=1	1.06	2	1	1	1	486	53.6	0
F5NZS4	Peptide chain release factor 2 OS=Shigella flexneri K-227 OX=766147 GN=prfB PE=3 SV=1	1.945	2	1	1	1	293	32.8	0
A0A6G5YV33	Sensor protein OS=Shigella flexneri OX=623 GN=cusS PE=4 SV=1	2.302	2	1	1	1	480	53.8	0
F5NRC2	Curved DNA-binding protein OS=Shigella flexneri K-227 OX=766147 GN=cbpA PE=3 SV=1	1.733	2	1	1	1	306	34.4	0
A0A4P7TKG0	Microcin C ABC transporter permease YejB OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_004585 PE=3 SV=1	1.095	2	1	1	1	364	40.4	0
A0A6N3QSQ6	Valine--pyruvate aminotransferase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00038 PE=4 SV=1	1.334	2	1	1	1	417	46.6	0
A0A0H2V319	Putative amino acid/amine transport protein OS=Shigella flexneri OX=623 GN=yifK PE=4 SV=1	1.2	2	1	1	1	417	45.3	0
D2A780	Galactose-proton symporter OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=galP PE=3 SV=1	1.501	2	1	1	1	464	51	0
A0A6N3R9J5	Pyridoxal kinase OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_2841 PE=4 SV=1	1.115	2	1	1	1	279	30.4	0
A0A6N3QI04	NiFe hydrogenase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03645 PE=3 SV=1	1.843	2	1	1	1	372	39.6	0
A0A6N3RFD0	Dihydrofolate synthase/folylpolyglutamate synthase OS=Shigella flexneri 1485-80 OX=766155 GN=folC PE=3 SV=1	0.921	2	1	1	1	422	45.3	0
A0A380AGV7	Multidrug resistance protein MdtB OS=Shigella flexneri OX=623 GN=mdtB PE=3 SV=1	0.972	2	1	1	1	1040	112	0
A0A6N3QQC4	Probable malate:quinone oxidoreductase OS=Shigella flexneri CDC 796-83 OX=945360 GN=mqo PE=3 SV=1	0.997	2	1	1	1	548	60.2	0
A0A4P7TKN2	O13/O129/O135 family O-antigen flippase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=wzx PE=4 SV=1	2.362	2	1	1	1	418	46.3	0
I6CMG4	Inner membrane protein YejM OS=Shigella flexneri K-315 OX=766150 GN=yejM PE=4 SV=1	1.466	2	1	1	1	586	67.2	0
Q0T5R2	Spermidine/putrescine import ATP-binding protein PotA OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=potA PE=3 SV=1	1.348	2	1	1	1	378	43.1	0
P40613	Surface presentation of antigens protein SpaN OS=Salmonella typhimurium (strain LT2 / SGSC1412 / ATCC 700720) OX=99287 GN=spaN PE=3 SV=1	1.041	2	1	1	1	336	36.4	0
A0A4S3P3G3	YccS/YhfK family integral membrane protein OS=Shigella flexneri OX=623 GN=yccS_2 PE=4 SV=1	3.417	2	1	1	1	721	82	0
A0A380AGY6	Iron-sulfur cluster carrier protein OS=Shigella flexneri OX=623 GN=minD_1 PE=3 SV=1	1.093	2	1	1	1	369	39.9	0
A0A0H2UZ50	Asparagine synthase (glutamine-hydrolyzing) OS=Shigella flexneri OX=623 GN=asnB PE=3 SV=1	0.997	1	1	1	1	515	58.4	0
F5P0N3	UvrABC system protein A OS=Shigella flexneri K-227 OX=766147 GN=uvrA PE=3 SV=1	1.181	1	1	1	1	940	103.8	0
A0A383JT60	Pitriylsin OS=Shigella flexneri OX=623 GN=ptrA PE=3 SV=1	1.254	1	1	1	1	962	107.7	0
A0A658Y4R6	Inner membrane protein YejM OS=Shigella flexneri OX=623 GN=yejM PE=4 SV=1	1.166	1	1	1	1	586	67.2	0
A0A6N3QUS5	Uncharacterized protein OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00296 PE=4 SV=1	1.167	1	1	1	1	631	71.4	0
I6D1F5	Chromosome partition protein MukB OS=Shigella flexneri K-315 OX=766150 GN=mukB PE=3 SV=1	1.763	1	1	1	1	1486	170.1	0



### Appx. D.3: Full protein identification from band 3 of Figure 5.2b.

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score Sequest HT
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=rfc PE=3 SV=1	32.896	18	8	108	8	382	43.7	203.14
A0A3U1DEW5	Formate C-acetyltransferase OS=Shigella flexneri OX=623 GN=pf1B PE=3 SV=1	147.656	46	31	44	31	760	85.3	128.51
A0A383JRI2	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=hflB PE=3 SV=1	89.465	37	24	44	24	647	71	82.66
A0A658XXS4	Outer membrane protein assembly factor YaeT OS=Shigella flexneri OX=623 GN=yaeT PE=4 SV=1	146.073	39	32	42	32	810	90.5	124.79
D2A6V5	Chain length determinant protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_2324 PE=4 SV=1	104.347	55	15	41	15	337	37.9	122.76
A0A379Z1T0	Fumarate reductase flavoprotein subunit OS=Shigella flexneri OX=623 GN=frdA PE=3 SV=1	133.692	49	24	37	24	602	66	102.97
Q0SY20	Elongation factor Tu 2 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tuf2 PE=3 SV=1	96.149	61	19	37	19	394	43.3	102.52
A0A4P7TR58	NAD(P)/FAD-dependent oxidoreductase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_010675 PE=4 SV=1	105.566	55	18	35	18	434	47.3	100.16
A0A1Q8MQG3	Chaperone protein DnaK OS=Shigella boydii OX=621 GN=dnaK PE=2 SV=1	109.25	46	26	31	26	638	69.1	95.65
A0A4P7TNK7	Aldehyde-alcohol dehydrogenase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=adhE PE=3 SV=1	131.473	34	22	29	22	891	96.1	93.34
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	77.589	46	19	29	19	513	55.2	88.51
I6D635	Peptidylprolyl isomerase OS=Shigella flexneri K-315 OX=766150 GN=ppiD PE=4 SV=1	91.369	49	21	29	1	597	65.3	78.39
A0A2Y4Y099	Glycerol-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=g1pD PE=3 SV=1	66.97	31	17	27	17	501	56.7	52.53
A0A2S4MYF8	Outer membrane protein C OS=Shigella flexneri OX=623 GN=ompC PE=1 SV=1	62.597	32	13	25	1	373	41.4	62.12
A0A6D2XMH9	ATP synthase subunit beta OS=Shigella flexneri OX=623 GN=atpD PE=3 SV=1	97.871	53	18	23	18	460	50.3	73.99
A0A658YVY8	L-tryptophan indole-lyase OS=Shigella flexneri OX=623 GN=tnaA PE=3 SV=1	76.644	41	18	23	18	471	52.8	70.63
A0A4P7TRN0	Pyruvate dehydrogenase E1 component OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=aceE PE=4 SV=1	64.805	27	19	22	19	887	99.6	50.96
Q0T1R3	Chaperone protein ClpB OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=clpB PE=3 SV=1	60.272	24	20	22	20	857	95.6	47.2
A0A379Z256	60 kDa chaperonin OS=Shigella flexneri OX=623 GN=groEL PE=3 SV=1	60.694	39	16	20	16	551	57.5	63.69
D2ABV5	ECA polysaccharide chain length modulation protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=wzzE PE=3 SV=1	49.463	38	11	20	11	349	39.6	58.26
A0A658Y1M8	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=gapA PE=3 SV=1	47.439	39	12	18	12	331	35.5	49.42
F5NPX4	LPS-assembly protein LptD OS=Shigella flexneri K-227 OX=766147 GN=lptD PE=3 SV=1	69.533	29	15	16	15	753	86.3	52.77
A0A6N3R3T7	Aspartate ammonia-lyase OS=Shigella flexneri 1485-80 OX=766155 GN=aspA PE=3 SV=1	42.251	31	13	16	13	478	52.3	43.03
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	59.75	25	11	15	11	474	49.3	51.12
A0A4P7TME0	Transketolase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tkt PE=3 SV=1	47.884	22	13	15	13	663	72.1	37.91
P0A852	Trigger factor OS=Shigella flexneri OX=623 GN=tig PE=3 SV=1	44.305	32	14	15	14	432	48.2	36.84
Q83SE6	Chaperone protein HtpG OS=Shigella flexneri OX=623 GN=htpG PE=3 SV=1	47.793	25	12	14	12	624	71.3	42.35
A0A4P7TNY5	Alkyl hydroperoxide reductase C OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=ahpC PE=3 SV=1	39.313	47	8	14	8	187	20.7	36.19
A0A6N3QN52	Lon protease OS=Shigella flexneri CDC 796-83 OX=945360 GN=lon PE=2 SV=1	34.035	17	12	14	12	799	89	29.23
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsC PE=3 SV=1	33.165	32	9	14	9	224	24.8	28.56
A0A1S9KGJ3	Peptidyl-prolyl cis-trans isomerase OS=Shigella dysenteriae OX=622 GN=fkpA PE=3 SV=1	31.997	47	9	14	9	270	28.9	24.78
Q0T4H5	NAD(P) transhydrogenase subunit beta OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pntB PE=3 SV=1	49.286	24	7	13	7	462	48.7	37.99
Q7BCK4	Outer membrane protein IcsA autotransporter OS=Shigella flexneri OX=623 GN=icsA PE=1 SV=1	37.633	10	9	13	9	1102	116.2	35.86
Q0SYD1	L-lactate dehydrogenase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lldD PE=3 SV=1	47.39	33	11	13	11	396	42.7	33.44
A0A6N3QD48	Succinate dehydrogenase flavoprotein subunit OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04889 PE=3 SV=1	30.275	16	10	13	10	588	64.4	32.87
A0A2S8E636	Acetyltransferase component of pyruvate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=aceF PE=3 SV=1	42.611	23	12	13	12	630	66.1	31.31
A0A379Z327	Adenylosuccinate synthetase OS=Shigella flexneri OX=623 GN=purA PE=3 SV=1	24.013	22	8	13	8	432	47.3	26.86
A0A2S8E1V7	Protein HflK OS=Shigella dysenteriae OX=622 GN=hflK PE=3 SV=1	44.039	24	9	12	9	419	45.5	38.87
A0A2S8D9Q6	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=sucB PE=3 SV=1	36.807	26	10	12	10	405	44.1	32.02
A0A3T2UWB2	ATP synthase gamma chain OS=Shigella flexneri OX=623 GN=atpG PE=3 SV=1	37.32	32	11	12	11	287	31.6	31.83
A0A6N3RKB3	KefA OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0517 PE=3 SV=1	38.187	10	11	12	11	1135	128.5	31.59
A0A2S8D6C0	Protein translocase subunit SecD OS=Shigella dysenteriae OX=622 GN=secD PE=3 SV=1	35.297	20	11	12	11	615	66.6	30.34

I6CMA1	Quinone-dependent D-lactate dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=dld PE=3 SV=1	33.363	20	11	12	11	571	64.6	24.12
A0A2S8DV74	Glycerol kinase OS=Shigella boydii OX=621 GN=glpK PE=3 SV=1	35.625	21	9	11	9	502	56.1	31.34
I6CG83	Phosphoglycerate kinase OS=Shigella flexneri K-315 OX=766150 GN=pgk PE=3 SV=1	34.209	27	9	11	9	382	40.5	30.31
I6D6T4	Dihydrolypolyl dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=lpdA PE=3 SV=1	33.739	21	10	11	10	474	50.7	27.16
D2ABG4	30S ribosomal protein S1 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsA PE=3 SV=1	23.252	19	10	11	10	557	61.1	26.07
A0A2Y4Y9J3	Pyruvate kinase OS=Shigella flexneri OX=623 GN=pykF PE=3 SV=1	27.099	19	11	11	11	542	58.7	24.8
Q7AK95	Chloramphenicol acetyltransferase OS=Shigella flexneri 2b OX=1288825 GN=cat PE=3 SV=1	26.694	17	3	10	3	219	25.6	35.4
A0A2S8DUZ3	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=Shigella boydii OX=621 GN=accA PE=3 SV=1	38.687	35	10	10	10	319	35.2	32.14
A0A2A2XR75	EIIAB-Man OS=Shigella flexneri OX=623 GN=manX_2 PE=4 SV=1	39.546	27	8	10	8	323	35	29.68
A0A0H2VVY4	Anaerobic dimethyl sulfoxide reductase subunit A OS=Shigella flexneri OX=623 GN=dmsA PE=3 SV=1	28.479	12	8	10	8	785	87.4	27.7
A0A380AZK5	D-amino acid dehydrogenase OS=Shigella flexneri OX=623 GN=dadA PE=3 SV=1	27.317	24	10	10	10	432	47.6	27.09
A0A6N3RB60	50S ribosomal protein L2 OS=Shigella flexneri 1485-80 OX=766155 GN=rplB PE=3 SV=1	34.038	36	8	10	2	273	29.8	26.6
D2AFY9	NADH-quinone oxidoreductase subunit C/D OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=nuoC PE=3 SV=1	28.82	18	10	10	10	600	68.7	25.78
A0A1S9KIA0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	29.367	27	9	10	9	419	47	25.21
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	21.481	26	8	10	8	329	36.5	24.31
A0A658YYQ4	NAD(P) transhydrogenase subunit alpha OS=Shigella flexneri OX=623 GN=pntA PE=3 SV=1	24.066	16	7	10	7	510	54.5	20.65
A0A658Y285	Protein ydgA OS=Shigella flexneri OX=623 GN=ydgA PE=4 SV=1	30.264	19	8	9	8	502	54.6	26.03
A0A4P7TTH9	30S ribosomal protein S2 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsB PE=3 SV=1	33.969	28	6	9	6	241	26.8	25.85
Q0T1P7	Enolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=eno PE=3 SV=1	21.796	16	7	9	7	431	45.7	24.69
A0A2Y5B4B8	Malate dehydrogenase OS=Shigella flexneri OX=623 GN=mdh PE=3 SV=1	25.32	29	8	9	8	312	32.3	24.23
F5P3A0	Elongation factor G OS=Shigella flexneri K-227 OX=766147 GN=fusA PE=3 SV=1	30.581	14	9	9	9	704	77.6	23.53
I6CBL5	ATP synthase FO, B subunit OS=Shigella flexneri K-315 OX=766150 GN=atpF PE=3 SV=1	30.577	64	9	9	9	127	14	23
F5NRH8	Efflux pump membrane transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0715 PE=3 SV=1	30.316	10	9	9	9	1049	113.6	20.59
F5NRX2	Serine-type D-Ala-D-Ala carboxypeptidase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0860 PE=3 SV=1	25.675	22	8	9	8	403	44.4	19.86
D2ADL5	Respiratory nitrate reductase 1 beta chain OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=narH PE=4 SV=1	17.096	15	8	9	8	512	58	18.53
A0A379Z9W6	Heme biosynthesis protein HemY OS=Shigella flexneri OX=623 GN=hemY PE=4 SV=1	17.528	13	7	9	7	398	45.2	18.38
D2AHW6	Peptidoglycan D,D-transpeptidase FtsI OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=ftsI PE=3 SV=1	33.096	21	8	8	8	588	63.9	28.01
A0A2Y4JWQ5	MbeB-like, N-term conserved region OS=Shigella flexneri OX=623 GN=SAMEA3710514_05138 PE=4 SV=1	23.017	32	6	8	6	176	19.9	23.57
A0A4P7TRQ9	Ribulokinase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=araB PE=3 SV=1	26.019	20	8	8	8	566	61.1	23.5
I6C8N1	Modulator of FtsH protease HflC OS=Shigella flexneri K-315 OX=766150 GN=hflC PE=3 SV=1	25.676	34	7	8	7	334	37.6	22.78
A0A2S8DA81	Succinate-CoA ligase [ADP-forming] subunit beta OS=Shigella dysenteriae OX=622 GN=sucC PE=3 SV=1	26.528	19	8	8	8	388	41.4	21.99
I6CJU2	Phosphate acetyltransferase OS=Shigella flexneri K-315 OX=766150 GN=pta PE=3 SV=1	23.125	11	7	8	7	709	76.5	19.83
I6D6H9	Transaldolase OS=Shigella flexneri K-315 OX=766150 GN=tal PE=3 SV=1	20.685	25	7	8	7	294	32.8	19.49
A0A4P7TQY3	Bifunctional purine biosynthesis protein PurH OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=purH PE=3 SV=1	29.151	19	7	8	7	529	57.3	19.15
Q0T4H3	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1619 PE=4 SV=1	20.743	25	8	8	8	314	33.9	18.44
Q2EUU0	AccD (Fragment) OS=Shigella flexneri OX=623 GN=accD PE=3 SV=1	18.556	23	6	8	6	296	32.6	18.07
A0A2Y4ZTN4	Xaa-Pro dipeptidase OS=Shigella flexneri OX=623 GN=pepQ PE=3 SV=1	18.14	16	7	8	7	443	50.2	16.82
A0A2S8DV96	50S ribosomal protein L5 OS=Shigella boydii OX=621 GN=rplE PE=3 SV=1	27.44	46	7	8	7	179	20.3	16.76
Q0T8D5	L-arabinose isomerase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=araA PE=3 SV=1	23.721	17	8	8	8	500	56	16.6
A0A2S8DKT5	NADH-quinone oxidoreductase OS=Shigella boydii OX=621 GN=nuoG PE=3 SV=1	26.288	10	6	7	6	908	100.3	23.78
A0A6N3RAZ3	Cell division protein FtsN OS=Shigella flexneri 1485-80 OX=766155 GN=ftsN PE=3 SV=1	23.76	35	7	7	7	284	31.8	21.09
A0A6N3R6K7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4998 PE=3 SV=1	22.397	9	6	7	6	402	42.7	19.38
I6CI36	Cytoskeleton protein RodZ OS=Shigella flexneri K-315 OX=766150 GN=rodZ PE=3 SV=1	26.307	18	6	7	6	337	36.2	19.31
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	20.567	16	6	7	6	477	50.7	19.24
D2AHG2	Autonomous glycol radical cofactor OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfiD PE=3 SV=1	24.479	46	5	7	5	127	14.2	19.04
F5NX93	EIII-Fru OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2769 PE=4 SV=1	19.145	23	7	7	7	376	39.7	18.16
I6C9W6	50S ribosomal protein L2 OS=Shigella flexneri K-315 OX=766150 GN=rplB PE=3 SV=1	22.606	33	7	7	1	255	27.9	17.97
A0A4V1CUD1	Histidine kinase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=cpxA PE=4 SV=1	16.332	15	7	7	7	457	51.6	17.78
A0A6N3RKA3	Succinate dehydrogenase iron-sulfur subunit OS=Shigella flexneri 1485-80 OX=766155 GN=sdhB PE=3 SV=1	18.869	24	6	7	6	238	26.8	17.63
A0A3U1D5H5	Alpha-D-glucose phosphate-specific phosphoglucomutase OS=Shigella flexneri OX=623 GN=pgm PE=3 SV=1	13.943	11	6	7	6	546	58.3	14.77
A0A379Z3L5	Glycerol-3-phosphate acyltransferase OS=Shigella flexneri OX=623 GN=plsB PE=3 SV=1	17.479	9	7	7	7	807	91.4	11.93

A0A6N3RM75	Oxoglutarate dehydrogenase (succinyl-transferring) OS=Shigella flexneri 1485-80 OX=766155 GN=sucA PE=3 SV=1	15.36	6	5	7	5	933	105	8.36
A0A2S8DDU2	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OS=Shigella dysenteriae OX=622 GN=murG PE=3 SV=1	21.109	22	6	6	6	355	37.8	20.27
A0A658Y983	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degQ PE=3 SV=1	24.554	16	6	6	6	455	47.2	19.87
Q0T6Q1	LPS-assembly lipoprotein LptE OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lptE PE=3 SV=1	21.135	27	5	6	5	193	21.4	18.59
F5NY87	Phosphoenolpyruvate-protein phosphotransferase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3048 PE=3 SV=1	23.326	13	6	6	6	585	64.5	18.58
A0A2S8DCH8	Cysteine synthase OS=Shigella dysenteriae OX=622 GN=cysK PE=3 SV=1	23.114	21	5	6	5	323	34.5	17.28
A0A2S8DUL5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase OS=Shigella boydii OX=621 GN=dapD PE=3 SV=1	20.426	25	6	6	6	274	29.9	17.23
A0A658YX10	Acriflavine resistance protein A OS=Shigella flexneri OX=623 GN=acrA PE=3 SV=1	23.053	22	6	6	6	397	42.1	16.39
A0A1S9JMU3	Cell shape-determining protein MreB OS=Shigella boydii OX=621 GN=mreB PE=3 SV=1	21.939	21	6	6	6	347	36.9	15.51
A0A658YTS0	Alcohol dehydrogenase yqhD OS=Shigella flexneri OX=623 GN=yqhD PE=4 SV=1	18.998	17	5	6	5	387	42.1	15.18
P0A6P4	Elongation factor Ts OS=Shigella flexneri OX=623 GN=tsf PE=3 SV=2	15.2	22	6	6	6	283	30.4	14.92
I6CDA5	ATP-dependent protease ATPase subunit HslU OS=Shigella flexneri K-315 OX=766150 GN=hslU PE=2 SV=1	19.399	14	6	6	6	443	49.6	14.8
A0A2S8DJU5	Polyribonucleotide nucleotidyltransferase OS=Shigella boydii OX=621 GN=pnp PE=3 SV=1	12.199	10	6	6	6	711	77.1	14.13
A0A2S8D3U0	Catabolite activator protein OS=Shigella dysenteriae OX=622 GN=crp PE=4 SV=1	14.11	24	5	6	5	210	23.7	13.97
I6D072	Outer membrane protein A OS=Shigella flexneri K-315 OX=766150 GN=ompA PE=3 SV=1	13.19	14	5	6	5	344	37	13.61
A0A3T2UY84	Cyclopropane-fatty-acyl-phospholipid synthase OS=Shigella flexneri OX=623 GN=DK174_15880 PE=3 SV=1	16.901	19	6	6	6	382	43.9	13.37
A0A2S8DR37	L-arabinose-binding periplasmic protein OS=Shigella boydii OX=621 GN=araF PE=3 SV=1	15.025	19	6	6	6	329	35.6	12.89
F5NUR0	Threonine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=thrS PE=3 SV=1	12.933	8	5	6	5	642	74	12.88
A0A3T2URE0	Catalase-peroxidase OS=Shigella flexneri OX=623 GN=katG PE=3 SV=1	17.179	10	5	6	5	726	80	12.6
A0A2X2I0N0	Cell division protein DamX OS=Shigella flexneri OX=623 GN=damX PE=3 SV=1	22.985	27	6	6	6	428	46.1	12.26
A0A383JWV6	Membrane protein insertase YidC OS=Shigella flexneri OX=623 GN=yidC PE=3 SV=1	13.002	7	4	6	4	548	61.5	12
F5P004	L-asparaginase 2 OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3757 PE=3 SV=1	25.086	28	5	6	5	235	25.2	11.64
D2A917	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	13.537	27	5	6	5	124	13.7	11.27
A0A2S8D9R8	Penicillin-binding protein 1B OS=Shigella dysenteriae OX=622 GN=mrcB PE=3 SV=1	13.675	7	6	6	6	840	93.8	8.64
Q0T6B3	Aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=aspC PE=3 SV=1	9.877	13	5	6	5	396	43.6	7.12
A0A6N3QJ73	Fructose-specific PTS system IIBC component OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03096 PE=4 SV=1	17.964	6	3	5	3	563	57.5	15.23
A0A2Y4XMF0	Sec-independent protein translocase protein TatA OS=Shigella flexneri OX=623 GN=tatA PE=3 SV=1	19.286	49	4	5	4	89	9.7	14.98
F5P2D1	Rhodanese-like domain protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4672 PE=4 SV=1	19.104	43	5	5	5	143	15.6	14.95
I6CXR3	Universal stress protein E OS=Shigella flexneri K-315 OX=766150 GN=uspE PE=4 SV=1	15.19	17	5	5	5	314	35.5	14.82
D2AGX5	Cysteine desulfurase IscS OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=iscS PE=3 SV=1	14.282	13	5	5	5	412	46	14.46
A0A6L6V006	Ribose-phosphate pyrophosphokinase OS=Shigella flexneri OX=623 GN=prs PE=3 SV=1	17.391	20	5	5	5	315	34.2	14.24
A0A6N3QME5	50S ribosomal protein L1 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rlpA PE=3 SV=1	15.614	20	4	5	4	234	24.7	14.19
A0A3T2UX48	Outer membrane protein F OS=Shigella flexneri OX=623 GN=ompF PE=3 SV=1	17.192	12	4	5	4	362	39.3	14.17
A0A6N3RCV1	Alanine--tRNA ligase OS=Shigella flexneri 1485-80 OX=766155 GN=alaS PE=3 SV=1	18.725	7	5	5	5	876	96	14.13
A0A2Y5C0F7	Putative uroporphyrinogen III C-methyltransferase OS=Shigella flexneri OX=623 GN=hemX PE=4 SV=1	17.518	15	5	5	5	401	43.6	13.36
Q0T8M9	Serine--tRNA ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=serS PE=3 SV=1	16.513	17	5	5	5	430	48.4	13.3
F5P3L0	Fumarate reductase iron-sulfur subunit OS=Shigella flexneri K-227 OX=766147 GN=SFK227_5105 PE=3 SV=1	20.355	25	5	5	5	244	27	13.28
A0A380B6P9	PTS N-acetylglucosamine EIICBA component OS=Shigella flexneri OX=623 GN=nagE PE=4 SV=1	16.964	9	5	5	5	648	68.3	13.2
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	12.196	14	5	5	5	376	41	13.04
A0A2S8DEF0	Chaperone SurA OS=Shigella dysenteriae OX=622 GN=surA PE=3 SV=1	10.515	10	4	5	4	428	47.3	12.35
F5P3C9	30S ribosomal protein S13 OS=Shigella flexneri K-227 OX=766147 GN=rpsM PE=3 SV=1	16.371	35	4	5	4	118	13.1	12.1
A0A6N3REQ7	Nickel-dependent hydrogenase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3604 PE=3 SV=1	10.662	8	4	5	4	567	62.5	11.86
I6D3P8	Cytochrome d ubiquinol oxidase subunit 1 OS=Shigella flexneri K-315 OX=766150 GN=cydA PE=3 SV=1	12.65	11	5	5	5	500	55.6	11.8
A0A6N3QUN6	Zinc/cadmium/mercury/lead-transporting ATPase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00074 PE=3 SV=1	12.834	8	5	5	5	732	76.7	9.9
A0A0H2VYI3	Nucleoside permease OS=Shigella flexneri OX=623 GN=nupC PE=3 SV=1	9.573	9	4	5	4	400	43.5	8.77
I6CX73	Cell division inhibitor MinD OS=Shigella flexneri K-315 OX=766150 GN=minD PE=3 SV=1	10.091	22	5	5	5	235	26.3	8.53
A0A2S4MYJ5	Glycerophosphodiester phosphodiesterase OS=Shigella flexneri OX=623 GN=glpQ PE=4 SV=1	7.998	16	5	5	5	358	40.9	8
P69785	PTS system glucose-specific EIIA component OS=Shigella flexneri OX=623 GN=crr PE=3 SV=2	9.907	20	2	4	2	169	18.2	15.27
A0A2S8DKX6	Insulinase family protein OS=Shigella boydii OX=621 GN=yhjJ PE=3 SV=1	18.452	12	4	4	4	498	55.4	13.39
Q0T240	Outer membrane protein assembly factor BamC OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nlpB PE=3 SV=1	13.16	14	4	4	4	344	36.8	12.37

I6CAV2	Cell division protein FtsX OS=Shigella flexneri K-315 OX=766150 GN=ftsX PE=3 SV=1	16.319	15	4	4	4	4	352	38.5	12.14
A0A6N3R4Q2	50S ribosomal protein L6 OS=Shigella flexneri 1485-80 OX=766155 GN=rlpF PE=3 SV=1	11.79	22	3	4	3		177	18.9	11.88
A0A3T2V040	Formate dehydrogenase-N subunit alpha OS=Shigella flexneri OX=623 GN=fdnG PE=3 SV=1	14.865	5	4	4	4		1016	112.5	11.74
A0A6N3RE59	S-adenosylmethionine synthase OS=Shigella flexneri 1485-80 OX=766155 GN=metK PE=3 SV=1	14.263	14	4	4	4		384	41.9	11.59
A0A383JTR9	Cardiolipin synthase A OS=Shigella flexneri OX=623 GN=cls PE=3 SV=1	12.256	7	3	4	3		486	54.8	11.49
F5NVZ8	Outer membrane porin protein LC OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2311 PE=3 SV=1	14.193	16	4	4	4		360	39.7	11.36
A0A6N3QM36	Protein QmcA OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01963 PE=3 SV=1	11.73	17	4	4	4		305	33.7	10.86
I6D632	ATP-dependent Clp protease ATP-binding subunit ClpX OS=Shigella flexneri K-315 OX=766150 GN=clpX PE=3 SV=1	11.449	11	4	4	4		424	46.4	10.76
F5P0M6	10 kDa chaperonin OS=Shigella flexneri K-227 OX=766147 GN=groS PE=3 SV=1	12.031	48	4	4	4		97	10.4	10.56
Q0T6G6	UPF0194 membrane protein YbhG OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ybhG PE=3 SV=1	10.5	14	4	4	4		331	36.3	10.15
F5NUC5	Tail-specific protease OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1725 PE=3 SV=1	10.125	6	4	4	4		680	76.5	10.05
D2AD66	DNA-directed RNA polymerase subunit beta OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpoB PE=3 SV=1	11.235	4	4	4	4		1342	150.5	9.37
A0A380A6R4	3-oxoacyl-ACP synthase OS=Shigella flexneri OX=623 GN=fabB PE=3 SV=1	12.1	8	4	4	4		406	42.7	8.74
I6CMC4	Lysine-specific permease OS=Shigella flexneri K-315 OX=766150 GN=lysP PE=4 SV=1	6.232	4	2	4	2		489	53.5	8.57
A0A6N3RKR4	Iron-sulfur cluster binding protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0392 PE=4 SV=1	10.064	8	4	4	4		475	53	7.75
A0A2S8DD45	Isocitrate dehydrogenase [NADP] OS=Shigella dysenteriae OX=622 GN=icd PE=3 SV=1	8.263	9	4	4	4		416	45.8	7.56
I6D059	Dihydroorotate dehydrogenase (quinone) OS=Shigella flexneri K-315 OX=766150 GN=pyrD PE=3 SV=1	11.372	12	4	4	4		336	36.8	7.52
A0A380B3G3	Asparagine--tRNA ligase OS=Shigella flexneri OX=623 GN=asnC_2 PE=3 SV=1	9.697	9	4	4	4		466	52.6	7.41
A0A2S8DJM4	Translation initiation factor IF-2 OS=Shigella boydii OX=621 GN=infB PE=3 SV=1	9.131	5	4	4	4		890	97.3	7.26
Q83IY3	Bifunctional protein GlmU OS=Shigella flexneri OX=623 GN=glmU PE=3 SV=1	10.562	9	4	4	4		456	49.2	6.98
A0A658YRM6	Peptidase, M48 family OS=Shigella flexneri OX=623 GN=SAMEA3710514_00010 PE=4 SV=1	10.317	10	4	4	4		487	53.9	6.91
A0A2Y4Y1C7	Fumarate hydratase class I OS=Shigella flexneri OX=623 GN=fumA PE=3 SV=1	7.532	5	3	4	1		548	60.3	6.3
I6CH97	AMP nucleosidase OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3709 PE=4 SV=1	8.595	9	4	4	4		444	49.9	6.23
A0A2S8DTV4	Phosphoenolpyruvate synthase OS=Shigella boydii OX=621 GN=ppsA PE=3 SV=1	6.352	5	4	4	4		792	87.4	6.15
A0A658Z505	Transporter, small conductance mechanosensitive ion channel (MscS) family OS=Shigella flexneri OX=623 GN=mscM PE=3 SV=1	6.505	9	4	4	4		415	46.6	5.77
I6D405	PhoH-like protein OS=Shigella flexneri K-315 OX=766150 GN=ybeZ PE=3 SV=1	5.387	9	3	4	3		346	39	4.15
Q0T239	4-hydroxy-tetrahydrodipicolinate synthase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dapA PE=3 SV=1	13.965	15	3	3	3		292	31.3	12.42
I6D602	Protein-export membrane protein SecF OS=Shigella flexneri K-315 OX=766150 GN=secF PE=3 SV=1	20.748	17	3	3	3		304	33.2	12.26
A0A658Y016	Glucan biosynthesis protein G OS=Shigella flexneri OX=623 GN=mdoG PE=3 SV=1	17.972	9	3	3	3		500	56.6	11.56
F5P2W0	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4852 PE=4 SV=1	14.433	23	3	3	3		185	19.8	11.35
A0A6A9TSA3	Bifunctional protein HldE OS=Shigella flexneri OX=623 GN=hldE PE=3 SV=1	14.186	11	3	3	3		477	51	10.68
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	14.741	25	3	3	3		129	13.8	9.9
A0A658YV21	Auxiliary transport protein, membrane fusion protein (MFP) family OS=Shigella flexneri OX=623 GN=macA_2 PE=4 SV=1	10.6	11	3	3	3		355	38.7	9.75
A0A6H1JH06	30S ribosomal protein S5 OS=Shigella flexneri OX=623 GN=rpsE PE=3 SV=1	7.281	19	2	3	2		167	17.6	9.67
A0A1Q8M8J0	50S ribosomal protein L7/L12 OS=Shigella boydii OX=621 GN=rlpL PE=3 SV=1	11.599	21	3	3	3		121	12.3	9.57
A0A6N3R6Z9	Lipopolysaccharide export system permease protein LptF OS=Shigella flexneri 1485-80 OX=766155 GN=yjgP PE=3 SV=1	6.466	7	2	3	2		335	36.6	9.39
I6CHF3	MFS transporter, sugar porter family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3768 PE=3 SV=1	11.54	5	2	3	2		472	51.7	9.34
A0A1Q8MQJ7	Protein RecA OS=Shigella boydii OX=621 GN=recA PE=3 SV=1	10.002	10	3	3	3		353	38	8.96
A0A6N3RBT1	GTP-binding protein TypA/BipA OS=Shigella flexneri 1485-80 OX=766155 GN=typA PE=4 SV=1	10.985	5	3	3	3		607	67.4	8.86
D2AAJ1	2,3-bisphosphoglycerate-independent phosphoglycerate mutase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=gpmM PE=3 SV=1	7.878	6	3	3	3		548	60.1	8.83
A0A6N3RB16	Protein translocase subunit SecY OS=Shigella flexneri 1485-80 OX=766155 GN=prlA PE=3 SV=1	8.249	5	2	3	2		443	48.5	8.41
Q0T2P1	sn-glycerol-3-phosphate permease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=glpT PE=3 SV=1	10.401	5	2	3	2		452	50.4	8.31
F5NZ29	Serine transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3429 PE=4 SV=1	7.382	7	3	3	3		429	46.9	8.22
F5P316	Fe/S biogenesis protein NfuA OS=Shigella flexneri K-227 OX=766147 GN=nfuA PE=3 SV=1	8.512	17	3	3	3		191	21	8.21
A0A4P7TP07	Tol-Pal system protein TolB OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tolB PE=3 SV=1	10.657	9	3	3	3		430	45.9	8.1
A0A2S8DRB8	Glucose-6-phosphate 1-dehydrogenase OS=Shigella boydii OX=621 GN=zwf PE=3 SV=1	7.062	6	3	3	3		491	55.7	7.99
I6CG61	Aminomethyltransferase OS=Shigella flexneri K-315 OX=766150 GN=gcvT PE=3 SV=1	11.253	10	3	3	3		347	38.2	7.98
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	5.773	14	3	3	3		206	23.4	7.8
A0A2S8DA55	SH3 domain-containing protein OS=Shigella dysenteriae OX=622 GN=ygiM PE=4 SV=1	7.434	14	3	3	3		206	23.1	7.75
F5NRS1	Oxygen-insensitive NAD(P)H nitroreductase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0809 PE=3 SV=1	8.394	15	3	3	3		217	23.8	7.69

A0A4P7TQT3	50S ribosomal protein L10 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpLJ PE=3 SV=1	7.987	19	3	3	3	165	17.7	7.53
Q83MF6	Cell division protein FtsZ OS=Shigella flexneri OX=623 GN=ftsZ PE=3 SV=2	11.774	9	3	3	3	383	40.1	7.47
A0A6N3RQG4	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydD OS=Shigella flexneri 1485-80 OX=766155 GN=cydD PE=4 SV=1	5.507	5	3	3	3	588	65	7.36
A0A3T2UY26	Beta-glucosidase BglX OS=Shigella flexneri OX=623 GN=DK174_15745 PE=3 SV=1	7.784	4	3	3	3	765	83.5	7.18
A0A2S8DI33	Sec translocon accessory complex subunit YajC OS=Shigella boydii OX=621 GN=yajC PE=3 SV=1	4.914	18	2	3	2	110	11.9	7.14
A0A3T3S7B2	ParB/RepB/SpoJ family partition protein OS=Shigella flexneri OX=623 GN=sopB_2 PE=3 SV=1	7.86	10	3	3	3	326	36.8	7.06
A0A2S8DNR9	Methionine--tRNA ligase OS=Shigella boydii OX=621 GN=metG PE=3 SV=1	6.929	5	3	3	3	677	76.2	7
I6CE82	Fumarate hydratase class I OS=Shigella flexneri K-315 OX=766150 GN=fumB PE=3 SV=1	5.828	5	3	3	1	548	60.1	6.9
F5NZV9	Mechanosensitive ion channel family protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3712 PE=3 SV=1	13.293	13	3	3	3	286	30.9	6.88
I6D3P2	Tol-Pal system protein TolR OS=Shigella flexneri K-315 OX=766150 GN=tolR PE=3 SV=1	8.37	17	2	3	2	142	15.4	6.83
F5P3D3	50S ribosomal protein L17 OS=Shigella flexneri K-227 OX=766147 GN=rpLQ PE=3 SV=1	3.906	21	3	3	3	127	14.4	6.79
A0A3T2UQA1	Glutamate-1-semialdehyde 2,1-aminomutase OS=Shigella flexneri OX=623 GN=hemL PE=3 SV=1	10.172	9	3	3	3	426	45.3	6.79
A0A6N3RJH6	Sulfatase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_1839 PE=3 SV=1	5.275	4	3	3	3	539	60.7	6.7
A0A6L6UZB9	Ribonuclease E OS=Shigella flexneri OX=623 GN=rne PE=3 SV=1	6.106	3	3	3	3	1063	118.4	6.68
I6CMG4	Inner membrane protein YejM OS=Shigella flexneri K-315 OX=766150 GN=yejM PE=4 SV=1	6.575	4	2	3	2	586	67.2	6.52
D2A8B5	Cytoplasmic membrane protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yrbD PE=4 SV=1	7.239	16	3	3	3	183	19.6	6.42
Q2EW05	Galactokinase OS=Shigella flexneri OX=623 GN=galK PE=3 SV=1	6.95	9	3	3	3	382	41.4	6.22
A0A658Z233	Inner membrane protein yjgQ OS=Shigella flexneri OX=623 GN=yjgQ PE=3 SV=1	7.342	8	3	3	3	360	39.6	6.01
A0A2Y4X2P5	Proline/glycine betaine transporter OS=Shigella flexneri OX=623 GN=prop_1 PE=4 SV=1	4.249	6	3	3	3	366	40.2	5.98
A0A6N3R9B1	PTS system, trehalose-specific IIB component OS=Shigella flexneri 1485-80 OX=766155 GN=treP PE=4 SV=1	5.342	5	2	3	2	472	51.1	5.95
I6CVF4	Protease 4 OS=Shigella flexneri K-315 OX=766150 GN=sppA PE=3 SV=1	6.516	4	2	3	2	618	67.2	5.77
F5P2R0	Oligopeptidase A OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4802 PE=3 SV=1	4.53	3	2	3	2	651	73.9	5.77
A0A6N3RFR1	6-phosphogluconate dehydrogenase, decarboxylating OS=Shigella flexneri 1485-80 OX=766155 GN=gnd PE=3 SV=1	6.667	7	3	3	3	468	51.5	5.56
A0A2S8DCH3	Lipid ABC transporter permease/ATP-binding protein (Fragment) OS=Shigella dysenteriae OX=622 GN=C5K18_12120 PE=4 SV=1	6.642	5	3	3	3	573	63.4	5.35
A0A658Z1V1	UPF0283 membrane protein YcjF OS=Shigella flexneri OX=623 GN=yjcF PE=3 SV=1	7.956	9	3	3	3	353	39.4	5.07
Q0T5J2	Ribosome-binding ATPase YchF OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ychF PE=3 SV=1	6.032	7	3	3	3	363	39.6	5.03
A0A2S8DPK5	Copper exporting ATPase OS=Shigella boydii OX=621 GN=copA PE=3 SV=1	6.682	4	3	3	3	834	87.9	4.89
A0A4P7TJ80	Glycine dehydrogenase (decarboxylating) OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=gcvP PE=3 SV=1	5.496	4	3	3	3	957	104.3	4.87
I6D448	Alkyl hydroperoxide reductase subunit F OS=Shigella flexneri K-315 OX=766150 GN=ahpF PE=3 SV=1	5.747	6	3	3	3	521	56.1	4.47
A0A6N3QHQ0	Ferrochelatase OS=Shigella flexneri CDC 796-83 OX=945360 GN=hemH PE=3 SV=1	13.807	10	2	3	2	320	35.9	4.12
Q0SYU1	ATP synthase subunit delta OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=atpH PE=3 SV=1	4.787	10	2	3	2	177	19.3	3.16
A0A2S8D3C6	S-ribosylhomocysteine lyase OS=Shigella dysenteriae OX=622 GN=luxS PE=3 SV=1	7.121	14	2	3	2	171	19.4	2.65
A0A658YZ45	Glutamine ABC transporter ATP-binding protein OS=Shigella flexneri OX=623 GN=glnQ PE=3 SV=1	5.726	12	3	3	3	240	26.6	2.62
F5P0R0	Maltodextrin-binding protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4033 PE=3 SV=1	7.849	10	3	3	3	396	43.4	2.6
A0A2S8DFP5	Cell division protein DedD OS=Shigella dysenteriae OX=622 GN=dedD PE=3 SV=1	4.417	10	2	3	2	220	22.9	2.4
A0A6N3QQ54	Histidine kinase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01033 PE=4 SV=1	4.57	5	2	3	2	450	50.3	1.74
A0A379ZH24	Glycine--tRNA ligase beta subunit OS=Shigella flexneri OX=623 GN=glyS PE=3 SV=1	3.977	6	3	3	3	689	76.7	0
I6CBM2	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS=Shigella flexneri K-315 OX=766150 GN=glnS PE=3 SV=1	4.354	5	3	3	3	609	66.9	0
A0A2S8DD79	Threonine/serine transporter TdcC OS=Shigella dysenteriae OX=622 GN=tdcC PE=3 SV=1	13.442	8	2	2	2	443	48.9	9.68
I6CJU1	Acetate kinase OS=Shigella flexneri K-315 OX=766150 GN=ackA PE=3 SV=1	12.677	11	2	2	2	400	43.3	8.6
I6CFU5	Peptidase Do OS=Shigella flexneri K-315 OX=766150 GN=degS PE=3 SV=1	7.886	10	2	2	2	355	37.6	7.31
Q0SZA9	Triosephosphate isomerase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tpiA PE=3 SV=1	10.977	16	2	2	2	255	27	7
A0A380AZ64	Phosphoenolpyruvate--glycerone phosphotransferase OS=Shigella flexneri OX=623 GN=ygcC PE=3 SV=1	7.689	5	2	2	2	472	51.4	6.9
Q83PC3	50S ribosomal protein L11 OS=Shigella flexneri OX=623 GN=rpLK PE=3 SV=3	7.946	17	2	2	2	142	14.9	6.72
D2AGV7	TPR_21 domain-containing protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfgM PE=3 SV=1	13.645	21	2	2	2	206	22.1	6.71
I6D070	Endopeptidase La OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0989 PE=3 SV=1	8.869	5	2	2	2	586	65.9	6.54
Q7UCQ9	Lipopolysaccharide assembly protein B OS=Shigella flexneri OX=623 GN=yCiM PE=3 SV=1	6.908	5	2	2	2	389	44.4	6.28
D2ACQ6	Sulfatase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yijP PE=4 SV=1	8.323	5	2	2	2	577	66.5	6.21
I6CVA3	L-serine dehydratase OS=Shigella flexneri K-315 OX=766150 GN=SFK315_1603 PE=3 SV=1	5.108	5	2	2	2	432	46.6	6.21
D2A8T7	Cytochrome d ubiquinol oxidase subunit III OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yhcB PE=4 SV=1	5.373	16	2	2	2	134	15.2	6.15

A0A4P7TSC3	3-octaprenyl-4-hydroxybenzoate carboxy-lyase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=ubiD PE=3 SV=1	8.531	5	2	2	2	2	497	55.6	6.01
A0A658XZH7	Oxidoreductase OS=Shigella flexneri OX=623 GN=NCTC8524_01188 PE=4 SV=1	7.092	3	2	2	2	2	804	89.3	5.93
Q0T0X8	Putative actin OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yggE PE=4 SV=1	3.259	9	2	2	2	2	235	25.4	5.9
A0A2S8DBJ8	Diguanylate cyclase OS=Shigella dysenteriae OX=622 GN=C5K18_12670 PE=4 SV=1	5.232	3	2	2	2	2	646	73.3	5.77
A0A379Z3I1	(Fe-S)-binding protein OS=Shigella flexneri OX=623 GN=ykgE PE=4 SV=1	7.129	10	2	2	2	2	239	26.1	5.67
A0A2S8DD51	Cell division protein ZipA OS=Shigella dysenteriae OX=622 GN=zipA PE=3 SV=1	4.488	3	1	2	1	1	328	36.5	5.65
A0A4P7TME5	Osmotic shock tolerance protein YggT OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=yggT PE=3 SV=1	6.389	6	2	2	2	2	188	21.1	5.63
F5NQ21	Cell division protein FtsQ OS=Shigella flexneri K-227 OX=766147 GN=ftsQ PE=3 SV=1	6.495	9	2	2	2	2	276	31.4	5.59
I6CBQ2	DNA gyrase subunit B OS=Shigella flexneri K-315 OX=766150 GN=gyrB PE=3 SV=1	7.053	3	2	2	2	2	804	89.9	5.49
D2A780	Galactose-proton symporter OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=galP PE=3 SV=1	4.326	4	2	2	2	2	464	51	5.34
F5NST7	Paraquat-inducible protein B OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1183 PE=4 SV=1	5.551	5	2	2	2	2	527	58.3	5.33
D2AEV8	Putative NADPH-dependent glutamate synthase beta chain-like oxidoreductase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yeiT PE=4 SV=1	7.047	6	2	2	2	2	412	44.4	5.32
A0A6D2W4F9	Chaperone protein Skp OS=Shigella flexneri OX=623 GN=skp PE=3 SV=1	6.951	14	2	2	2	2	161	17.7	5.24
Q0T5R2	Spermidine/putrescine import ATP-binding protein PotA OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=potA PE=3 SV=1	4.669	5	2	2	2	2	378	43.1	5.23
A0A2Y4YUV3	Peptidyl-prolyl cis-trans isomerase OS=Shigella flexneri OX=623 GN=fklB PE=3 SV=1	7.365	12	2	2	2	2	206	22.2	5.21
A0A6N3RIF3	Enoyl-[acyl-carrier-protein] reductase [NADH] OS=Shigella flexneri 1485-80 OX=766155 GN=fabI PE=3 SV=1	4.563	8	2	2	2	2	262	27.8	5.1
A0A1S9KGS8	Phosphatidylserine decarboxylase proenzyme OS=Shigella dysenteriae OX=622 GN=psd PE=3 SV=1	3.898	6	2	2	2	2	322	35.9	5.09
Q0T4A8	Possible enzyme OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1686 PE=4 SV=1	4.595	6	2	2	2	2	418	42.9	5.09
A0A6N3RAY3	50S ribosomal protein L15 OS=Shigella flexneri 1485-80 OX=766155 GN=rp10 PE=3 SV=1	6.52	21	2	2	2	2	126	13.1	5.05
F5P300	Glucose-1-phosphate adenyllyltransferase OS=Shigella flexneri K-227 OX=766147 GN=glgC PE=3 SV=1	2.301	2	1	2	1	1	431	48.7	4.95
A0A2Y4XSH5	Periplasmic protein of mal regulon OS=Shigella flexneri OX=623 GN=malM PE=4 SV=1	3.059	5	2	2	2	2	306	31.9	4.93
Q0T2K3	NADH-quinone oxidoreductase subunit F OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nuoF PE=3 SV=1	4.387	5	2	2	2	2	445	49.4	4.86
I6D6Z7	Zinc metalloprotease OS=Shigella flexneri K-315 OX=766150 GN=rseP PE=3 SV=1	3.738	5	2	2	2	2	450	49.1	4.77
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	5.138	24	2	2	2	2	78	8.3	4.75
A0A6N3QSE0	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00807 PE=3 SV=1	6.415	6	2	2	2	2	401	41.7	4.74
A0A3T3S2E7	Pyruvate kinase OS=Shigella flexneri OX=623 GN=pyk PE=3 SV=1	5.054	4	2	2	2	2	480	51.3	4.69
I6CI34	Histidine--tRNA ligase OS=Shigella flexneri K-315 OX=766150 GN=hisS PE=3 SV=1	3.337	3	1	2	1	1	424	47.1	4.64
I6CVL0	Phenylalanine--tRNA ligase beta subunit OS=Shigella flexneri K-315 OX=766150 GN=pheT PE=3 SV=1	4.802	3	2	2	2	2	795	87.3	4.59
A0A6N3R946	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin) OS=Shigella flexneri 1485-80 OX=766155 GN=ispG PE=3 SV=1	5.565	6	2	2	2	2	372	40.7	4.58
A0A2Y4XSL4	Peptidyl-prolyl cis-trans isomerase OS=Shigella flexneri OX=623 GN=ppiB PE=3 SV=1	1.892	7	1	2	1	1	164	18.2	4.56
Q0SZ45	Thiol:disulfide interchange protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dsbA PE=3 SV=1	3.215	8	2	2	2	2	208	23.1	4.52
Q0T7Q8	Aminoacyl-histidine dipeptidase (Peptidase D) OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pepD PE=4 SV=1	2.956	4	2	2	2	2	485	52.9	4.45
D2AHT6	Co-chaperone protein DjlA OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=djlA PE=3 SV=1	2.519	6	2	2	2	2	271	30.5	4.42
A0A2Y4XRM3	CTP synthase OS=Shigella flexneri OX=623 GN=pyrG PE=3 SV=1	3.87	3	2	2	2	2	545	60.4	4.41
F5NPS2	Lipoprotein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0205 PE=3 SV=1	5.011	7	2	2	2	2	271	29.4	4.3
I6CY67	UPF0227 protein YcfP OS=Shigella flexneri K-315 OX=766150 GN=ycfP PE=3 SV=1	3.355	9	2	2	2	2	180	21.2	4.19
Q0SZA6	ATP-dependent 6-phosphofructokinase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pfkA PE=3 SV=1	4.047	5	2	2	2	2	340	37.1	4.19
I6CBY6	Lipopolysaccharide heptosyltransferase II OS=Shigella flexneri K-315 OX=766150 GN=rfaF PE=4 SV=1	3.95	5	2	2	2	2	348	39	4.15
A0A2S8DXT0	High frequency lysogenization protein HflD homolog OS=Shigella boydii OX=621 GN=hflD PE=3 SV=1	2.966	9	2	2	2	2	213	22.9	4.02
I6C193	Serine hydroxymethyltransferase OS=Shigella flexneri K-315 OX=766150 GN=glyA PE=3 SV=1	3.278	2	2	2	2	2	417	45.3	4
A0A6N3QIL7	NADP-dependent malic enzyme OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02679 PE=3 SV=1	2.394	5	2	2	2	2	279	30.5	3.85
A0A4P7TKN2	O13/O129/O135 family O-antigen flippase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=wzx PE=4 SV=1	4.089	5	2	2	2	2	418	46.3	3.84
A0A658Z0B8	AsmA family protein OS=Shigella flexneri OX=623 GN=SAMEA3710514_03157 PE=4 SV=1	1.599	2	1	2	1	1	569	62.3	3.78
F5NVN9	Glycosyl transferases group 1 family protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2195 PE=4 SV=1	3.801	6	2	2	2	2	341	38.2	3.77
P0A6L6	N-acetylneuraminatase lyase OS=Shigella flexneri OX=623 GN=nanA PE=3 SV=2	4.449	8	2	2	2	2	297	32.6	3.71
A0A6G5Z3B5	Biotin carboxylase OS=Shigella flexneri OX=623 GN=accC PE=4 SV=1	3.822	4	2	2	2	2	449	49.3	3.68
F5NZS4	Peptide chain release factor 2 OS=Shigella flexneri K-227 OX=766147 GN=prfB PE=3 SV=1	2.714	5	2	2	2	2	293	32.8	3.63
A0A2S8DKK2	Ribonucleoside-diphosphate reductase OS=Shigella boydii OX=621 GN=rdA PE=3 SV=1	3.217	3	2	2	2	2	761	85.7	3.36
F5NPR9	Proline--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=proS PE=3 SV=1	4.365	5	2	2	2	2	572	63.6	3.29

Q83SN2	Protein translocase subunit SecA OS=Shigella flexneri OX=623 GN=secA PE=3 SV=1	4.981	2	2	2	2	901	102	3.28
D2AC55	Putative lipoprotein yceB OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yceB PE=4 SV=1	5.542	11	2	2	2	205	22.6	2.98
I6C174	Signal peptidase I OS=Shigella flexneri K-315 OX=766150 GN=lepB PE=3 SV=1	4.188	6	2	2	2	324	36	2.81
I6CXD2	UTP--glucose-1-phosphate uridylyltransferase OS=Shigella flexneri K-315 OX=766150 GN=galU PE=3 SV=1	5.388	8	2	2	2	302	33	2.78
A0A380B6A5	BAX inhibitor (BI)-1/YccA family protein OS=Shigella flexneri OX=623 GN=ybhL_2 PE=3 SV=1	2.721	6	2	2	2	234	25.9	2.54
A0A383JTM9	Periplasmic glutamine-binding protein permease OS=Shigella flexneri OX=623 GN=fljY PE=3 SV=1	2.803	6	2	2	2	285	31	2.45
I6CB18	Transcriptional regulatory protein ompR OS=Shigella flexneri K-315 OX=766150 GN=ompR PE=4 SV=1	4.416	8	2	2	2	239	27.4	2.42
A0A6N3R6J7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4987 PE=3 SV=1	3.892	2	1	2	1	446	47.8	2.41
A0A658Y003	DNA-directed RNA polymerase subunit OS=Shigella flexneri OX=623 GN=rpoC PE=3 SV=1	4.13	2	2	2	2	1407	155.1	2.4
I6C9W2	30S ribosomal protein S10 OS=Shigella flexneri K-315 OX=766150 GN=rpsJ PE=3 SV=1	2.683	10	1	2	1	103	11.7	2.2
Q83Q07	30S ribosomal protein S9 OS=Shigella flexneri OX=623 GN=rpsI PE=3 SV=3	4.155	15	2	2	2	130	14.9	2.17
A0A4P7TRM2	Lipopolysaccharide N-acetylglucosaminyltransferase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_019395 PE=4 SV=1	3.767	4	2	2	2	380	43.5	2.12
F5NZF4	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3556 PE=3 SV=1	3.965	4	2	2	2	493	53.7	2.02
A0A4P7TQF0	50S ribosomal protein L9 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rplI PE=3 SV=1	1.687	7	1	2	1	149	15.8	1.89
A0A2Y4XYI3	Esterase FrsA OS=Shigella flexneri OX=623 GN=frsA PE=3 SV=1	3.821	5	2	2	2	414	47	1.87
I6CGI6	1-acyl-sn-glycerol-3-phosphate acyltransferase OS=Shigella flexneri K-315 OX=766150 GN=plsC PE=3 SV=1	2.768	9	2	2	2	211	23.4	1.73
A0A2S8D5N6	Aspartate carbamoyltransferase OS=Shigella dysenteriae OX=622 GN=pyrB PE=3 SV=1	2.758	6	2	2	2	311	34.4	1.72
A0A2S8D523	Anaerobic glycerol-3-phosphate dehydrogenase subunit B OS=Shigella dysenteriae OX=622 GN=glpB PE=3 SV=1	1.666	2	1	2	1	419	45.3	1.63
A0A6G5YYG3	Lipoprotein-releasing system ATP-binding protein LolD OS=Shigella flexneri OX=623 GN=lolD PE=3 SV=1	2.645	6	2	2	2	233	25.5	1.61
Q0T2J7	Putative aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_2357 PE=4 SV=1	3.071	4	2	2	2	405	45.5	0
D2ADW1	Putative transport protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yegH PE=3 SV=1	3.127	3	2	2	2	549	62.1	0
I6D6M8	L-ribulose-5-phosphate 4-epimerase OS=Shigella flexneri K-315 OX=766150 GN=araD PE=3 SV=1	1.362	3	1	2	1	231	25.5	0
A0A4P7TS84	Probable cytosol aminopeptidase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=pepA PE=3 SV=1	3.016	3	2	2	2	503	54.8	0
A0A6N3R9K0	Phosphotransferase system, EIIc family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_2851 PE=4 SV=1	7.559	7	1	1	1	345	36.5	4.86
A0A1Q8M4D3	30S ribosomal protein S18 OS=Shigella boydii OX=621 GN=rpsR PE=3 SV=1	8.114	24	1	1	1	75	9	4.39
I6CAR9	Universal stress protein OS=Shigella flexneri K-315 OX=766150 GN=uspA PE=3 SV=1	6.028	14	1	1	1	144	16.1	4.31
Q5IPI8	Heat shock protein (Fragment) OS=Shigella flexneri OX=623 GN=grpE PE=3 SV=1	6.467	9	1	1	1	158	17.6	4.01
A0A6L6UVT0	Lipid A biosynthesis lauroyltransferase OS=Shigella flexneri OX=623 GN=lpXL PE=3 SV=1	4.788	4	1	1	1	306	35.4	3.89
A0A2S8DCN0	Lipoprotein-releasing ABC transporter permease subunit LolE OS=Shigella dysenteriae OX=622 GN=C5K18_10460 PE=3 SV=1	3.957	2	1	1	1	414	45.3	3.78
A0A3Y2RM52	Phosphoglucosamine mutase OS=Shigella flexneri OX=623 GN=glmM PE=3 SV=1	4.421	3	1	1	1	445	47.5	3.57
I6CFV1	Putative stringent starvation protein A OS=Shigella flexneri K-315 OX=766150 GN=sspA PE=3 SV=1	6.005	7	1	1	1	212	24.3	3.55
I6D700	UDP-3-O-(3-hydroxymyristoyl)glucosamine N-acyltransferase OS=Shigella flexneri K-315 OX=766150 GN=lpxD PE=3 SV=1	4.794	8	1	1	1	341	36	3.54
A0A0H2VZX0	Thiol:disulfide interchange protein DsbD OS=Shigella flexneri OX=623 GN=dsbD PE=3 SV=1	4.343	2	1	1	1	565	61.8	3.4
A0A6N3QLC2	Universal stress protein G OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02439 PE=3 SV=1	2.508	10	1	1	1	142	15.9	3.36
A0A2Y4XVQ2	Outer membrane protein assembly complex subunit YfgL OS=Shigella flexneri OX=623 GN=yfgL PE=4 SV=1	4.113	7	1	1	1	184	20.1	3.36
A0A6N3RRG2	Outer membrane lipoprotein RcsF OS=Shigella flexneri 1485-80 OX=766155 GN=rscF PE=3 SV=1	4.786	12	1	1	1	117	12.4	3.33
A0A3T2UUK6	Energy-dependent translational throttle protein EttA OS=Shigella flexneri OX=623 GN=yjyK PE=3 SV=1	3.716	2	1	1	1	555	62.4	3.32
A0A2Y5ARN5	DNA-binding transcriptional regulator TyrR OS=Shigella flexneri OX=623 GN=tyrR PE=4 SV=1	2.89	5	1	1	1	219	25.1	3.31
A0A379ZAE3	Dihydroxy-acid dehydratase OS=Shigella flexneri OX=623 GN=ilvD PE=3 SV=1	3.439	2	1	1	1	616	65.5	3.3
A0A2S8DKM1	Putrescine-binding periplasmic protein OS=Shigella boydii OX=621 GN=potD PE=3 SV=1	3.638	3	1	1	1	348	38.9	3.29
Q0T5C6	Putative protease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=sohB PE=3 SV=1	4.437	4	1	1	1	296	33.4	3.21
F5P2M7	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4769 PE=4 SV=1	4.626	6	1	1	1	236	25.6	3.2
A0A2Y4Y2T5	2,5-diketo-D-gluconate reductase A OS=Shigella flexneri OX=623 GN=dkgA PE=4 SV=1	3.461	8	1	1	1	275	31	3.19
I6C9X6	30S ribosomal protein S14 OS=Shigella flexneri K-315 OX=766150 GN=rpsN PE=3 SV=1	2.813	10	1	1	1	96	11	3.15
F5NV08	Tyrosine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=tyrS PE=3 SV=1	2.705	3	1	1	1	424	47.6	3.11
A0A2S8DCL9	Adenylosuccinate lyase OS=Shigella dysenteriae OX=622 GN=purB PE=3 SV=1	2.536	2	1	1	1	456	51.6	3.1
A0A2Y4XZK1	Citrate synthase OS=Shigella flexneri OX=623 GN=gltA PE=3 SV=1	2.372	3	1	1	1	427	48	2.98
A0A6N3QGJ9	UPF0301 protein YqgE OS=Shigella flexneri CDC 796-83 OX=945360 GN=yqgE PE=3 SV=1	3.623	6	1	1	1	187	20.7	2.97
F5NY98	3-oxoacyl-[acyl-carrier-protein] reductase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3059 PE=4 SV=1	2.609	4	1	1	1	263	27.8	2.95
Q0T3Y0	Fumarate and nitrate reduction Regulatory protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fnr PE=4 SV=1	3.498	4	1	1	1	250	27.9	2.94

A0A2S8DJM8	Na+/H+ antiporter OS=Shigella boydii OX=621 GN=C5K23_27965 PE=3 SV=1	3.807	4	1	1	1	517	56.9	2.94
D2AG45	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfcZ PE=3 SV=1	4.212	13	1	1	1	102	11.3	2.89
I6D6C2	Peptidyl-prolyl cis-trans isomerase OS=Shigella flexneri K-315 OX=766150 GN=ppiB PE=3 SV=1	3.342	7	1	1	1	164	18.2	2.87
A0A658Y5D6	Arginyl-tRNA synthetase OS=Shigella flexneri OX=623 GN=argS PE=3 SV=1	3.44	2	1	1	1	577	64.7	2.87
A0A1Q8NKE7	Phosphoheptose isomerase OS=Shigella dysenteriae OX=622 GN=gmbA PE=3 SV=1	2.715	5	1	1	1	192	20.8	2.85
A0A6N3RI00	Alpha-D-glucosyl-1-phosphate uridylyltransferase OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_2337 PE=3 SV=1	3.17	12	1	1	1	198	22	2.83
Q0SZX1	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yheO PE=4 SV=1	3.715	5	1	1	1	240	26.8	2.83
A0A6N3RJ20	Pyridoxal kinase PdxY OS=Shigella flexneri 1485-80 OX=766155 GN=pxdY PE=3 SV=1	4.169	5	1	1	1	286	31.2	2.81
D2AE64	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4630 PE=3 SV=1	3.158	9	1	1	1	141	15.2	2.8
I6CG52	tRNA-modifying protein YgfZ OS=Shigella flexneri K-315 OX=766150 GN=ygfZ PE=3 SV=1	2.607	3	1	1	1	326	36.1	2.78
A0A2S4MTA8	Lactate utilization protein C OS=Shigella flexneri OX=623 GN=ykgG PE=4 SV=1	2.074	4	1	1	1	231	25.2	2.77
A0A6N3QU25	Thiol peroxidase OS=Shigella flexneri CDC 796-83 OX=945360 GN=tpx PE=3 SV=1	5.169	14	1	1	1	168	17.8	2.7
A0A2S8DI03	Signal recognition particle-docking protein FtsY (Fragment) OS=Shigella boydii OX=621 GN=C5K23_31365 PE=3 SV=1	2.544	2	1	1	1	486	53.2	2.69
Q0SYE6	2-amino-3-ketobutyrate coenzyme A ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=kbl PE=3 SV=1	2.908	3	1	1	1	398	43.2	2.67
A0A383JXY5	NAD dependent epimerase/dehydratase family OS=Shigella flexneri OX=623 GN=DOD76_09530 PE=4 SV=1	3.499	4	1	1	1	337	38	2.66
A0A1Q8M6D1	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase OS=Shigella boydii OX=621 GN=gpmA PE=3 SV=1	4.042	5	1	1	1	250	28.5	2.64
A0A237F1C5	Arginine ABC transporter ATP-binding protein ArtP OS=Shigella boydii OX=621 GN=artP PE=3 SV=1	2.827	4	1	1	1	242	27	2.63
A0A4V1CUA5	50S ribosomal protein L28 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpmB PE=3 SV=1	1.872	13	1	1	1	78	9	2.62
D2AD60	Protein translocase subunit SecE OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=secE PE=3 SV=1	1.766	7	1	1	1	127	13.6	2.58
A0A6G5Z373	Sensor protein QseC OS=Shigella flexneri OX=623 GN=qseC PE=4 SV=1	3.12	2	1	1	1	449	50.3	2.58
Q83PS4	C4-dicarboxylate transport protein OS=Shigella flexneri OX=623 GN=dctA PE=3 SV=1	1.319	2	1	1	1	428	45.4	2.58
Q83Q93	Biosynthetic arginine decarboxylase OS=Shigella flexneri OX=623 GN=speA PE=3 SV=2	1.916	1	1	1	1	662	74.3	2.57
A0A6N3RR48	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0546 PE=3 SV=1	2.293	4	1	1	1	259	28.2	2.56
A0A2S4N174	50S ribosomal protein L22 OS=Shigella flexneri OX=623 GN=rplV PE=3 SV=1	5.372	13	1	1	1	110	12.2	2.54
D2A8B1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=murA PE=3 SV=1	4.09	3	1	1	1	419	44.8	2.54
A0A2S8DWY7	Lysine-tRNA ligase OS=Shigella boydii OX=621 GN=lysS PE=3 SV=1	3.795	2	1	1	1	505	57.6	2.54
I6CGI0	Bifunctional glutathionylspermidine synthetase/amidase OS=Shigella flexneri K-315 OX=766150 GN=gsp PE=3 SV=1	3.421	2	1	1	1	619	70.5	2.53
Q0SZX6	30S ribosomal protein S7 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsG PE=3 SV=1	2.981	9	1	1	1	179	20	2.52
A0A2S8D7U4	Phosphoribosylaminoimidazole-succinocarboxamide synthase OS=Shigella dysenteriae OX=622 GN=purC PE=3 SV=1	2.081	5	1	1	1	237	27	2.52
A0A658Z2S5	GMP synthase OS=Shigella flexneri OX=623 GN=guaA PE=4 SV=1	2.78	2	1	1	1	525	58.7	2.52
F5P455	Carbon starvation protein A OS=Shigella flexneri K-227 OX=766147 GN=SFK227_5301 PE=3 SV=1	2.179	1	1	1	1	716	77.3	2.51
A0A2S8DU80	Agmatinase OS=Shigella boydii OX=621 GN=speB PE=3 SV=1	2.346	3	1	1	1	306	33.5	2.5
A0A2Y5A0I3	Repressor protein for FtsI OS=Shigella flexneri OX=623 GN=sufI PE=4 SV=1	3.129	4	1	1	1	370	40.8	2.49
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=Igkc PE=1 SV=1	1.33	6	1	1	1	219	24.2	2.48
A0A0H2V0V9	50S ribosomal protein L3 glutamine methyltransferase OS=Shigella flexneri OX=623 GN=yfcB PE=3 SV=1	2.627	2	1	1	1	421	47.8	2.48
A0A3T2USI5	Glucokinase OS=Shigella flexneri OX=623 GN=glk PE=3 SV=1	4.151	4	1	1	1	321	34.7	2.45
A0A6N3QV54	YciO family OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00295 PE=4 SV=1	2.505	5	1	1	1	218	24.5	2.42
I6CAS1	Phosphate transporter OS=Shigella flexneri K-315 OX=766150 GN=pitA PE=3 SV=1	2.321	2	1	1	1	499	53.4	2.42
A0A3U1D8Q6	C4-dicarboxylic acid transporter DauA OS=Shigella flexneri OX=623 GN=dauA PE=3 SV=1	2.51	2	1	1	1	559	59.4	2.39
A0A088CPT0	Scaffold protein OS=Enterobacteria phage Sfi101 OX=1524881 GN=Sfi101_0004 PE=4 SV=1	1.11	7	1	1	1	297	33.6	2.37
A0A2S8DET8	Galactose-1-phosphate uridylyltransferase OS=Shigella dysenteriae OX=622 GN=C5K18_07390 PE=3 SV=1	1.493	3	1	1	1	348	39.6	2.37
Q0SY57	ATP-dependent protease subunit HslV OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hslV PE=3 SV=1	2.205	6	1	1	1	176	19.1	2.36
A0A0H2V2Y1	Inositol-1-monophosphatase OS=Shigella flexneri OX=623 GN=suhB PE=3 SV=1	1.938	4	1	1	1	267	29.1	2.35
D2ADZ6	Glucose-6-phosphate isomerase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=pgi PE=3 SV=1	3.259	2	1	1	1	549	61.5	2.35
A0A4P7TM74	30S ribosomal protein S21 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsU PE=3 SV=1	1.134	11	1	1	1	71	8.5	2.34
I6CFZ0	50S ribosomal protein L27 OS=Shigella flexneri K-315 OX=766150 GN=rpmA PE=3 SV=1	3.531	16	1	1	1	85	9.1	2.33
A0A658Z1Y3	D-lactate dehydrogenase OS=Shigella flexneri OX=623 GN=idhA PE=3 SV=1	3.716	4	1	1	1	329	36.5	2.33
A0A6N3RN87	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ OS=Shigella flexneri 1485-80 OX=766155 GN=fabZ PE=3 SV=1	1.845	5	1	1	1	151	17	2.31
A0A6N3QNZ8	Inorganic pyrophosphatase OS=Shigella flexneri CDC 796-83 OX=945360 GN=ppa PE=3 SV=1	2.084	5	1	1	1	176	19.7	2.31
F5NQK6	Glutamine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=glnS PE=3 SV=1	3.019	2	1	1	1	554	63.4	2.31



F5P3V8	Fructose-1,6-bisphosphatase class I OS=Shigella flexneri K-227 OX=766147 GN=fbp PE=3 SV=1	3.437	3	1	1	1	332	36.8	2.3
A0A6N3RJ42	Glutaredoxin OS=Shigella flexneri 1485-80 OX=766155 GN=ydhD PE=3 SV=1	1.727	7	1	1	1	115	12.9	2.27
F5P3W0	UDP-N-acetylmuramate--L-alanyl-gamma-D-glutamyl-meso-2,6-diaminoheptandioate ligase OS=Shigella flexneri K-227 OX=766147 GN=mpI PE=3 SV=1	2.259	2	1	1	1	457	49.9	2.27
I6CFX7	Lipopolysaccharide export system protein LptC OS=Shigella flexneri K-315 OX=766150 GN=lptC PE=3 SV=1	2.133	4	1	1	1	191	21.7	2.25
A0A6N3QPX8	Prophage P4 integrase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03306 PE=4 SV=1	1.181	2	1	1	1	422	48.2	2.24
A0A383JXW9	UPF0145 protein YbjQ OS=Shigella flexneri OX=623 GN=ybjQ PE=3 SV=1	2.362	11	1	1	1	107	11.4	2.23
A0A658YTG8	Putative F0F1-type ATP synthase, subunit b OS=Shigella flexneri OX=623 GN=SAMEA3710514_00636 PE=4 SV=1	1.398	6	1	1	1	160	18.9	2.23
Q0SY18	Transcription termination/antitermination protein NusG OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nusG PE=3 SV=1	3.256	6	1	1	1	181	20.5	2.22
A0A2S8DRJ3	Tryptophan--tRNA ligase OS=Shigella boydii OX=621 GN=trpS PE=3 SV=1	1.441	3	1	1	1	334	37.4	2.22
A0A2S8DRX8	Sulfate ABC transporter substrate-binding protein OS=Shigella boydii OX=621 GN=cysP PE=3 SV=1	2.481	3	1	1	1	338	37.7	2.22
P0A7Z3	Ribose-5-phosphate isomerase A OS=Shigella flexneri OX=623 GN=rpiA PE=3 SV=1	2.251	5	1	1	1	219	22.8	2.2
F5P3E9	3'(2),5'-bisphosphate nucleotidase CysQ OS=Shigella flexneri K-227 OX=766147 GN=cysQ PE=3 SV=1	1.963	4	1	1	1	230	25.4	2.17
A0A6N3RQ35	DNA protection during starvation protein OS=Shigella flexneri 1485-80 OX=766155 GN=dps PE=3 SV=1	1.396	5	1	1	1	167	18.7	2.15
A0A3T2UTU6	Sensor protein OS=Shigella flexneri OX=623 GN=DK174_07305 PE=4 SV=1	2	2	1	1	1	598	67.1	2.13
A0A6N3RQ53	Molybdenum cofactor biosynthesis protein B OS=Shigella flexneri 1485-80 OX=766155 GN=moaB PE=3 SV=1	1.985	5	1	1	1	170	18.7	2.09
I6C8L3	Fumarate reductase subunit C OS=Shigella flexneri K-315 OX=766150 GN=frdC PE=3 SV=1	1.596	7	1	1	1	120	13.6	2.05
Q83J96	Phosphoenolpyruvate carboxykinase (ATP) OS=Shigella flexneri OX=623 GN=pckA PE=3 SV=4	2.58	2	1	1	1	540	59.6	2.03
A0A2S4MZ01	Co-chaperone protein HscB OS=Shigella flexneri OX=623 GN=hscB PE=3 SV=1	2.819	6	1	1	1	171	20.1	2.01
A0A658YV63	Bifunctional acyl-[acyl carrier protein] synthetase/2-acylglycerophosphoethanolamine acyltransferase OS=Shigella flexneri OX=623 GN=aas PE=4 SV=1	2.796	2	1	1	1	719	80.6	2.01
A0A2S8DHN0	Ubiquinone/menaquinone biosynthesis C-methyltransferase UbiE OS=Shigella dysenteriae OX=622 GN=ubiE PE=3 SV=1	3.237	5	1	1	1	251	28.1	1.99
I6C9X3	50S ribosomal protein L14 OS=Shigella flexneri K-315 OX=766150 GN=rpL14 PE=3 SV=1	1.922	8	1	1	1	117	12.8	1.95
A0A6N3REG1	NADH-quinone oxidoreductase subunit H OS=Shigella flexneri 1485-80 OX=766155 GN=nuoH PE=3 SV=1	1.139	2	1	1	1	325	36.2	1.95
D2A949	Bifunctional protein f0LD OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=f0LD PE=3 SV=1	1.277	3	1	1	1	288	30.9	1.93
I6CVK9	Phenylalanine--tRNA ligase alpha subunit OS=Shigella flexneri K-315 OX=766150 GN=pheS PE=3 SV=1	1.133	2	1	1	1	327	36.8	1.93
A0A6N3QEL1	50S ribosomal protein L19 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rpL19 PE=3 SV=1	1.44	7	1	1	1	115	13.1	1.92
A0A3T2UZZ4	Glucans biosynthesis glucosyltransferase H OS=Shigella flexneri OX=623 GN=mdoH PE=3 SV=1	1.516	1	1	1	1	847	96.9	1.92
F5NTG0	2-dehydro-3-deoxyphosphooctonate aldolase OS=Shigella flexneri K-227 OX=766147 GN=kdsA PE=3 SV=1	2.871	4	1	1	1	284	30.8	1.91
F5P090	50S ribosomal protein L21 OS=Shigella flexneri K-227 OX=766147 GN=rpL21 PE=3 SV=1	1.654	12	1	1	1	103	11.5	1.88
A0A1W2MD82	Protease HtpX OS=Shigella flexneri OX=623 GN=htpX PE=3 SV=1	2.949	4	1	1	1	293	31.9	1.88
A0A2S8DX13	Aromatic amino acid transporter AroP OS=Shigella boydii OX=621 GN=C5K23_02025 PE=4 SV=1	0.99	2	1	1	1	457	49.7	1.86
I6D6Z3	Ribosome-recycling factor OS=Shigella flexneri K-315 OX=766150 GN=frr PE=3 SV=1	1.108	4	1	1	1	185	20.6	1.82
Q0T0X5	Fructose-bisphosphate aldolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fba PE=3 SV=1	1.84	2	1	1	1	387	42.7	1.82
A0A2Y4XGG6	Transcriptional regulatory protein RcsB OS=Shigella flexneri OX=623 GN=rbsB PE=3 SV=1	1.055	3	1	1	1	216	23.6	1.8
A0A4P7TPX8	Ferrichrome porin FhuA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=fhuA PE=3 SV=1	2.108	2	1	1	1	747	82.1	1.77
I6D6W3	Poly(A) polymerase I OS=Shigella flexneri K-315 OX=766150 GN=pcnB PE=3 SV=1	1.257	2	1	1	1	454	52.5	1.77
A0A2A2XF62	Cell division ATP-binding protein FtsE OS=Shigella flexneri OX=623 GN=ftsE PE=3 SV=1	1.011	3	1	1	1	222	24.4	1.76
A0A6N3RFD0	Dihydrofolate synthase/folypolyglutamate synthase OS=Shigella flexneri 1485-80 OX=766155 GN=folC PE=3 SV=1	1.865	2	1	1	1	422	45.3	1.76
D2ABV0	ATP-dependent RNA helicase RhlB OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rhlB PE=3 SV=1	1.482	3	1	1	1	421	47.1	1.7
A0A2S8E9Y4	Acid-resistance membrane protein OS=Shigella dysenteriae OX=622 GN=hdeD_2 PE=4 SV=1	1.198	4	1	1	1	190	20.9	1.66
A0A2Y4XP64	Oligopeptide ABC transporter periplasmic oligopeptide-binding protein OS=Shigella flexneri OX=623 GN=oppA PE=4 SV=1	4.138	3	1	1	1	442	50.4	1.66
A0A6N3RBA5	4-alpha-L-fucosyltransferase domain protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4232 PE=4 SV=1	2.478	15	1	1	1	94	10.3	0
A0A1Z1VVH5	Protein QmcA OS=Shigella flexneri Y OX=424720 PE=3 SV=1	1.084	10	1	1	1	325	35.8	0
I6CEP4	Cytochrome c-type biogenesis protein CcmF OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3858 PE=3 SV=1	1.004	9	1	1	1	532	59	0
A0A6G5Z524	ParA family plasmid-partitioning AAA ATPase OS=Shigella flexneri OX=623 GN=GE167_23555 PE=4 SV=1	1.094	9	1	1	1	208	23	0
A0A6N3RBN2	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3951 PE=4 SV=1	1.156	9	1	1	1	101	11.2	0
I6CCX3	Sec-independent protein translocase protein TatC OS=Shigella flexneri K-315 OX=766150 GN=tatC PE=3 SV=1	1.335	9	1	1	1	258	28.9	0
Q5IPA5	Aerobic respiratory control protein (Fragment) OS=Shigella flexneri OX=623 GN=arcA PE=4 SV=1	2.585	8	1	1	1	182	21	0
Q0T2S7	Cytochrome c-type biogenesis protein CcmE OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ccmE PE=3 SV=1	1.167	7	1	1	1	159	17.7	0

D2AH71	Universal stress protein F OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=uspF PE=3 SV=1	2.098	6	1	1	1	168	18.4	0
A0A2Y4YFW7	Adenylate kinase OS=Shigella flexneri OX=623 GN=adk PE=3 SV=1	1.126	5	1	1	1	234	25.8	0
A0A6N3QPL6	Low affinity tryptophan permease OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01246 PE=4 SV=1	1.414	5	1	1	1	298	32.6	0
I6CPK6	Attaching and effacing protein OS=Shigella flexneri K-315 OX=766150 GN=eaeH PE=3 SV=1	1.632	4	1	1	1	583	64.8	0
A0A2S8DBY7	Iron ABC transporter ATP-binding protein FetA OS=Shigella dysenteriae OX=622 GN=C5K18_13250 PE=4 SV=1	1.573	4	1	1	1	225	25.4	0
A0A658Z3M3	Mce-like protein OS=Shigella flexneri OX=623 GN=SAMEA3710514_04035 PE=4 SV=1	2.288	4	1	1	1	227	24.9	0
D2ABQ4	Transposon Tn7 transposition protein tnsC OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4148 PE=4 SV=1	1.379	4	1	1	1	555	63	0
A0A2S8DH97	Pyridoxine/pyridoxamine 5'-phosphate oxidase OS=Shigella dysenteriae OX=622 GN=pxdH PE=3 SV=1	1.735	4	1	1	1	218	25.6	0
A0A380B7X8	Carbonic anhydrase OS=Shigella flexneri OX=623 GN=can PE=3 SV=1	1.776	4	1	1	1	220	25.1	0
A0A6L6UWY8	Flavodoxin family protein OS=Shigella flexneri OX=623 GN=mdaB PE=4 SV=1	1.007	4	1	1	1	193	22	0
A0A658YXQ5	Dihydropyrimidine dehydrogenase OS=Shigella flexneri OX=623 GN=preA PE=4 SV=1	1.451	3	1	1	1	289	31.2	0
A0A6N3QEH3	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04791 PE=3 SV=1	1.103	3	1	1	1	391	42.9	0
A0A2Y4ELK4	Phosphate import ATP-binding protein PstB OS=Shigella flexneri OX=623 GN=pstB PE=3 SV=1	1.233	3	1	1	1	257	29	0
A0A6N3QWE0	33 kDa chaperonin OS=Shigella flexneri CDC 796-83 OX=945360 GN=hsIO PE=3 SV=1	1.557	3	1	1	1	294	32.7	0
A0A4P7TPX0	Acyl-[acyl-carrier-protein]-UDP-N-acetylglucosamine O-acyltransferase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=lpxA PE=3 SV=1	1.231	3	1	1	1	262	28.1	0
D2AJ35	Delta-aminolevulinic acid dehydratase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=hemB PE=3 SV=1	2.353	3	1	1	1	335	36.9	0
Q32SZ3	Acyl_transf_3 domain-containing protein OS=Shigella flexneri OX=623 PE=4 SV=1	2.198	3	1	1	1	349	40.1	0
Q0T4N5	Putative oxidoreductase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ydfG PE=3 SV=1	1.037	3	1	1	1	248	27.2	0
A0A6N3RM87	Lipoyl synthase OS=Shigella flexneri 1485-80 OX=766155 GN=lipA PE=3 SV=1	1.374	3	1	1	1	321	36.1	0
A0A6N3RFW5	Penicillin-binding protein 7 OS=Shigella flexneri 1485-80 OX=766155 GN=pbpG PE=3 SV=1	1.772	3	1	1	1	293	32.5	0
A0A6N3QJ05	Macrolide-specific efflux protein MacA OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04622 PE=3 SV=1	1.813	3	1	1	1	371	40.6	0
D2AAC5	Putative lipase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yhjY PE=4 SV=1	0.997	3	1	1	1	234	26.2	0
Q0T3A5	Histidine biosynthesis bifunctional protein HisB OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hisB PE=3 SV=1	1.075	3	1	1	1	355	40.1	0
F5P2C5	L-threonine 3-dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=tdh PE=3 SV=1	2.152	2	1	1	1	341	37.3	0
I6CDH9	DNA polymerase I OS=Shigella flexneri K-315 OX=766150 GN=polA PE=3 SV=1	2.934	2	1	1	1	605	68.1	0
F5P301	Glycogen synthase OS=Shigella flexneri K-227 OX=766147 GN=glgA PE=3 SV=1	1.152	2	1	1	1	477	52.8	0
A0A2S8DL49	NADH-quinone oxidoreductase subunit N OS=Shigella boydii OX=621 GN=nuoN PE=3 SV=1	2.436	2	1	1	1	485	52	0
A0A6N3RBM8	Diguanylate cyclase OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4553 PE=4 SV=1	2.148	2	1	1	1	633	71.2	0
A0A0H2V319	Putative amino acid/amine transport protein OS=Shigella flexneri OX=623 GN=yifK PE=4 SV=1	1.219	2	1	1	1	417	45.3	0
A0A6N3QI04	NiFe hydrogenase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03645 PE=3 SV=1	1.228	2	1	1	1	372	39.6	0
A0A3U1D6H5	Erythronate-4-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=pxdB PE=3 SV=1	1.061	2	1	1	1	378	41.3	0
A0A6N3RLZ1	Cytochrome d ubiquinol oxidase, subunit II OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0759 PE=3 SV=1	1.489	2	1	1	1	379	42.4	0
A0A658Y5Y7	Phosphatidylserine synthase OS=Shigella flexneri OX=623 GN=pssA PE=3 SV=1	1.103	2	1	1	1	451	52.9	0
A0A2S8DDN5	Glutamine synthetase OS=Shigella dysenteriae OX=622 GN=glnA PE=3 SV=1	1.16	2	1	1	1	461	51	0
A0A4P7TR77	Maltoporin OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=lamB PE=2 SV=1	1.447	2	1	1	1	446	50	0
D2AE43	DUF853 domain-containing protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4608 PE=4 SV=1	1.034	2	1	1	1	516	56.2	0

**Appx. D.4: Full protein identification from band 4 of Figure 5.2b.**

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score	Sequest HT
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=rfc PE=3 SV=1	28.508	18	9	128	9	382	43.7	273.61	
I6C8N1	Modulator of FtsH protease HflC OS=Shigella flexneri K-315 OX=766150 GN=hflC PE=3 SV=1	84.418	68	26	40	26	334	37.6	111.58	
Q0T240	Outer membrane protein assembly factor BamC OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nlpB PE=3 SV=1	83.12	43	12	38	12	344	36.8	126.93	
P0AG02	ECA polysaccharide chain length modulation protein OS=Shigella flexneri OX=623 GN=wzzE PE=3 SV=2	46.595	51	14	24	14	348	39.5	64.52	
I6CHF3	MFS transporter, sugar porter family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3768 PE=3 SV=1	14.977	11	6	14	6	472	51.7	42.9	
A0A6N3QM36	Protein QmcA OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01963 PE=3 SV=1	33.743	53	13	13	13	305	33.7	41.53	
Q0T6G6	UPF0194 membrane protein YbhG OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ybhG PE=3 SV=1	27.996	31	9	13	9	331	36.3	38.17	
A0A2S4MYF8	Outer membrane protein C OS=Shigella flexneri OX=623 GN=ompC PE=1 SV=1	23.252	23	9	12	9	373	41.4	32.2	
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	27.828	20	10	12	10	474	49.3	34.77	
D2A6V5	Chain length determinant protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_2324 PE=4 SV=1	17.931	33	8	9	8	337	37.9	26.9	
A0A4P7TR77	Maltoporin OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=lamB PE=2 SV=1	27.202	21	4	9	4	446	50	32.05	
Q0SY20	Elongation factor Tu 2 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tuf2 PE=3 SV=1	14.419	21	7	8	7	394	43.3	19.81	
A0A6N3RQ35	DNA protection during starvation protein OS=Shigella flexneri 1485-80 OX=766155 GN=dps PE=3 SV=1	15.989	37	6	7	6	167	18.7	22.11	
A0A2A2XR75	EIIAB-Man OS=Shigella flexneri OX=623 GN=manX_2 PE=4 SV=1	15.543	27	6	6	6	323	35	17.21	
A0A2S8DDU2	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OS=Shigella dysenteriae OX=622 GN=murG PE=3 SV=1	15.271	23	6	6	6	355	37.8	19.67	
Q0T4H3	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1619 PE=4 SV=1	12.028	20	6	6	6	314	33.9	14.92	
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	14.571	19	6	6	6	329	36.5	16.97	
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	10.866	13	5	6	5	376	41	18.63	
Q0T2PI	sn-glycerol-3-phosphate permease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=glpT PE=3 SV=1	11.165	12	4	6	4	452	50.4	15.2	
A0A6N3R6K7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4998 PE=3 SV=1	11.779	9	6	6	6	402	42.7	17.8	
A0A6N3R6J7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4987 PE=3 SV=1	10.846	8	4	6	4	446	47.8	13.87	
Q0SYD1	L-lactate dehydrogenase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lldD PE=3 SV=1	14.088	15	5	5	5	396	42.7	14.51	
A0A0H2V319	Putative amino acid/amine transport protein OS=Shigella flexneri OX=623 GN=yifK PE=4 SV=1	7.811	9	4	5	4	417	45.3	10.82	
A0A383JRI2	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=hflB PE=3 SV=1	9.319	9	5	5	5	647	71	13.86	
A0A2S8DFP5	Cell division protein DedD OS=Shigella dysenteriae OX=622 GN=dedD PE=3 SV=1	8.336	34	4	4	4	220	22.9	11.64	
I6CFU5	Peptidase Do OS=Shigella flexneri K-315 OX=766150 GN=degS PE=3 SV=1	10.911	17	4	4	4	355	37.6	12.27	
A0A6N3QLJ7	Magnesium transport protein CorA OS=Shigella flexneri CDC 796-83 OX=945360 GN=corA PE=3 SV=1	8.098	16	4	4	4	316	36.6	11.09	
I6CAV2	Cell division protein FtsX OS=Shigella flexneri K-315 OX=766150 GN=ftsX PE=3 SV=1	8.071	16	4	4	4	352	38.5	11.23	
A0A4P7TR58	NAD(P)/FAD-dependent oxidoreductase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_010675 PE=4 SV=1	13.461	10	2	4	2	434	47.3	13.71	
A0A2Y5B4B8	Malate dehydrogenase OS=Shigella flexneri OX=623 GN=mdh PE=3 SV=1	3.051	9	3	4	3	312	32.3	5.55	
A0A3U1DEW5	Formate C-acetyltransferase OS=Shigella flexneri OX=623 GN=pflB PE=3 SV=1	8.791	8	4	4	4	760	85.3	11.12	
A0A0H2VYI3	Nucleoside permease OS=Shigella flexneri OX=623 GN=nupC PE=3 SV=1	4.792	7	3	4	3	400	43.5	7.08	
I6D3P2	Tol-Pal system protein TolR OS=Shigella flexneri K-315 OX=766150 GN=tolR PE=3 SV=1	5.697	23	3	3	3	142	15.4	5.09	
A0A6N3QME5	50S ribosomal protein L1 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rlpA PE=3 SV=1	7.75	15	3	3	3	234	24.7	8.97	
A0A6N3RAZ3	Cell division protein FtsN OS=Shigella flexneri 1485-80 OX=766155 GN=ftsN PE=3 SV=1	8.2	12	3	3	3	284	31.8	9.65	
A0A1S9JMU3	Cell shape-determining protein MreB OS=Shigella boydii OX=621 GN=mreB PE=3 SV=1	6.986	11	3	3	3	347	36.9	8.92	
A0A2S8DKM8	Lipoprotein-releasing ABC transporter permease subunit LolC OS=Shigella boydii OX=621 GN=lolC PE=3 SV=1	5.335	10	3	3	3	399	43.3	6.69	
A0A1Q8MQJ7	Protein RecA OS=Shigella boydii OX=621 GN=recA PE=3 SV=1	3.218	10	3	3	3	353	38	6.66	
A0A2S8DD79	Threonine/serine transporter TdcC OS=Shigella dysenteriae OX=622 GN=tdcC PE=3 SV=1	9.808	9	3	3	3	443	48.9	10.51	
A0A383JXY5	NAD dependent epimerase/dehydratase family OS=Shigella flexneri OX=623 GN=DOD76_09530 PE=4 SV=1	7.506	9	3	3	3	337	38	8.56	
A0A658YV21	Auxiliary transport protein, membrane fusion protein (MFP) family OS=Shigella flexneri OX=623 GN=macA_2 PE=4 SV=1	6.738	9	3	3	3	355	38.7	8.15	
A0A658Z1V1	UPF0283 membrane protein YcjF OS=Shigella flexneri OX=623 GN=ycjF PE=3 SV=1	7.008	9	3	3	3	353	39.4	4.82	
A0A1S9KIA0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	4.582	8	3	3	3	419	47	7.43	

D2A917	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	3.498	18	2	2	2	2	124	13.7	5.75
F5NUJ0	Glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1790 PE=4 SV=1	8.143	18	2	2	2	2	244	25.7	7.84
A0A4P7TQT3	50S ribosomal protein L10 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpLJ PE=3 SV=1	3.116	12	2	2	2	2	165	17.7	4.15
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	2.044	9	2	2	2	2	206	23.4	5.1
A0A6H1JMP7	33 kDa chaperonin OS=Shigella flexneri OX=623 GN=hsI0 PE=3 SV=1	2.093	6	2	2	2	2	289	32.2	4.32
A0A379Z1S8	Protein HflK OS=Shigella flexneri OX=623 GN=hflK PE=3 SV=1	4.575	6	2	2	2	2	419	45.6	5.71
I6D072	Outer membrane protein A OS=Shigella flexneri K-315 OX=766150 GN=ompA PE=3 SV=1	3.663	6	2	2	2	2	344	37	5.1
A0A3T2UX48	Outer membrane protein F OS=Shigella flexneri OX=623 GN=ompF PE=3 SV=1	5.007	6	2	2	2	2	362	39.3	4.62
A0A2Y4B9I6	Outer membrane-specific lipoprotein transporter subunit LolE OS=Shigella flexneri OX=623 GN=lolE PE=3 SV=1	4.183	6	2	2	2	2	412	45.1	7.66
A0A658Y983	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degQ PE=3 SV=1	1.995	5	2	2	2	2	455	47.2	4.77
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	3.082	4	2	2	2	2	477	50.7	4.67
A0A658YVY8	L-tryptophan indole-lyase OS=Shigella flexneri OX=623 GN=tnaA PE=3 SV=1	2.432	4	2	2	2	2	471	52.8	4.97
F5NZ29	Serine transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3429 PE=4 SV=1	2.571	3	2	2	2	2	429	46.9	4.55
A0A2S8D6C3	Proline-specific permease ProY OS=Shigella dysenteriae OX=622 GN=C5K18_23740 PE=4 SV=1	4.572	3	1	2	1	1	457	50.1	4.31
A0A6N3RKB3	KefA OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0517 PE=3 SV=1	2.908	2	2	2	2	2	1135	128.5	4.77
A0A2Y4XMF0	Sec-independent protein translocase protein TatA OS=Shigella flexneri OX=623 GN=tatA PE=3 SV=1	0.769	42	1	1	1	1	89	9.7	0
A0A4P7TN66	TfoX_C domain-containing protein OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_011525 PE=4 SV=1	0.769	19	1	1	1	1	83	9.2	0
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	3.125	15	1	1	1	1	78	8.3	3.05
A0A2S8DV96	50S ribosomal protein L5 OS=Shigella boydii OX=621 GN=rpLE PE=3 SV=1	6.08	12	1	1	1	1	179	20.3	4.51
A0A2Y4XR11	Chaperonin GroEL OS=Shigella flexneri OX=623 GN=groEL PE=4 SV=1	2.132	11	1	1	1	1	160	16.5	2.89
A0A6N3QHY3	Inner membrane component of tripartite multidrug resistance system OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04692 PE=3 SV=1	0.773	10	1	1	1	1	343	36.9	0
A0A6N3RAY3	50S ribosomal protein L15 OS=Shigella flexneri 1485-80 OX=766155 GN=rpIO PE=3 SV=1	2.055	10	1	1	1	1	126	13.1	3.4
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	2.434	9	1	1	1	1	129	13.8	2.3
F5P3C9	30S ribosomal protein S13 OS=Shigella flexneri K-227 OX=766147 GN=rpsM PE=3 SV=1	1.231	8	1	1	1	1	118	13.1	2.2
A0A6H1JH06	30S ribosomal protein S5 OS=Shigella flexneri OX=623 GN=rpsE PE=3 SV=1	2.625	7	1	1	1	1	167	17.6	2.78
Q83PC3	50S ribosomal protein L11 OS=Shigella flexneri OX=623 GN=rpLK PE=3 SV=3	2.057	7	1	1	1	1	142	14.9	3.12
A0A6N3R9K0	Phosphotransferase system, EII family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_2851 PE=4 SV=1	5.943	7	1	1	1	1	345	36.5	4.49
A0A2Y4XZJ9	Ferrochelatase OS=Shigella flexneri OX=623 GN=hemH PE=3 SV=1	1.861	7	1	1	1	1	320	35.9	2.54
A0A2Y4XVQ2	Outer membrane protein assembly complex subunit YfgL OS=Shigella flexneri OX=623 GN=yfgL PE=4 SV=1	1.748	7	1	1	1	1	184	20.1	3.54
F5NQ21	Cell division protein FtsQ OS=Shigella flexneri K-227 OX=766147 GN=ftsQ PE=3 SV=1	1.495	7	1	1	1	1	276	31.4	2.14
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	4.427	6	1	1	1	1	513	55.2	3.93
I6C9W3	50S ribosomal protein L3 OS=Shigella flexneri K-315 OX=766150 GN=rpLC PE=3 SV=1	1.524	6	1	1	1	1	209	22.2	0
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=Igkc PE=1 SV=1	3.462	6	1	1	1	1	219	24.2	3.48
A0A4P7TME5	Osmotic shock tolerance protein YggT OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=yggT PE=3 SV=1	1.616	6	1	1	1	1	188	21.1	3.12
A0A4P7TTH9	30S ribosomal protein S2 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsB PE=3 SV=1	1.914	6	1	1	1	1	241	26.8	2.29
F5NUR0	Threonine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=thrS PE=3 SV=1	0.802	5	1	1	1	1	642	74	0
I6CBV0	Uracil-xanthine permease family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_4394 PE=3 SV=1	4.579	5	1	1	1	1	463	48.9	3.4
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsC PE=3 SV=1	1.524	5	1	1	1	1	224	24.8	2.43
Q7AK95	Chloramphenicol acetyltransferase OS=Shigella flexneri 2b OX=1288825 GN=cat PE=3 SV=1	1.351	4	1	1	1	1	219	25.6	2.58
I6CVK9	Phenylalanine--tRNA ligase alpha subunit OS=Shigella flexneri K-315 OX=766150 GN=pheS PE=3 SV=1	2.029	4	1	1	1	1	327	36.8	2.49
Q83MF6	Cell division protein FtsZ OS=Shigella flexneri OX=623 GN=ftsZ PE=3 SV=2	4.548	3	1	1	1	1	383	40.1	3.8
A0A2S8DK39	Adenosine deaminase OS=Shigella boydii OX=621 GN=add PE=3 SV=1	1.327	3	1	1	1	1	333	36.3	1.82
A0A658XXS4	Outer membrane protein assembly factor YaeT OS=Shigella flexneri OX=623 GN=yaeT PE=4 SV=1	2.291	3	1	1	1	1	810	90.5	2.4
A0A6N3R832	Fumarate reductase flavoprotein subunit OS=Shigella flexneri 1485-80 OX=766155 GN=frdA PE=3 SV=1	5.976	3	1	1	1	1	561	62	4.71
A0A2Y4Y1X7	D-alanine/D-serine/glycine permease OS=Shigella flexneri OX=623 GN=cycA PE=4 SV=1	1.168	3	1	1	1	1	470	51.7	0
Q0T5R2	Spermidine/putrescine import ATP-binding protein PotA OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=potA PE=3 SV=1	2.998	3	1	1	1	1	378	43.1	2.9
A0A6N3RBI6	Protein translocase subunit SecY OS=Shigella flexneri 1485-80 OX=766155 GN=prlA PE=3 SV=1	0.934	3	1	1	1	1	443	48.5	2.19
F5NVZ8	Outer membrane porin protein LC OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2311 PE=3 SV=1	2.386	3	1	1	1	1	360	39.7	2.3
A0A0H2V3T2	L-lactate permease OS=Shigella flexneri OX=623 GN=SF4369 PE=3 SV=1	0.985	3	1	1	1	1	300	32.5	2.29

I6D6T0	Aromatic amino acid transport protein AroP OS=Shigella flexneri K-315 OX=766150 GN=aroP PE=4 SV=1	0.878	3	1	1	1	456	49.6	2.59
A0A6A9TRT7	ATP synthase subunit beta OS=Shigella flexneri OX=623 GN=atpD PE=3 SV=1	2.174	2	1	1	1	460	50.3	2.76
A0A6N3QQN1	Trehalosemaltose-specific PTS system components IIBC OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02485 PE=4 SV=1	2.424	2	1	1	1	473	51.2	1.85
A0A6N3QJ73	Fructose-specific PTS system IIBC component OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03096 PE=4 SV=1	2.931	2	1	1	1	563	57.5	3.71
A0A2S8D4S3	Aldehyde-alcohol dehydrogenase OS=Shigella dysenteriae OX=622 GN=C5K18_27725 PE=3 SV=1	3.659	2	1	1	1	891	96.1	3.67
Q83PS4	C4-dicarboxylate transport protein OS=Shigella flexneri OX=623 GN=dctA PE=3 SV=1	0.837	2	1	1	1	428	45.4	2.24
I6CMC4	Lysine-specific permease OS=Shigella flexneri K-315 OX=766150 GN=lysP PE=4 SV=1	2.049	2	1	1	1	489	53.5	2.6
A0A383JWV6	Membrane protein insertase YidC OS=Shigella flexneri OX=623 GN=yidC PE=3 SV=1	1.539	2	1	1	1	548	61.5	1.88
A0A2Y4X2P5	Proline/glycine betaine transporter OS=Shigella flexneri OX=623 GN=proP_1 PE=4 SV=1	0.796	2	1	1	1	366	40.2	0
Q0T0J7	DNA primase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dnaG PE=3 SV=1	1.017	2	1	1	1	581	65.6	1.65
A0A2S8DPK5	Copper exporting ATPase OS=Shigella boydii OX=621 GN=copA PE=3 SV=1	1.131	1	1	1	1	834	87.9	2.42
A0A658Y8X2	Outer membrane protein IcsA OS=Shigella flexneri OX=623 GN=icsA_2 PE=4 SV=1	0.769	1	1	1	1	1092	114.9	1.94

**Appx. D.5: Full protein identification from band 5 of Figure 5.2b.**

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score	Sequest HT
F5NRI1	Potassium efflux system kefA OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0718 PE=3 SV=1	223.39	40	41	104	41	1120	127.1	314.98	
A0A383JRI2	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=hflB PE=3 SV=1	82.001	34	21	37	21	647	71	95.25	
A0A2S8D4S3	Aldehyde-alcohol dehydrogenase OS=Shigella dysenteriae OX=622 GN=C5K18_27725 PE=3 SV=1	89.934	31	23	29	23	891	96.1	76.4	
F5NPX4	LPS-assembly protein LptD OS=Shigella flexneri K-227 OX=766147 GN=lptD PE=3 SV=1	79.392	36	21	24	21	753	86.3	69.63	
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=rfc PE=3 SV=1	22.457	18	9	24	9	382	43.7	42.12	
A0A2S4MYF8	Outer membrane protein C OS=Shigella flexneri OX=623 GN=ompC PE=1 SV=1	45.992	24	11	21	1	373	41.4	46.92	
Q0T1R3	Chaperone protein ClpB OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=clpB PE=3 SV=1	59.493	21	17	21	17	857	95.6	55.12	
A0A4P7TRN0	Pyruvate dehydrogenase E1 component OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=aceE PE=4 SV=1	52.867	22	17	19	17	887	99.6	51.34	
A0A658XXS4	Outer membrane protein assembly factor YaeT OS=Shigella flexneri OX=623 GN=yaeT PE=4 SV=1	49.043	21	15	18	15	810	90.5	51.25	
A0A380B6Y2	Efflux pump membrane transporter OS=Shigella flexneri OX=623 GN=acrB PE=3 SV=1	47.541	19	16	17	16	1049	113.5	47.35	
D2AD66	DNA-directed RNA polymerase subunit beta OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpoB PE=3 SV=1	29.463	11	14	15	14	1342	150.5	32.77	
A0A3U1DEW5	Formate C-acetyltransferase OS=Shigella flexneri OX=623 GN=pfIB PE=3 SV=1	30.805	16	11	13	11	760	85.3	29.39	
A0A658YYQ4	NAD(P) transhydrogenase subunit alpha OS=Shigella flexneri OX=623 GN=pntA PE=3 SV=1	22.244	19	8	11	8	510	54.5	29.17	
D2ABV5	ECA polysaccharide chain length modulation protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=wzzE PE=3 SV=1	23.228	23	8	10	8	349	39.6	29.21	
Q0SZX8	Elongation factor Tu 1 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tuf1 PE=3 SV=1	24.453	22	8	10	8	394	43.3	27.86	
A0A2S8DV74	Glycerol kinase OS=Shigella boydii OX=621 GN=glpK PE=3 SV=1	22.13	19	8	10	8	502	56.1	28.02	
A0A6N3QN52	Lon protease OS=Shigella flexneri CDC 796-83 OX=945360 GN=lon PE=2 SV=1	21.682	14	10	10	10	799	89	22.86	
A0A2S8E636	Acetyltransferase component of pyruvate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=aceF PE=3 SV=1	18.932	13	7	10	7	630	66.1	23.74	
F5P070	Translation initiation factor IF-2 OS=Shigella flexneri K-227 OX=766147 GN=infB PE=3 SV=1	18.914	10	10	10	10	890	97.3	18.92	
A0A658YVY8	L-tryptophan indole-lyase OS=Shigella flexneri OX=623 GN=tnaA PE=3 SV=1	15.154	21	9	9	9	471	52.8	19.25	
Q0T4H5	NAD(P) transhydrogenase subunit beta OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pntB PE=3 SV=1	29.064	18	6	9	6	462	48.7	28.96	
A0A658Y003	DNA-directed RNA polymerase subunit OS=Shigella flexneri OX=623 GN=rpoC PE=3 SV=1	19.009	7	9	9	9	1407	155.1	13.94	
A0A2Y5I422	Diguanylate cyclase OS=Shigella flexneri OX=623 GN=yfgF_2 PE=4 SV=1	16.125	14	7	8	7	505	57.7	12.67	
A0A658YRQ2	Efflux pump membrane transporter OS=Shigella flexneri OX=623 GN=acrD PE=3 SV=1	22.106	8	7	8	7	1037	113.1	20.31	
F5P2J3	C4-dicarboxylate transport protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4734 PE=4 SV=1	14.171	13	5	7	5	395	41.7	18.54	
A0A2S8D6C0	Protein translocase subunit SecD OS=Shigella dysenteriae OX=622 GN=secD PE=3 SV=1	21.058	13	7	7	7	615	66.6	19.39	
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	12.115	13	6	7	6	513	55.2	9.92	
A0A6N3QUN6	Zinc/cadmium/mercury/lead-transporting ATPase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00074 PE=3 SV=1	22.03	12	7	7	7	732	76.7	17.7	
F5NRF0	Peptidylprolyl isomerase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0687 PE=4 SV=1	18.057	12	6	7	6	623	68.1	18.5	
F5P3A0	Elongation factor G OS=Shigella flexneri K-227 OX=766147 GN=fusA PE=3 SV=1	24.619	12	7	7	7	704	77.6	17.39	
A0A6N3R6K7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4998 PE=3 SV=1	14.002	9	7	7	7	402	42.7	18.03	
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	15.557	40	3	6	3	78	8.3	14.84	
D2A917	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	13.159	27	6	6	6	124	13.7	15.27	
A0A379Z256	60 kDa chaperonin OS=Shigella flexneri OX=623 GN=groEL PE=3 SV=1	17.394	16	6	6	6	551	57.5	19.15	
I6CBQ2	DNA gyrase subunit B OS=Shigella flexneri K-315 OX=766150 GN=gyrB PE=3 SV=1	19.189	8	5	6	5	804	89.9	17.08	
A0A380B6V8	Copper exporting ATPase OS=Shigella flexneri OX=623 GN=copA PE=3 SV=1	12.093	8	6	6	6	834	87.9	15.9	
A0A2S8DTV4	Phosphoenolpyruvate synthase OS=Shigella boydii OX=621 GN=ppsA PE=3 SV=1	11.88	7	6	6	6	792	87.4	10.85	
A0A6N3RB60	50S ribosomal protein L2 OS=Shigella flexneri 1485-80 OX=766155 GN=rpLB PE=3 SV=1	11.463	23	5	5	1	273	29.8	12.52	
I6C9W6	50S ribosomal protein L2 OS=Shigella flexneri K-315 OX=766150 GN=rpLB PE=3 SV=1	11.222	22	5	5	1	255	27.9	14.48	
A0A6A9TRT7	ATP synthase subunit beta OS=Shigella flexneri OX=623 GN=atpD PE=3 SV=1	10.573	12	5	5	5	460	50.3	11.84	
A0A3T2UX48	Outer membrane protein F OS=Shigella flexneri OX=623 GN=ompF PE=3 SV=1	16.268	12	4	5	4	362	39.3	13.78	
I6CJU2	Phosphate acetyltransferase OS=Shigella flexneri K-315 OX=766150 GN=pta PE=3 SV=1	14.035	8	5	5	5	709	76.5	13.15	
A0A6N3QJ73	Fructose-specific PTS system IIBC component OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03096 PE=4 SV=1	25.083	7	4	5	4	563	57.5	16.88	

A0A6N3R8Q1	Mechanosensitive ion channel family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_5021 PE=3 SV=1	7.939	6	5	5	5	5	1107	123.9	6.71
D2ABG4	30S ribosomal protein S1 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsA PE=3 SV=1	9.254	5	3	5	3	3	557	61.1	10.49
Q7BCK4	Outer membrane protein IcsA autotransporter OS=Shigella flexneri OX=623 GN=icsA PE=1 SV=1	11.773	5	5	5	5	5	1102	116.2	14.36
A0A2Y4MDE1	Bifunctional aspartokinase/homoserine dehydrogenase OS=Shigella flexneri OX=623 GN=thrA PE=3 SV=1	5.226	4	3	5	3	3	820	89	10.6
A0A6N3RAY3	50S ribosomal protein L15 OS=Shigella flexneri 1485-80 OX=766155 GN=rplO PE=3 SV=1	13.517	39	4	4	4	4	126	13.1	12.61
D2A8B5	Cytoplasmic membrane protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yrbD PE=4 SV=1	8.184	21	4	4	4	4	183	19.6	9.33
A0A1S9JMU3	Cell shape-determining protein MreB OS=Shigella boydii OX=621 GN=mreB PE=3 SV=1	10.472	15	4	4	4	4	347	36.9	10.84
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	12.202	9	4	4	4	4	474	49.3	9.78
A0A658YSU3	Bifunctional NADH:ubiquinone oxidoreductase subunit C/D OS=Shigella flexneri OX=623 GN=nuoC PE=3 SV=1	8.859	7	4	4	4	4	600	68.7	6.93
A0A380B6P9	PTS N-acetylglucosamine EIICBA component OS=Shigella flexneri OX=623 GN=nagE PE=4 SV=1	7.885	7	4	4	4	4	648	68.3	7.18
I6D3R0	Succinate dehydrogenase flavoprotein subunit OS=Shigella flexneri K-315 OX=766150 GN=sdhA PE=3 SV=1	7.163	6	3	4	3	3	588	64.4	8.98
A0A6N3RCV1	Alanine--tRNA ligase OS=Shigella flexneri 1485-80 OX=766155 GN=alaS PE=3 SV=1	11.589	5	4	4	4	4	876	96	11.46
A0A4V1CTY5	ATP-dependent Clp protease ATP-binding subunit ClpA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=clpA PE=3 SV=1	6.619	5	3	4	3	3	758	84.2	9.47
A0A2S8DUB6	Glycerol-3-phosphate acyltransferase (Fragment) OS=Shigella boydii OX=621 GN=C5K23_08135 PE=3 SV=1	4.823	5	3	4	3	3	667	75.5	6.58
Q0T6W4	Oxoglutarate dehydrogenase (succinyl-transferring) OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=sucA PE=3 SV=1	7.273	4	4	4	4	4	933	105	5.68
A0A4P7TP15	Cu(+)/Ag(+) efflux RND transporter permease subunit CusA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=cusA PE=3 SV=1	7.985	4	4	4	4	4	1059	115.8	8.99
Q83SN2	Protein translocase subunit SecA OS=Shigella flexneri OX=623 GN=secA PE=3 SV=1	6.624	4	3	4	3	3	901	102	9.58
Q5INY3	Clp protease ATP-binding subunit (Fragment) OS=Shigella flexneri OX=623 GN=clpX PE=4 SV=1	9.324	13	2	3	2	2	203	21.7	9.38
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	5.861	11	3	3	3	3	329	36.5	7.19
I6CXA4	Ribose-phosphate pyrophosphokinase OS=Shigella flexneri K-315 OX=766150 GN=prs PE=3 SV=1	6.911	11	3	3	3	3	312	33.9	7.31
F5P2M7	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4769 PE=4 SV=1	7.927	10	3	3	3	3	236	25.6	8.21
A0A1Q8MQJ7	Protein RecA OS=Shigella boydii OX=621 GN=recA PE=3 SV=1	7.56	10	3	3	3	3	353	38	9.14
D2A6V5	Chain length determinant protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_2324 PE=4 SV=1	5.433	9	3	3	3	3	337	37.9	8.37
I6D3P8	Cytochrome d ubiquinol oxidase subunit 1 OS=Shigella flexneri K-315 OX=766150 GN=cydA PE=3 SV=1	5.978	7	3	3	3	3	500	55.6	5.36
A0A0H2VYI3	Nucleoside permease OS=Shigella flexneri OX=623 GN=nupC PE=3 SV=1	6.397	7	3	3	3	3	400	43.5	6.97
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	7.291	7	3	3	3	3	477	50.7	7.21
Q0SXC2	Fumarate reductase flavoprotein subunit OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=frdA PE=3 SV=1	8.607	7	3	3	3	3	602	65.9	8.43
A0A658Z2B7	GDP/GTP pyrophosphokinase OS=Shigella flexneri OX=623 GN=reIA PE=3 SV=1	9.504	7	3	3	3	3	744	83.8	9.71
Q0T2Q0	DNA gyrase subunit A OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=gyrA PE=3 SV=1	7.356	4	3	3	3	3	875	96.9	6.56
F5P0N3	UvrABC system protein A OS=Shigella flexneri K-227 OX=766147 GN=uvrA PE=3 SV=1	8.103	4	3	3	3	3	940	103.8	7
A0A2S8DUS9	Phosphotransferase RcsD OS=Shigella boydii OX=621 GN=yojN PE=3 SV=1	8.385	3	2	3	2	2	886	99.8	5.79
A0A4P7TTY8	Autotransporter assembly complex protein TamB OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tamB PE=4 SV=1	8.973	3	3	3	3	3	1259	136.8	7.9
A0A4V1CUA5	50S ribosomal protein L28 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpmB PE=3 SV=1	3.352	26	2	2	2	2	78	9	4.69
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	7.204	24	2	2	2	2	129	13.8	6.11
F5NUJ0	Glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1790 PE=4 SV=1	2.365	11	2	2	2	2	244	25.7	1.86
A0A2S8D3U0	Catabolite activator protein OS=Shigella dysenteriae OX=622 GN=crp PE=4 SV=1	3.874	10	2	2	2	2	210	23.7	1.76
A0A6N3R9K0	Phosphotransferase system, EIIC family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_2851 PE=4 SV=1	11.152	10	2	2	2	2	345	36.5	9.03
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsC PE=3 SV=1	4.045	9	2	2	2	2	224	24.8	3.44
A0A380B9X9	Putative pyruvate/2-oxoglutarate dehydrogenase complex, dihydroliipoamide dehydrogenase (E3) component,-like enzyme OS=Shigella flexneri OX=623 GN=lpd_4 PE=4 SV=1	2.731	9	2	2	2	2	157	17.6	3.42
A0A658YVE7	Arabinose-proton symporter OS=Shigella flexneri OX=623 GN=araE_1 PE=4 SV=1	5.762	9	1	2	1	1	147	15.6	5.87
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	3.052	9	2	2	2	2	206	23.4	5.22
A0A4P7TR58	NAD(P)/FAD-dependent oxidoreductase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_010675 PE=4 SV=1	3.508	7	2	2	2	2	434	47.3	4.49
I6CI36	Cytoskeleton protein RodZ OS=Shigella flexneri K-315 OX=766150 GN=rodZ PE=3 SV=1	4.139	7	2	2	2	2	337	36.2	5.21
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	4.525	6	2	2	2	2	376	41	2.5
A0A3T2UQA1	Glutamate-1-semialdehyde 2,1-aminomutase OS=Shigella flexneri OX=623 GN=hemL PE=3 SV=1	5.678	6	2	2	2	2	426	45.3	3.91
A0A1S9KIA0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	4.582	6	2	2	2	2	419	47	4.91
Q6R2C3	Isocitrate dehydrogenase [NADP] (Fragment) OS=Shigella flexneri OX=623 GN=icd PE=3 SV=1	3.571	6	2	2	2	2	389	42.8	2.63
F5NVZ8	Outer membrane porin protein LC OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2311 PE=3 SV=1	3.986	5	2	2	1	1	360	39.7	3.21

A0A2S8DD79	Threonine/serine transporter TdcC OS=Shigella dysenteriae OX=622 GN=tdcC PE=3 SV=1	5.945	5	2	2	2	443	48.9	5.25
A0A3T2URE0	Catalase-peroxidase OS=Shigella flexneri OX=623 GN=katG PE=3 SV=1	5.081	4	2	2	2	726	80	4.84
A0A6N3R3T7	Aspartate ammonia-lyase OS=Shigella flexneri 1485-80 OX=766155 GN=aspA PE=3 SV=1	3.788	4	2	2	2	478	52.3	4.53
A0A383JVV6	Membrane protein insertase YidC OS=Shigella flexneri OX=623 GN=yidC PE=3 SV=1	5.54	4	2	2	2	548	61.5	2.78
A0A6N3R842	Adenylosuccinate synthetase OS=Shigella flexneri 1485-80 OX=766155 GN=purA PE=3 SV=1	2.368	4	2	2	2	432	47.3	1.86
I6CI73	Elongation factor 4 OS=Shigella flexneri K-315 OX=766150 GN=lepA PE=3 SV=1	3.478	3	2	2	2	599	66.6	4.23
A0A2Y4XRI8	Chaperone protein DnaK OS=Shigella flexneri OX=623 GN=dnaK PE=2 SV=1	3.405	3	2	2	2	626	67.7	5.19
F5NUR0	Threonine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=thrS PE=3 SV=1	2.739	3	2	2	2	642	74	2.19
A0A379ZPY0	Aerobic respiration control sensor protein OS=Shigella flexneri OX=623 GN=arcB PE=4 SV=1	4.868	3	2	2	2	778	87.9	4
Q32SZ3	Acyl_transf_3 domain-containing protein OS=Shigella flexneri OX=623 PE=4 SV=1	1.82	3	1	2	1	349	40.1	2.6
I6CDQ5	Bifunctional aspartokinase/homoserine dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=metL PE=3 SV=1	3.011	3	2	2	2	810	88.8	3.62
A0A4P7TVF5	Cyclic di-GMP-binding protein OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=bcsB PE=3 SV=1	3.347	3	2	2	2	779	86.1	1.65
D2ACF0	Fatty acid oxidation complex subunit alpha OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=fadB PE=3 SV=1	4.195	2	2	2	2	729	79.6	4.2
I6D1F5	Chromosome partition protein MukB OS=Shigella flexneri K-315 OX=766150 GN=mukB PE=3 SV=1	3.332	1	2	2	2	1486	170.1	4.16
A0A3Y2RQZ3	TRAP transporter small permease OS=Shigella flexneri OX=623 GN=DTY77_11710 PE=4 SV=1	1.602	16	1	1	1	170	18.6	0
Q8GKU0	DNA-binding protein OS=Shigella flexneri 2a OX=42897 GN=sfh PE=1 SV=1	1.106	15	1	1	1	134	15.1	2.51
A0A2S8DV96	50S ribosomal protein L5 OS=Shigella boydii OX=621 GN=rplE PE=3 SV=1	2.562	12	1	1	1	179	20.3	3.02
A0A4P7TM74	30S ribosomal protein S21 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsU PE=3 SV=1	1.199	11	1	1	1	71	8.5	0
I6C8W6	PTS system, glucose-like IIB component domain protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_4976 PE=4 SV=1	2.25	11	1	1	1	99	10.9	1.88
A0A6N3QMX0	Serine transporter OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02214 PE=4 SV=1	1.749	7	1	1	1	134	14.6	0
F5NXP5	Transposase DDE domain protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2922 PE=4 SV=1	1.053	7	1	1	1	448	51	0
A0A1Q8N7Q2	50S ribosomal protein L11 OS=Shigella dysenteriae OX=622 GN=rplK PE=3 SV=1	1.122	7	1	1	1	142	14.9	2.98
A0A4P7TQT3	50S ribosomal protein L10 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rplJ PE=3 SV=1	2.051	7	1	1	1	165	17.7	1.67
P84077	ADP-ribosylation factor 1 OS=Homo sapiens OX=9606 GN=ARF1 PE=1 SV=2	1.705	6	1	1	1	181	20.7	1.97
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=lgkc PE=1 SV=1	4.988	6	1	1	1	219	24.2	3.71
A0A4P7TTH9	30S ribosomal protein S2 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsB PE=3 SV=1	3.512	6	1	1	1	241	26.8	2.91
A0A6N3QME5	50S ribosomal protein L1 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rplA PE=3 SV=1	5.212	6	1	1	1	234	24.7	3.3
A0A2Y5ARN5	DNA-binding transcriptional regulator TyrR OS=Shigella flexneri OX=623 GN=tyrR PE=4 SV=1	3.514	5	1	1	1	219	25.1	2.91
A0A6N3QPL6	Low affinity tryptophan permease OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01246 PE=4 SV=1	2.216	5	1	1	1	298	32.6	1.8
Q0SZX1	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yheO PE=4 SV=1	2.297	5	1	1	1	240	26.8	2.67
I6CIP8	Oxidoreductase ucpA OS=Shigella flexneri K-315 OX=766150 GN=ucpA PE=4 SV=1	1.567	5	1	1	1	263	27.8	2.09
A0A2S8DNI4	RNA-binding transcriptional accessory protein (Fragment) OS=Shigella boydii OX=621 GN=C5K23_19395 PE=4 SV=1	2.916	4	1	1	1	304	34.4	1.93
A0A6N3RLN2	PotC OS=Shigella flexneri 1485-80 OX=766155 GN=potC PE=3 SV=1	1.421	4	1	1	1	264	29.1	0
A0A6N3RIF3	Enoyl-[acyl-carrier-protein] reductase [NADH] OS=Shigella flexneri 1485-80 OX=766155 GN=fabI PE=3 SV=1	2.49	4	1	1	1	262	27.8	1.99
A0A6N3QSE0	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00807 PE=3 SV=1	1.702	3	1	1	1	401	41.7	0
D2A8B1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=murA PE=3 SV=1	1.917	3	1	1	1	419	44.8	2.24
A0A6N3R6Z9	Lipopolysaccharide export system permease protein LptF OS=Shigella flexneri 1485-80 OX=766155 GN=yjgP PE=3 SV=1	3.698	3	1	1	1	335	36.6	3.11
Q0T2K3	NADH-quinone oxidoreductase subunit F OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nuoF PE=3 SV=1	4.163	3	1	1	1	445	49.4	2.71
A0A3Y2RM52	Phosphoglucosamine mutase OS=Shigella flexneri OX=623 GN=glmM PE=3 SV=1	1.635	3	1	1	1	445	47.5	1.76
A0A6N3RCM6	3-ketoacyl-CoA thiolase OS=Shigella flexneri 1485-80 OX=766155 GN=fadA PE=3 SV=1	1.269	3	1	1	1	387	40.8	2.38
A0A2X2I0N0	Cell division protein DamX OS=Shigella flexneri OX=623 GN=damX PE=3 SV=1	2.343	3	1	1	1	428	46.1	2
F5NZ29	Serine transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3429 PE=4 SV=1	3.266	3	1	1	1	429	46.9	3.02
I6CC01	L-lactate dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=lldD PE=3 SV=1	1.629	3	1	1	1	396	42.7	0
A0A4P7TKA6	Glycerol-3-phosphate dehydrogenase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=glpA PE=3 SV=1	1.47	3	1	1	1	542	58.9	1.78
Q0T2G2	3-oxoacyl-[acyl-carrier-protein] synthase I OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fabB PE=3 SV=1	1.212	3	1	1	1	370	39	1.67
I6D072	Outer membrane protein A OS=Shigella flexneri K-315 OX=766150 GN=ompA PE=3 SV=1	1.409	3	1	1	1	344	37	2.4
Q0SYE6	2-amino-3-ketobutyrate coenzyme A ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=klb PE=3 SV=1	1.473	3	1	1	1	398	43.2	1.61
A0A6N3RBT1	GTP-binding protein TypA/BipA OS=Shigella flexneri 1485-80 OX=766155 GN=typA PE=4 SV=1	1.599	2	1	1	1	607	67.4	0
Q0T2J7	Putative aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SfV_2357 PE=4 SV=1	3.363	2	1	1	1	405	45.5	2.46
A0A2S8DMU6	NAD-dependent epimerase OS=Shigella boydii OX=621 GN=C5K23_21730 PE=4 SV=1	1.14	2	1	1	1	334	37.6	0



A0A380AWS0	Diguanylate cyclase OS=Shigella flexneri OX=623 GN=yeaP PE=4 SV=1	1.294	2	1	1	1	341	38.5	0
A0A0H2VZX0	Thiol:disulfide interchange protein DsbD OS=Shigella flexneri OX=623 GN=dsbD PE=3 SV=1	2.155	2	1	1	1	565	61.8	2.18
A0A6N3R6J7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4987 PE=3 SV=1	1.602	2	1	1	1	446	47.8	2.28
A0A0H2V319	Putative amino acid/amine transport protein OS=Shigella flexneri OX=623 GN=yifK PE=4 SV=1	1.564	2	1	1	1	417	45.3	0
A0A379ZAE3	Dihydroxy-acid dehydratase OS=Shigella flexneri OX=623 GN=ilvD PE=3 SV=1	1.388	2	1	1	1	616	65.5	1.77
I6CVF4	Protease 4 OS=Shigella flexneri K-315 OX=766150 GN=sppA PE=3 SV=1	1.746	2	1	1	1	618	67.2	1.87
A0A6G5Z3B5	Biotin carboxylase OS=Shigella flexneri OX=623 GN=accC PE=4 SV=1	2.715	2	1	1	1	449	49.3	1.73
A0A4P7TKN2	O13/O129/O135 family O-antigen flippase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=wzx PE=4 SV=1	1.605	2	1	1	1	418	46.3	1.85
F5NY87	Phosphoenolpyruvate-protein phosphotransferase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3048 PE=3 SV=1	1.635	2	1	1	1	585	64.5	1.82
A0A3T2V110	Aspartate-tRNA ligase OS=Shigella flexneri OX=623 GN=aspS PE=3 SV=1	2.757	2	1	1	1	590	65.8	1.8
A0A2S8DX13	Aromatic amino acid transporter AroP OS=Shigella boydii OX=621 GN=C5K23_02025 PE=4 SV=1	1.36	2	1	1	1	457	49.7	1.66
A0A2S8D8F6	DNA topoisomerase 4 subunit B OS=Shigella dysenteriae OX=622 GN=parE PE=3 SV=1	1.259	2	1	1	1	630	70.2	2.05
A0A6N3REQ7	Nickel-dependent hydrogenase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3604 PE=3 SV=1	1.319	2	1	1	1	567	62.5	1.93
A0A380AGV7	Multidrug resistance protein MdtB OS=Shigella flexneri OX=623 GN=mdtB PE=3 SV=1	2.526	2	1	1	1	1040	112	1.75
A0A658YS67	Nitrite reductase [NAD(P)H], large subunit OS=Shigella flexneri OX=623 GN=nirB PE=4 SV=1	1.621	2	1	1	1	847	93	2.15
A0A658YVC1	Aconitate hydratase B OS=Shigella flexneri OX=623 GN=acnB PE=3 SV=1	2.594	1	1	1	1	874	94.5	3.65
A0A4P7TRQ9	Ribulokinase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=araB PE=3 SV=1	1.174	1	1	1	1	566	61.1	1.64
A0A3T2UYA0	Carbamoyl-phosphate synthase large chain OS=Shigella flexneri OX=623 GN=carB PE=3 SV=1	4.753	1	1	1	1	1073	117.8	3.35
A0A658YV63	Bifunctional acyl-[acyl carrier protein] synthetase/2-acylglycerophosphoethanolamine acyltransferase OS=Shigella flexneri OX=623 GN=aaS PE=4 SV=1	1.924	1	1	1	1	719	80.6	1.79
I6CDA5	ATP-dependent protease ATPase subunit HslU OS=Shigella flexneri K-315 OX=766150 GN=hslU PE=2 SV=1	1.066	1	1	1	1	443	49.6	1.79
A0A2S8DC69	AsmA2 domain-containing protein OS=Shigella dysenteriae OX=622 GN=C5K18_12630 PE=4 SV=1	2.593	1	1	1	1	1266	139	3.27
A0A379ZZL4	DNA mismatch repair protein MutS OS=Shigella flexneri OX=623 GN=mutS PE=3 SV=1	1.294	1	1	1	1	853	95.2	1.84
Q83Q93	Biosynthetic arginine decarboxylase OS=Shigella flexneri OX=623 GN=speA PE=3 SV=2	1.998	1	1	1	1	662	74.3	2.24
A0A380B802	DNA polymerase III subunit alpha OS=Shigella flexneri OX=623 GN=dnaE PE=4 SV=1	1.642	1	1	1	1	1160	129.7	1.96
A0A4P7TUP9	Efflux pump membrane transporter OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_018995 PE=3 SV=1	1.528	1	1	1	1	1037	111.5	1.94
A0A2S8DHT0	Methionine synthase OS=Shigella dysenteriae OX=622 GN=C5K18_00150 PE=3 SV=1	1.141	1	1	1	1	1227	136	1.66
A0A6L6UZB9	Ribonuclease E OS=Shigella flexneri OX=623 GN=me PE=3 SV=1	1.462	1	1	1	1	1063	118.4	1.92
A0A2S8D9R8	Penicillin-binding protein 1B OS=Shigella dysenteriae OX=622 GN=mrCB PE=3 SV=1	1.15	1	1	1	1	840	93.8	1.9

### Appx. D.6: Full protein identification from band 6 of Figure 5.2b.

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score Sequest HT
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=rfc PE=3 SV=1	27.751	18	9	72	9	382	43.7	145.8
A0A383JRI2	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=ftsH PE=3 SV=1	122.479	39	31	71	31	647	71	182.8
A0A658XXS4	Outer membrane protein assembly factor YaeT OS=Shigella flexneri OX=623 GN=yaeT PE=4 SV=1	131.501	43	32	57	32	810	90.5	170.8
A0A2S4MYF8	Outer membrane protein C OS=Shigella flexneri OX=623 GN=ompC PE=1 SV=1	78.413	40	15	33	1	373	41.4	82.9
A0A1Q8MQG3	Chaperone protein DnaK OS=Shigella boydii OX=621 GN=dnaK PE=2 SV=1	61.708	37	22	26	22	638	69.1	69.62
A0A380B9F2	Peptidylprolyl isomerase OS=Shigella flexneri OX=623 GN=ppiD PE=4 SV=1	72.389	43	19	25	1	623	68.1	84.09
D2ABV5	ECA polysaccharide chain length modulation protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=wzzE PE=3 SV=1	46.867	38	12	24	12	349	39.6	60.41
F5NPX4	LPS-assembly protein LptD OS=Shigella flexneri K-227 OX=766147 GN=lptD PE=3 SV=1	61.442	37	20	23	20	753	86.3	73.79
Q7BCK4	Outer membrane protein IcsA autotransporter OS=Shigella flexneri OX=623 GN=icsA PE=1 SV=1	38.452	12	10	18	10	1102	116.2	47.69
A0A2S8D4S3	Aldehyde-alcohol dehydrogenase OS=Shigella dysenteriae OX=622 GN=C5K18_27725 PE=3 SV=1	28.927	15	12	16	12	891	96.1	32.88
A0A379Z256	60 kDa chaperonin OS=Shigella flexneri OX=623 GN=groEL PE=3 SV=1	41.924	35	14	15	14	551	57.5	41.67
Q0SZX8	Elongation factor Tu 1 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tuf1 PE=3 SV=1	35.672	30	11	15	11	394	43.3	35.34
Q0T1R3	Chaperone protein ClpB OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=clpB PE=3 SV=1	34.629	16	13	14	13	857	95.6	34.4
A0A658YYQ4	NAD(P) transhydrogenase subunit alpha OS=Shigella flexneri OX=623 GN=pntA PE=3 SV=1	22.075	19	8	13	8	510	54.5	26.83
A0A2S8E636	Acetyltransferase component of pyruvate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=aceF PE=3 SV=1	21.914	16	9	11	9	630	66.1	26.3
A0A2Y5J422	Diguanylate cyclase OS=Shigella flexneri OX=623 GN=yfgF_2 PE=4 SV=1	23.911	16	8	11	8	505	57.7	22.56
A0A6N3RKB3	KefA OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0517 PE=3 SV=1	31.723	11	11	11	11	1135	128.5	30.74
A0A2S8DV74	Glycerol kinase OS=Shigella boydii OX=621 GN=glpK PE=3 SV=1	23.657	22	10	10	10	502	56.1	26.55
A0A3U1DEW5	Formate C-acetyltransferase OS=Shigella flexneri OX=623 GN=pflB PE=3 SV=1	23.937	14	10	10	10	760	85.3	21.35
A0A2S8D9R8	Penicillin-binding protein 1B OS=Shigella dysenteriae OX=622 GN=mrcB PE=3 SV=1	15.496	10	7	10	7	840	93.8	15.98
Q0T6Q1	LPS-assembly lipoprotein LptE OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lptE PE=3 SV=1	25.627	27	6	9	6	193	21.4	29.77
A0A658YVY8	L-tryptophan indole-lyase OS=Shigella flexneri OX=623 GN=tnaA PE=3 SV=1	18.147	20	9	9	9	471	52.8	19.7
A0A4P7TPX8	Ferrichrome porin FhuA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=fhuA PE=3 SV=1	32.225	13	8	9	8	747	82.1	25.82
F5NVZ8	Outer membrane porin protein LC OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2311 PE=3 SV=1	14.092	19	5	8	5	360	39.7	21.77
Q0T4H5	NAD(P) transhydrogenase subunit beta OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pntB PE=3 SV=1	22.809	16	5	8	5	462	48.7	26.37
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	17.313	16	8	8	8	513	55.2	20.37
A0A2S8D6C0	Protein translocase subunit SecD OS=Shigella dysenteriae OX=622 GN=secD PE=3 SV=1	20.139	15	8	8	8	615	66.6	19.12
D2ABG4	30S ribosomal protein S1 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsA PE=3 SV=1	16.037	13	7	8	7	557	61.1	11.34
A0A2Y5AZK8	Lon protease OS=Shigella flexneri OX=623 GN=lon PE=2 SV=1	19.752	13	8	8	8	654	72.9	17.32
A0A6N3RB60	50S ribosomal protein L2 OS=Shigella flexneri 1485-80 OX=766155 GN=rlpB PE=3 SV=1	16.344	21	5	7	1	273	29.8	17.47
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	13.179	15	6	7	6	477	50.7	19.13
A0A3T2UX48	Outer membrane protein F OS=Shigella flexneri OX=623 GN=ompF PE=3 SV=1	17.167	15	6	7	6	362	39.3	18.63
A0A380B6V8	Copper exporting ATPase OS=Shigella flexneri OX=623 GN=copA PE=3 SV=1	14.7	9	7	7	7	834	87.9	15.36
A0A6GSZ0X2	Autotransporter YejO OS=Shigella flexneri OX=623 GN=yejO PE=4 SV=1	10.85	6	5	7	5	863	91.2	17.32
I6C9W6	50S ribosomal protein L2 OS=Shigella flexneri K-315 OX=766150 GN=rlpB PE=3 SV=1	13.905	22	5	6	1	255	27.9	15.14
A0A0H2VYI3	Nucleoside permease OS=Shigella flexneri OX=623 GN=nupC PE=3 SV=1	8.641	11	5	6	5	400	43.5	9.52
A0A6N3R6K7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4998 PE=3 SV=1	13.586	9	6	6	6	402	42.7	18.38
A0A2X2I0N0	Cell division protein DamX OS=Shigella flexneri OX=623 GN=damX PE=3 SV=1	14.341	12	5	5	5	428	46.1	14.88
F5P3A0	Elongation factor G OS=Shigella flexneri K-227 OX=766147 GN=fusA PE=3 SV=1	14.541	9	5	5	5	704	77.6	13.94
A0A4V1CTY5	ATP-dependent Clp protease ATP-binding subunit ClpA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=clpA PE=3 SV=1	9.503	6	4	5	4	758	84.2	12.62
A0A4P7TRN0	Pyruvate dehydrogenase E1 component OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=aceE PE=4 SV=1	7.715	6	5	5	5	887	99.6	8.06
A0A4P7TQT3	50S ribosomal protein L10 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rlpJ PE=3 SV=1	10.652	25	4	4	4	165	17.7	10.14

I6CI36	Cytoskeleton protein RodZ OS=Shigella flexneri K-315 OX=766150 GN=rodZ PE=3 SV=1	8.883	15	4	4	4	4	337	36.2	12.95
A0A1S9JMU3	Cell shape-determining protein MreB OS=Shigella boydii OX=621 GN=mreB PE=3 SV=1	13.252	15	4	4	4	4	347	36.9	12.39
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	10.323	15	4	4	4	4	329	36.5	10.93
D2A6V5	Chain length determinant protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_2324 PE=4 SV=1	9.774	14	4	4	4	4	337	37.9	11.75
I6D3P8	Cytochrome d ubiquinol oxidase subunit 1 OS=Shigella flexneri K-315 OX=766150 GN=cydA PE=3 SV=1	10.3	10	4	4	4	4	500	55.6	10.75
A0A2S8DD79	Threonine/serine transporter TdcC OS=Shigella dysenteriae OX=622 GN=tdcC PE=3 SV=1	12.674	9	3	4	3	3	443	48.9	10.8
A0A6G5Z4D2	Ferric aerobactin receptor IutA OS=Shigella flexneri OX=623 GN=iutA PE=3 SV=1	12.031	7	4	4	4	4	731	80.9	10.9
I6CHF3	MFS transporter, sugar porter family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3768 PE=3 SV=1	10.661	7	3	4	3	3	472	51.7	9.56
I6CJU2	Phosphate acetyltransferase OS=Shigella flexneri K-315 OX=766150 GN=pta PE=3 SV=1	11.109	6	4	4	4	4	709	76.5	10.24
A0A6N3RAY3	50S ribosomal protein L15 OS=Shigella flexneri 1485-80 OX=766155 GN=rpLO PE=3 SV=1	8.303	28	3	3	3	3	126	13.1	8.93
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	7.621	24	2	3	2	2	78	8.3	8.32
D2A917	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	6.89	19	3	3	3	3	124	13.7	7.73
Q5INY3	Clp protease ATP-binding subunit (Fragment) OS=Shigella flexneri OX=623 GN=clpX PE=4 SV=1	6.069	13	2	3	2	2	203	21.7	9.4
I6CXA4	Ribose-phosphate pyrophosphokinase OS=Shigella flexneri K-315 OX=766150 GN=prs PE=3 SV=1	9.154	11	3	3	3	3	312	33.9	7.89
A0A1Q8MQJ7	Protein RecA OS=Shigella boydii OX=621 GN=recA PE=3 SV=1	8.29	10	3	3	3	3	353	38	9.43
A0A3T2UQA1	Glutamate-1-semialdehyde 2,1-aminomutase OS=Shigella flexneri OX=623 GN=hemL PE=3 SV=1	5.32	9	3	3	3	3	426	45.3	6.51
F5P2J3	C4-dicarboxylate transport protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4734 PE=4 SV=1	5.601	8	3	3	3	3	395	41.7	3.02
Q6R2C3	Isocitrate dehydrogenase [NADP] (Fragment) OS=Shigella flexneri OX=623 GN=icd PE=3 SV=1	4.227	8	3	3	3	3	389	42.8	6.68
A0A658Y983	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degQ PE=3 SV=1	6.182	8	3	3	3	3	455	47.2	6.28
I6D070	Endopeptidase La OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0989 PE=3 SV=1	4.127	6	3	3	3	3	586	65.9	5.56
A0A4P7TR58	NAD(P)/FAD-dependent oxidoreductase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_010675 PE=4 SV=1	3.896	6	3	3	3	3	434	47.3	0
A0A6A9TRT7	ATP synthase subunit beta OS=Shigella flexneri OX=623 GN=atpD PE=3 SV=1	5.637	6	3	3	3	3	460	50.3	7.23
I6D6T4	Dihydrolipoyl dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=lpdA PE=3 SV=1	5.337	6	3	3	3	3	474	50.7	7.11
I6D3R0	Succinate dehydrogenase flavoprotein subunit OS=Shigella flexneri K-315 OX=766150 GN=sdhA PE=3 SV=1	5.076	6	3	3	3	3	588	64.4	6.51
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	6.257	5	2	3	2	2	474	49.3	8.33
A0A2S8DUB6	Glycerol-3-phosphate acyltransferase (Fragment) OS=Shigella boydii OX=621 GN=C5K23_08135 PE=3 SV=1	4.026	5	3	3	3	3	667	75.5	7.06
A0A6N3QJ73	Fructose-specific PTS system IIBC component OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03096 PE=4 SV=1	11.664	5	2	3	2	2	563	57.5	11
I6CBQ2	DNA gyrase subunit B OS=Shigella flexneri K-315 OX=766150 GN=gyrB PE=3 SV=1	6.145	4	3	3	3	3	804	89.9	6.08
A0A6N3QUN6	Zinc/cadmium/mercury/lead-transporting ATPase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00074 PE=3 SV=1	4.853	4	3	3	3	3	732	76.7	6.28
A0A6N3RCV1	Alanine--tRNA ligase OS=Shigella flexneri 1485-80 OX=766155 GN=alaS PE=3 SV=1	7.897	4	3	3	3	3	876	96	7.12
A0A383JWV6	Membrane protein insertase YidC OS=Shigella flexneri OX=623 GN=yidC PE=3 SV=1	5.967	4	2	3	2	2	548	61.5	7.29
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	6.023	24	2	2	2	2	129	13.8	5.44
I6C9X6	30S ribosomal protein S14 OS=Shigella flexneri K-315 OX=766150 GN=rpsN PE=3 SV=1	4.143	19	2	2	2	2	96	11	3.26
A0A6N3R4Q2	50S ribosomal protein L6 OS=Shigella flexneri 1485-80 OX=766155 GN=rpIF PE=3 SV=1	3.434	13	2	2	2	2	177	18.9	4.7
F5NUJ0	Glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1790 PE=4 SV=1	5.519	11	2	2	2	2	244	25.7	5.41
A0A6N3RN87	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ OS=Shigella flexneri 1485-80 OX=766155 GN=fabZ PE=3 SV=1	2.756	11	2	2	2	2	151	17	2.23
A0A6N3QME5	50S ribosomal protein L1 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rpIA PE=3 SV=1	6.172	10	2	2	2	2	234	24.7	5.79
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	3.324	9	2	2	2	2	206	23.4	4.72
A0A6N3R6Z9	Lipopolysaccharide export system permease protein LptF OS=Shigella flexneri 1485-80 OX=766155 GN=yjgP PE=3 SV=1	6.225	9	2	2	2	2	335	36.6	5.49
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	3.942	6	2	2	2	2	376	41	2.91
A0A4P7TME5	Osmotic shock tolerance protein YggT OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=yggT PE=3 SV=1	4.207	6	2	2	2	2	188	21.1	5.33
I6D072	Outer membrane protein A OS=Shigella flexneri K-315 OX=766150 GN=ompA PE=3 SV=1	2.905	6	2	2	2	2	344	37	2.37
A0A1S9KIA0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	5.267	6	2	2	2	2	419	47	5.6
Q0T2G2	3-oxoacyl-[acyl-carrier-protein] synthase I OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fabB PE=3 SV=1	2.931	5	2	2	2	2	370	39	4.12
D2AGX5	Cysteine desulfurase IscS OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=iscS PE=3 SV=1	2.592	5	2	2	2	2	412	46	2.16
I6CVA3	L-serine dehydratase OS=Shigella flexneri K-315 OX=766150 GN=SFK315_1603 PE=3 SV=1	4.337	5	2	2	2	2	432	46.6	5.33
A0A4P7TR77	Maltoporin OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=lamB PE=2 SV=1	2.613	4	2	2	2	2	446	50	1.96
I6CDA5	ATP-dependent protease ATPase subunit HslU OS=Shigella flexneri K-315 OX=766150 GN=hslU PE=2 SV=1	4.335	4	2	2	2	2	443	49.6	1.95
A0A6N3REQ7	Nickel-dependent hydrogenase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3604 PE=3 SV=1	3.3	3	2	2	2	2	567	62.5	4.56
A0A658YSU3	Bifunctional NADH:ubiquinone oxidoreductase subunit C/D OS=Shigella flexneri OX=623 GN=nuoC PE=3 SV=1	3.629	3	2	2	2	2	600	68.7	4.1

A0A380B6P9	PTS N-acetylglucosamine EIICBA component OS=Shigella flexneri OX=623 GN=nagE PE=4 SV=1	5.927	3	2	2	2	648	68.3	5.04
I6CC01	L-lactate dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=lldD PE=3 SV=1	2.081	3	1	2	1	396	42.7	0
D2ACF0	Fatty acid oxidation complex subunit alpha OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=fadB PE=3 SV=1	2.781	3	2	2	2	729	79.6	3.48
A0A380D533	Outer membrane protein OS=Shigella flexneri OX=623 GN=caf1A_3 PE=3 SV=1	2.195	3	2	2	2	812	85.6	4.13
A0A658XZH7	Oxidoreductase OS=Shigella flexneri OX=623 GN=NCTC8524_01188 PE=4 SV=1	4.348	3	2	2	2	804	89.3	5.44
F5NRH8	Efflux pump membrane transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0715 PE=3 SV=1	5.072	3	2	2	2	1049	113.6	5.65
A0A4P7TMI4	Autotransporter outer membrane beta-barrel domain-containing protein OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_010170 PE=4 SV=1	5.027	2	1	2	1	955	99.8	5.5
I6CBX0	50S ribosomal protein L28 OS=Shigella flexneri K-315 OX=766150 GN=rpmB PE=3 SV=1	2.166	15	1	1	1	66	7.7	2.71
I6CFZ0	50S ribosomal protein L27 OS=Shigella flexneri K-315 OX=766150 GN=rpmA PE=3 SV=1	0.996	13	1	1	1	85	9.1	0
A0A2S8DV96	50S ribosomal protein L5 OS=Shigella boydii OX=621 GN=rplE PE=3 SV=1	6.439	12	1	1	1	179	20.3	5.69
A0A4P7TM74	30S ribosomal protein S21 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsU PE=3 SV=1	1.859	11	1	1	1	71	8.5	1.82
F5P3C9	30S ribosomal protein S13 OS=Shigella flexneri K-227 OX=766147 GN=rpsM PE=3 SV=1	1.507	8	1	1	1	118	13.1	0
A0A1Q8N7Q2	50S ribosomal protein L11 OS=Shigella dysenteriae OX=622 GN=rplK PE=3 SV=1	2.142	7	1	1	1	142	14.9	3.03
A0A6N3R9K0	Phosphotransferase system, EIIC family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_2851 PE=4 SV=1	5.784	7	1	1	1	345	36.5	5.58
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=IgcK PE=1 SV=1	3.68	6	1	1	1	219	24.2	3.65
F5P2M7	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4769 PE=4 SV=1	2.383	6	1	1	1	236	25.6	2.46
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsC PE=3 SV=1	4.429	5	1	1	1	224	24.8	3.51
A0A2Y5ARN5	DNA-binding transcriptional regulator TyrR OS=Shigella flexneri OX=623 GN=tyrR PE=4 SV=1	1.91	5	1	1	1	219	25.1	2.4
A0A2S8D3U0	Catabolite activator protein OS=Shigella dysenteriae OX=622 GN=crp PE=4 SV=1	3.403	5	1	1	1	210	23.7	2.64
Q0SZX1	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yheO PE=4 SV=1	1.578	5	1	1	1	240	26.8	0
I6CIP8	Oxidoreductase ucpA OS=Shigella flexneri K-315 OX=766150 GN=ucpA PE=4 SV=1	1.556	5	1	1	1	263	27.8	2.3
A0A6N3R842	Adenylosuccinate synthetase OS=Shigella flexneri 1485-80 OX=766155 GN=purA PE=3 SV=1	3.928	4	1	1	1	432	47.3	2.99
A0A6N3RKA3	Succinate dehydrogenase iron-sulfur subunit OS=Shigella flexneri 1485-80 OX=766155 GN=sdhB PE=3 SV=1	1.153	4	1	1	1	238	26.8	0
A0A4P7TTH9	30S ribosomal protein S2 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsB PE=3 SV=1	1.386	4	1	1	1	241	26.8	0
A0A3T3S7B2	ParB/RepB/Spo0J family partition protein OS=Shigella flexneri OX=623 GN=sopB_2 PE=3 SV=1	2.085	3	1	1	1	326	36.8	2.97
A0A4P7TNZ8	Cell envelope integrity protein TolA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tolA PE=4 SV=1	1.857	3	1	1	1	392	40.3	2.17
A0A2S8DK39	Adenosine deaminase OS=Shigella boydii OX=621 GN=add PE=3 SV=1	1.627	3	1	1	1	333	36.3	1.76
A0A6N3QM36	Protein QmcA OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01963 PE=3 SV=1	3.032	3	1	1	1	305	33.7	2.83
Q0T5R2	Spermidine/putrescine import ATP-binding protein PotA OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=potA PE=3 SV=1	2.981	3	1	1	1	378	43.1	2.61
D2A8B1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=murA PE=3 SV=1	3.526	3	1	1	1	419	44.8	2.29
A0A4V1CTN6	dTDP-4-dehydrorhamnose reductase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rfbD PE=3 SV=1	1.272	3	1	1	1	299	32.5	2.68
Q7BEG5	ParA family protein OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=parA PE=4 SV=1	2.525	3	1	1	1	399	44.4	2.25
Q32SZ3	Acyl_transf_3 domain-containing protein OS=Shigella flexneri OX=623 PE=4 SV=1	2.656	3	1	1	1	349	40.1	2.25
A0A2S8D6C3	Proline-specific permease ProY OS=Shigella dysenteriae OX=622 GN=C5K18_23740 PE=4 SV=1	0.97	3	1	1	1	457	50.1	0
A0A6N3RCM6	3-ketoacyl-CoA thiolase OS=Shigella flexneri 1485-80 OX=766155 GN=fadA PE=3 SV=1	0.947	3	1	1	1	387	40.8	0
B5L3Q6	Glycosyltransferase OS=Shigella dysenteriae OX=622 GN=wfeY PE=4 SV=1	1.212	3	1	1	1	248	28.3	0
F5NZ29	Serine transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3429 PE=4 SV=1	3.783	3	1	1	1	429	46.9	3.44
F5POQ9	Maltose/maltodextrin import ATP-binding protein MalK OS=Shigella flexneri K-227 OX=766147 GN=malK PE=3 SV=1	1.62	3	1	1	1	371	41	2.09
Q83J96	Phosphoenolpyruvate carboxykinase (ATP) OS=Shigella flexneri OX=623 GN=pckA PE=3 SV=4	0.984	3	1	1	1	540	59.6	0
A0A380AZ64	Phosphoenolpyruvate--glycerone phosphotransferase OS=Shigella flexneri OX=623 GN=ycgC PE=3 SV=1	1.642	3	1	1	1	472	51.4	2.54
Q7UBI8	Bifunctional protein HldE OS=Shigella flexneri OX=623 GN=hldE PE=3 SV=2	1.064	3	1	1	1	477	51.1	0
Q0SYE6	2-amino-3-ketobutyrate coenzyme A ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=klb PE=3 SV=1	1.166	3	1	1	1	398	43.2	0
Q0T2J7	Putative aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_2357 PE=4 SV=1	2.303	2	1	1	1	405	45.5	2.61
F5P3V8	Fructose-1,6-bisphosphatase class 1 OS=Shigella flexneri K-227 OX=766147 GN=fbp PE=3 SV=1	0.961	2	1	1	1	332	36.8	0
Q0T2K3	NADH-quinone oxidoreductase subunit F OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nuoF PE=3 SV=1	1.446	2	1	1	1	445	49.4	2.48
A0A6N3R6J7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4987 PE=3 SV=1	2.854	2	1	1	1	446	47.8	2.74
I6D089	Hydrogenase-1 large chain OS=Shigella flexneri K-315 OX=766150 GN=hyaB PE=4 SV=1	1.431	2	1	1	1	425	47.7	0
I6CVF4	Protease 4 OS=Shigella flexneri K-315 OX=766150 GN=sppA PE=3 SV=1	4.257	2	1	1	1	618	67.2	3.27

Q0T1P7	Enolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=eno PE=3 SV=1	2.727	2	1	1	1	431	45.7	2.08
A0A2S8D890	Gluconate transporter OS=Shigella dysenteriae OX=622 GN=C5K18_19750 PE=4 SV=1	1.741	2	1	1	1	438	45.9	1.62
A0A6G5Z3B5	Biotin carboxylase OS=Shigella flexneri OX=623 GN=accC PE=4 SV=1	1.416	2	1	1	1	449	49.3	1.71
A0A6N3RL91	L-arabinose isomerase OS=Shigella flexneri 1485-80 OX=766155 GN=araA PE=3 SV=1	2.342	2	1	1	1	500	56.1	1.93
Q0T2P1	sn-glycerol-3-phosphate permease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=glpT PE=3 SV=1	1.841	2	1	1	1	452	50.4	1.62
A0A4P7TKN2	O13/O129/O135 family O-antigen flippase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=wzx PE=4 SV=1	1.77	2	1	1	1	418	46.3	1.93
I6CI73	Elongation factor 4 OS=Shigella flexneri K-315 OX=766150 GN=lepA PE=3 SV=1	4.243	2	1	1	1	599	66.6	3.89
A0A3T2UY84	Cyclopropane-fatty-acyl-phospholipid synthase OS=Shigella flexneri OX=623 GN=DK174_15880 PE=3 SV=1	1.426	2	1	1	1	382	43.9	0
I6D6Z7	Zinc metalloprotease OS=Shigella flexneri K-315 OX=766150 GN=rseP PE=3 SV=1	1.39	2	1	1	1	450	49.1	1.7
I6D681	Chaperone protein HtpG OS=Shigella flexneri K-315 OX=766150 GN=htpG PE=3 SV=1	0.961	2	1	1	1	624	71.5	2.63
A0A658Z2E9	Inorganic anion transporter, sulfate permease (SulP) family OS=Shigella flexneri OX=623 GN=purP PE=3 SV=1	1.054	2	1	1	1	445	46.9	0
I6D6W3	Poly(A) polymerase I OS=Shigella flexneri K-315 OX=766150 GN=pcnB PE=3 SV=1	0.968	2	1	1	1	454	52.5	1.84
A0A658YSH2	Dehydrogenase OS=Shigella flexneri OX=623 GN=yrfF PE=3 SV=1	1.449	1	1	1	1	711	79.5	1.98
A0A2S8DBJ8	Diguanylate cyclase OS=Shigella dysenteriae OX=622 GN=C5K18_12670 PE=4 SV=1	1.195	1	1	1	1	646	73.3	0
F5NUR0	Threonine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=thrS PE=3 SV=1	1.571	1	1	1	1	642	74	2.15
Q83SN2	Protein translocase subunit SecA OS=Shigella flexneri OX=623 GN=secA PE=3 SV=1	2.602	1	1	1	1	901	102	1.73
A0A658YRQ2	Efflux pump membrane transporter OS=Shigella flexneri OX=623 GN=acrD PE=3 SV=1	1.945	1	1	1	1	1037	113.1	0
A0A6L6UZB9	Ribonuclease E OS=Shigella flexneri OX=623 GN=rne PE=3 SV=1	1.888	1	1	1	1	1063	118.4	1.94

**Appx. D.7: Full protein identification from band 7 of Figure 5.2b.**

Accession	Description	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	calc. pI	Score	Sequest HT
A0A1Z1VVU8	Histidine kinase OS=Shigella flexneri Y OX=424720 PE=4 SV=1	1	2	1	572	63.1	8.18	1.95	
A0A3T2URR1	TDP-N-acetylfucosamine:lipid II N-acetylfucosaminyltransferase OS=Shigella flexneri OX=623 GN=wecF PE=3 SV=1	1	1	1	359	40.5	7.27	2.94	
F5NUJ0	Glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1790 PE=4 SV=1	1	1	1	244	25.7	6.55	2.9	
A0A658Y8X2	Outer membrane protein IcsA OS=Shigella flexneri OX=623 GN=icsA_2 PE=4 SV=1	1	1	1	1092	114.9	5.47	2.82	
D2ABF6	Formate acetyltransferase 1 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=pfIB PE=4 SV=1	1	1	1	397	44.3	6.16	2.55	
A0A379Z1S8	Protein HflK OS=Shigella flexneri OX=623 GN=hflK PE=3 SV=1	1	1	1	419	45.6	6.43	2.51	
A0A6N3QGR9	Uncharacterized protein OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04420 PE=4 SV=1	1	1	1	114	13	7.52	1.6	
A0A4P7TR97	MFS transporter OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_019835 PE=4 SV=1	1	1	1	430	47	9.36	0	

### Appx. D.8: Full protein identification from band 8 of Figure 5.2b.

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score Sequest HT
A0A4P7TP61	Mechanosensitive channel MscK OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=mscK PE=3 SV=1	135.432	36	32	80	32	1120	127.2	222.09
A0A4P7TRN0	Pyruvate dehydrogenase E1 component OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=aceE PE=4 SV=1	28.336	17	13	15	13	887	99.6	29.55
A0A6N3R3T7	Aspartate ammonia-lyase OS=Shigella flexneri 1485-80 OX=766155 GN=aspA PE=3 SV=1	25.446	27	11	13	11	478	52.3	29.54
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	32.627	16	9	12	9	474	49.3	37.31
A0A3U1DEW5	Formate C-acetyltransferase OS=Shigella flexneri OX=623 GN=pfIB PE=3 SV=1	23.821	16	10	12	10	760	85.3	27.99
A0A2S8D4S3	Aldehyde-alcohol dehydrogenase OS=Shigella dysenteriae OX=622 GN=C5K18_27725 PE=3 SV=1	27.98	13	10	11	10	891	96.1	27.58
A0A658YVY8	L-tryptophan indole-lyase OS=Shigella flexneri OX=623 GN=tnaA PE=3 SV=1	17.671	22	11	11	11	471	52.8	22.8
A0A2Y5AZK8	Lon protease OS=Shigella flexneri OX=623 GN=lon PE=2 SV=1	21.746	19	10	10	10	654	72.9	26.17
A0A6A9TRT7	ATP synthase subunit beta OS=Shigella flexneri OX=623 GN=atpD PE=3 SV=1	20.733	32	10	10	10	460	50.3	23.74
A0A379Z256	60 kDa chaperonin OS=Shigella flexneri OX=623 GN=groEL PE=3 SV=1	24.54	21	8	9	8	551	57.5	25.4
A0A1Q8MQG3	Chaperone protein DnaK OS=Shigella boydii OX=621 GN=dnaK PE=2 SV=1	22.079	16	8	9	8	638	69.1	20.29
I6D6T4	Dihydrolipoyl dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=lpdA PE=3 SV=1	21.522	19	8	9	8	474	50.7	24.52
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	21.369	18	9	9	9	513	55.2	27.15
A0A6N3RM75	Oxoglutarate dehydrogenase (succinyl-transferring) OS=Shigella flexneri 1485-80 OX=766155 GN=sucA PE=3 SV=1	16.711	10	8	9	8	933	105	17.76
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=rfc PE=3 SV=1	14.279	18	8	8	8	382	43.7	20.04
A0A2S8D9Q6	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=sucB PE=3 SV=1	17.454	19	7	7	7	405	44.1	18.45
Q0SXC2	Fumarate reductase flavoprotein subunit OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=frdA PE=3 SV=1	16.717	14	7	7	7	602	65.9	18.82
Q0SY20	Elongation factor Tu 2 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tuf2 PE=3 SV=1	15.805	22	6	7	1	394	43.3	20.46
Q0SZX8	Elongation factor Tu 1 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tuf1 PE=3 SV=1	14.781	22	6	7	1	394	43.3	18.74
F5P3A0	Elongation factor G OS=Shigella flexneri K-227 OX=766147 GN=fusA PE=3 SV=1	10.557	11	7	7	7	704	77.6	10.03
Q0T1R3	Chaperone protein ClpB OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=clpB PE=3 SV=1	9.624	9	7	7	7	857	95.6	11.1
A0A2S4MYJ5	Glycerophosphodiester phosphodiesterase OS=Shigella flexneri OX=623 GN=glpQ PE=4 SV=1	9.544	16	6	7	6	358	40.9	7.58
F5P004	L-asparaginase 2 OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3757 PE=3 SV=1	16.584	24	5	6	5	235	25.2	14.52
A0A2S8E636	Acetyltransferase component of pyruvate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=aceF PE=3 SV=1	14.028	11	6	6	6	630	66.1	14.68
A0A4P7TQY3	Bifunctional purine biosynthesis protein PurH OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=purH PE=3 SV=1	13.224	13	6	6	6	529	57.3	13.94
A0A3T2URE0	Catalase-peroxidase OS=Shigella flexneri OX=623 GN=katG PE=3 SV=1	12.946	12	6	6	6	726	80	12.35
Q7BCK4	Outer membrane protein IcsA autotransporter OS=Shigella flexneri OX=623 GN=icsA PE=1 SV=1	11.798	5	6	6	6	1102	116.2	14.96
A0A2Y5B4B8	Malate dehydrogenase OS=Shigella flexneri OX=623 GN=mdh PE=3 SV=1	10.305	22	6	6	6	312	32.3	11.65
Q7UB84	Glycerol kinase OS=Shigella flexneri OX=623 GN=glpK PE=3 SV=2	9.266	15	6	6	6	502	56.2	12.88
A0A2Y4Y2H2	Transketolase OS=Shigella flexneri OX=623 GN=tktA PE=3 SV=1	9.115	8	5	6	5	633	69	12.01
F5NUJ0	Glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1790 PE=4 SV=1	12.168	23	5	5	5	244	25.7	13.79
Q0T001	50S ribosomal protein L15 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpL15 PE=3 SV=1	11.732	42	5	5	5	144	15	12.02
A0A2S8DWS3	Uroporphyrinogen-III C-methyltransferase OS=Shigella boydii OX=621 GN=C5K23_02405 PE=4 SV=1	10.381	14	4	5	4	411	44.4	13.46
F5NRF0	Peptidylprolyl isomerase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0687 PE=4 SV=1	9.545	8	5	5	5	623	68.1	11.44
D2AD66	DNA-directed RNA polymerase subunit beta OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpoB PE=3 SV=1	7.971	4	5	5	5	1342	150.5	8.85
Q0T1P7	Enolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=eno PE=3 SV=1	7.657	13	5	5	5	431	45.7	10.73
A0A2S8D6C0	Protein translocase subunit SecD OS=Shigella dysenteriae OX=622 GN=secD PE=3 SV=1	12.887	8	4	4	4	615	66.6	10.4
A0A6N3QD48	Succinate dehydrogenase flavoprotein subunit OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04889 PE=3 SV=1	9.916	7	4	4	4	588	64.4	8.42
P0A852	Trigger factor OS=Shigella flexneri OX=623 GN=tig PE=3 SV=1	8.955	11	4	4	4	432	48.2	9.69
D2A8B5	Cytoplasmic membrane protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yrbD PE=4 SV=1	8.079	21	4	4	4	183	19.6	8.84
A0A2Y4ZTN4	Xaa-Pro dipeptidase OS=Shigella flexneri OX=623 GN=pepQ PE=3 SV=1	7.774	10	4	4	4	443	50.2	7.83
I6CJU2	Phosphate acetyltransferase OS=Shigella flexneri K-315 OX=766150 GN=pta PE=3 SV=1	7.461	6	4	4	4	709	76.5	9
A0A6N3RB60	50S ribosomal protein L2 OS=Shigella flexneri 1485-80 OX=766155 GN=rpL2 PE=3 SV=1	7.014	16	4	4	4	273	29.8	6.6

A0A658Y983	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degQ PE=3 SV=1	6.813	9	4	4	4	455	47.2	9.68
A0A2S8DCH8	Cysteine synthase OS=Shigella dysenteriae OX=622 GN=cysK PE=3 SV=1	7.107	11	3	3	3	323	34.5	7.46
D2AHG2	Autonomous glycol radical cofactor OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfid PE=3 SV=1	6.457	24	3	3	3	127	14.2	7.17
A0A658YSU3	Bifunctional NADH:ubiquinone oxidoreductase subunit C/D OS=Shigella flexneri OX=623 GN=nuoC PE=3 SV=1	6.37	5	3	3	3	600	68.7	5.78
A0A6N3RFP3	Chaperone protein HtpG OS=Shigella flexneri 1485-80 OX=766155 GN=htpG PE=3 SV=1	5.967	6	3	3	3	624	71.4	4.37
A0A658Y003	DNA-directed RNA polymerase subunit OS=Shigella flexneri OX=623 GN=rpoC PE=3 SV=1	4.335	2	3	3	3	1407	155.1	6.42
Q0T8G8	Isoleucine--tRNA ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ileS PE=3 SV=1	4.172	3	3	3	3	922	102.5	5.64
A0A1S9KIA0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	3.656	6	2	3	2	419	47	4.07
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsC PE=3 SV=1	6.402	10	2	2	2	224	24.8	5.6
A0A658YVC1	Aconitate hydratase B OS=Shigella flexneri OX=623 GN=acnB PE=3 SV=1	5.093	3	2	2	2	874	94.5	5.28
D2A6V5	Chain length determinant protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_2324 PE=4 SV=1	4.83	6	2	2	2	337	37.9	6.32
I6CE16	Isocitrate OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3962 PE=4 SV=1	4.263	11	2	2	2	237	26	4.14
Q7AK95	Chloramphenicol acetyltransferase OS=Shigella flexneri 2b OX=1288825 GN=cat PE=3 SV=1	4.164	4	1	2	1	219	25.6	5.62
P0A6P4	Elongation factor Ts OS=Shigella flexneri OX=623 GN=tsf PE=3 SV=2	3.851	6	2	2	2	283	30.4	3.43
D2A917	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	3.567	18	2	2	2	124	13.7	5.22
A0A2S4MYF8	Outer membrane protein C OS=Shigella flexneri OX=623 GN=ompC PE=1 SV=1	3.418	6	2	2	2	373	41.4	4.43
A0A6N3RJH6	Sulfatase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_1839 PE=3 SV=1	3.178	3	2	2	2	539	60.7	5.08
I6CG83	Phosphoglycerate kinase OS=Shigella flexneri K-315 OX=766150 GN=pgk PE=3 SV=1	3.13	4	1	2	1	382	40.5	6.12
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	2.986	9	2	2	2	206	23.4	4.86
A0A4P7TJ80	Glycine dehydrogenase (decarboxylating) OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=gcvP PE=3 SV=1	2.785	3	2	2	2	957	104.3	5.06
I6CI73	Elongation factor 4 OS=Shigella flexneri K-315 OX=766150 GN=lepA PE=3 SV=1	2.757	3	2	2	2	599	66.6	2.19
D2ABG4	30S ribosomal protein S1 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsA PE=3 SV=1	2.707	3	2	2	2	557	61.1	1.8
Q0T4H3	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1619 PE=4 SV=1	2.465	7	2	2	2	314	33.9	2.21
A0A3T2V040	Formate dehydrogenase-N subunit alpha OS=Shigella flexneri OX=623 GN=fdnG PE=3 SV=1	2.39	2	2	2	2	1016	112.5	2.05
A0A2Y4XXM5	Uncharacterized protein OS=Shigella flexneri OX=623 GN=SAMEA3710404_03077 PE=4 SV=1	1.413	25	1	2	1	87	9	0
F5NRH8	Efflux pump membrane transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0715 PE=3 SV=1	4.477	1	1	1	1	1049	113.6	2.71
A0A658XXS4	Outer membrane protein assembly factor YaeT OS=Shigella flexneri OX=623 GN=yaeT PE=4 SV=1	3.797	2	1	1	1	810	90.5	3.02
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	3.144	15	1	1	1	78	8.3	3.05
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	3.143	9	1	1	1	129	13.8	2.57
Q83SN2	Protein translocase subunit SecA OS=Shigella flexneri OX=623 GN=secA PE=3 SV=1	3.058	1	1	1	1	901	102	2.61
A0A2A2XR75	EIAB-Man OS=Shigella flexneri OX=623 GN=manX_2 PE=4 SV=1	2.783	6	1	1	1	323	35	3.29
A0A2S8DUZ3	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=Shigella boydii OX=621 GN=accA PE=3 SV=1	2.736	4	1	1	1	319	35.2	2.4
A0A658Y6P0	Translation initiation factor IF-2 OS=Shigella flexneri OX=623 GN=infB PE=3 SV=1	2.543	1	1	1	1	882	96.4	2.32
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=Igkc PE=1 SV=1	2.261	6	1	1	1	219	24.2	2.46
A0A2S8D3U0	Catabolite activator protein OS=Shigella dysenteriae OX=622 GN=crp PE=4 SV=1	2.226	5	1	1	1	210	23.7	0
A0A380B6P9	PTS N-acetylglucosamine EIICBA component OS=Shigella flexneri OX=623 GN=nagE PE=4 SV=1	2.207	2	1	1	1	648	68.3	1.8
A0A2S8DSA5	Valine--tRNA ligase OS=Shigella boydii OX=621 GN=valS PE=3 SV=1	2.141	1	1	1	1	951	108.1	2.48
A0A6N3RCV1	Alanine--tRNA ligase OS=Shigella flexneri 1485-80 OX=766155 GN=alaS PE=3 SV=1	2.1	1	1	1	1	876	96	2.28
A0A1S9KGJ3	Peptidyl-prolyl cis-trans isomerase OS=Shigella dysenteriae OX=622 GN=fkpA PE=3 SV=1	2.099	4	1	1	1	270	28.9	2.37
A0A6N3QGJ9	UPF0301 protein YqgE OS=Shigella flexneri CDC 796-83 OX=945360 GN=yqgE PE=3 SV=1	2.014	6	1	1	1	187	20.7	1.99
I6CC01	L-lactate dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=lldD PE=3 SV=1	1.969	3	1	1	1	396	42.7	2.56
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	1.932	3	1	1	1	376	41	2.6
A0A3T2UWB2	ATP synthase gamma chain OS=Shigella flexneri OX=623 GN=atpG PE=3 SV=1	1.767	3	1	1	1	287	31.6	2.24
I6CAV2	Cell division protein FtsX OS=Shigella flexneri K-315 OX=766150 GN=ftsX PE=3 SV=1	1.766	3	1	1	1	352	38.5	1.88
I6CBL5	ATP synthase FO, B subunit OS=Shigella flexneri K-315 OX=766150 GN=atpF PE=3 SV=1	1.765	10	1	1	1	127	14	1.82
Q0T2G2	3-oxoacyl-[acyl-carrier-protein] synthase I OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fabB PE=3 SV=1	1.671	4	1	1	1	370	39	2.21
D2ABV5	ECA polysaccharide chain length modulation protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=wzzE PE=3 SV=1	1.664	4	1	1	1	349	39.6	0
A0A2S4N174	50S ribosomal protein L22 OS=Shigella flexneri OX=623 GN=rplV PE=3 SV=1	1.637	13	1	1	1	110	12.2	0
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	1.611	4	1	1	1	329	36.5	2.02
F5NRH9	Periplasmic linker protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0716 PE=3 SV=1	1.543	4	1	1	1	361	38.4	1.89



Q0SYE6	2-amino-3-ketobutyrate coenzyme A ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=kl PE=3 SV=1	1.524	3	1	1	1	398	43.2	1.84
Q2EVP9	Isocitrate dehydrogenase [NADP] (Fragment) OS=Shigella flexneri OX=623 GN=icdA PE=3 SV=1	1.48	4	1	1	1	318	35.2	2.12
A0A2S8DDU2	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OS=Shigella dysenteriae OX=622 GN=murG PE=3 SV=1	1.459	3	1	1	1	355	37.8	0
Q0T2K3	NADH-quinone oxidoreductase subunit F OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nuoF PE=3 SV=1	1.445	3	1	1	1	445	49.4	1.72
A0A6N3RKA3	Succinate dehydrogenase iron-sulfur subunit OS=Shigella flexneri 1485-80 OX=766155 GN=sdhB PE=3 SV=1	1.419	4	1	1	1	238	26.8	0
A0A0H2UYV7	Nitrate reductase (quinone) OS=Shigella flexneri OX=623 GN=narG PE=3 SV=1	1.391	1	1	1	1	1247	140.4	1.89
A0A6N3QQN1	Trehalosemaltose-specific PTS system components IIBC OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02485 PE=4 SV=1	1.306	2	1	1	1	473	51.2	0
A0A6N3R842	Adenylosuccinate synthetase OS=Shigella flexneri 1485-80 OX=766155 GN=purA PE=3 SV=1	1.291	2	1	1	1	432	47.3	0
A0A383JRI2	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=hflB PE=3 SV=1	1.258	2	1	1	1	647	71	0
A0A3Y2RQZ3	TRAP transporter small permease OS=Shigella flexneri OX=623 GN=DTY77_11710 PE=4 SV=1	1.238	16	1	1	1	170	18.6	0
D2A9E7	Cytochrome d ubiquinol oxidase subunit 1 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=cydA PE=3 SV=1	1.187	2	1	1	1	523	58.3	1.96
F5NUC5	Tail-specific protease OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1725 PE=3 SV=1	1.178	1	1	1	1	680	76.5	0
A0A6N3REQ7	Nickel-dependent hydrogenase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3604 PE=3 SV=1	1.171	2	1	1	1	567	62.5	1.94
F5P106	Sulfatase domain-containing protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4163 PE=4 SV=1	1.143	5	1	1	1	577	66.5	0
A0A2Y4Y099	Glycerol-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=glpD PE=3 SV=1	1.132	2	1	1	1	501	56.7	1.68
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	1.127	3	1	1	1	477	50.7	2.38
A0A1Q8M8J0	50S ribosomal protein L7/L12 OS=Shigella boydii OX=621 GN=rpL PE=3 SV=1	1.118	10	1	1	1	121	12.3	1.83
A0A0A7NPN4	Uncharacterized protein OS=Shigella phage pSf-2 OX=1572702 GN=pSf2_026 PE=4 SV=1	1.1	18	1	1	1	117	13	0
D2ABQ4	Transposon Tn7 transposition protein tnsC OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4148 PE=4 SV=1	1.034	4	1	1	1	555	63	0
A0A2S8DH39	UPF0319 protein YccT OS=Shigella dysenteriae OX=622 GN=yccT PE=3 SV=1	0.993	14	1	1	1	220	24.6	0

### Appx. D.9: Full protein identification from band 9 of Figure 5.2b.

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score	Sequest HT
F5NRI1	Potassium efflux system kefA OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0718 PE=3 SV=1	90.423	29	27	32	27	1120	127.1	84.28	
A0A3U1DEW5	Formate C-acetyltransferase OS=Shigella flexneri OX=623 GN=pfIB PE=3 SV=1	50.179	30	20	24	20	760	85.3	52.66	
Q7BCK4	Outer membrane protein IcsA autotransporter OS=Shigella flexneri OX=623 GN=icsA PE=1 SV=1	34.902	10	9	20	9	1102	116.2	44.1	
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	50.52	21	12	19	12	474	49.3	55.28	
A0A4P7TRN0	Pyruvate dehydrogenase E1 component OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=aceE PE=4 SV=1	28.688	15	14	18	14	887	99.6	25.47	
A0A1Q8MQG3	Chaperone protein DnaK OS=Shigella boydii OX=621 GN=dnaK PE=2 SV=1	45.228	29	16	17	16	638	69.1	40.44	
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	42.775	32	14	15	14	513	55.2	43.01	
A0A6N3R3T7	Aspartate ammonia-lyase OS=Shigella flexneri 1485-80 OX=766155 GN=aspA PE=3 SV=1	38.911	29	13	15	13	478	52.3	38.94	
Q0SY20	Elongation factor Tu 2 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tuf2 PE=3 SV=1	36.045	26	9	14	9	394	43.3	35.5	
A0A6D2XMH9	ATP synthase subunit beta OS=Shigella flexneri OX=623 GN=atpD PE=3 SV=1	41.431	38	13	14	13	460	50.3	28.41	
A0A658YVY8	L-tryptophan indole-lyase OS=Shigella flexneri OX=623 GN=tnaA PE=3 SV=1	30.99	26	14	14	14	471	52.8	27.98	
A0A6L6UYG8	Dihydrolipoyl dehydrogenase OS=Shigella flexneri OX=623 GN=lpdA PE=3 SV=1	24.335	22	10	14	10	474	50.7	22.63	
A0A379Z256	60 kDa chaperonin OS=Shigella flexneri OX=623 GN=groEL PE=3 SV=1	33.218	25	11	12	11	551	57.5	30.01	
A0A4P7TQY3	Bifunctional purine biosynthesis protein PurH OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=purH PE=3 SV=1	29.246	22	11	12	11	529	57.3	27.51	
A0A6N3QN52	Lon protease OS=Shigella flexneri CDC 796-83 OX=945360 GN=lon PE=2 SV=1	27.364	16	12	12	12	799	89	22.23	
A0A4P7TSA3	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=odhB PE=3 SV=1	31.507	22	9	11	9	405	44	27.21	
A0A2S8E636	Acetyltransferase component of pyruvate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=aceF PE=3 SV=1	28.825	17	11	11	11	630	66.1	26.77	
P0A9B5	Glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=gapA PE=3 SV=2	26.714	34	9	11	9	331	35.5	25.92	
A0A2S8D4S3	Aldehyde-alcohol dehydrogenase OS=Shigella dysenteriae OX=622 GN=C5K18_27725 PE=3 SV=1	32.083	11	8	10	8	891	96.1	30.07	
A0A4P7TME0	Transketolase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tkt PE=3 SV=1	28.768	17	10	10	10	663	72.1	22.54	
Q0SXC2	Fumarate reductase flavoprotein subunit OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=frdA PE=3 SV=1	32.239	18	8	8	8	602	65.9	23.17	
A0A6N3QD48	Succinate dehydrogenase flavoprotein subunit OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04889 PE=3 SV=1	18.69	9	5	8	5	588	64.4	18.93	
P0A852	Trigger factor OS=Shigella flexneri OX=623 GN=tig PE=3 SV=1	20.763	20	8	8	8	432	48.2	17.6	
F5NRF0	Peptidylprolyl isomerase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0687 PE=4 SV=1	14.085	13	7	8	7	623	68.1	15.25	
A0A2S8D6C0	Protein translocase subunit SecD OS=Shigella dysenteriae OX=622 GN=secD PE=3 SV=1	16.224	14	8	8	8	615	66.6	14.62	
A0A3T2URE0	Catalase-peroxidase OS=Shigella flexneri OX=623 GN=katG PE=3 SV=1	19.867	13	7	8	7	726	80	14.34	
A0A6N3RB60	50S ribosomal protein L2 OS=Shigella flexneri 1485-80 OX=766155 GN=rpIB PE=3 SV=1	15.556	26	6	8	2	273	29.8	11.95	
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=rfc PE=3 SV=1	15.433	16	6	7	6	382	43.7	16.97	
A0A380B750	Succinate--CoA ligase [ADP-forming] subunit beta OS=Shigella flexneri OX=623 GN=sucC PE=3 SV=1	20.729	18	7	7	7	388	41.4	15.04	
Q49TD7	Malate dehydrogenase (Fragment) OS=Shigella flexneri OX=623 GN=mdh PE=3 SV=1	17.272	29	7	7	7	265	27.5	14.98	
A0A2S8DV74	Glycerol kinase OS=Shigella boydii OX=621 GN=glpK PE=3 SV=1	13.261	13	6	7	6	502	56.1	13.43	
Q0T4H3	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1619 PE=4 SV=1	13.31	22	7	7	7	314	33.9	10.59	
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsC PE=3 SV=1	14.883	23	5	7	5	224	24.8	8.44	
A0A658Y983	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degQ PE=3 SV=1	18.651	17	6	6	6	455	47.2	15.23	
A0A6N3RFP3	Chaperone protein HtpG OS=Shigella flexneri 1485-80 OX=766155 GN=htpG PE=3 SV=1	14.144	10	6	6	6	624	71.4	12.15	
I6C9W6	50S ribosomal protein L2 OS=Shigella flexneri K-315 OX=766150 GN=rpIB PE=3 SV=1	12.042	22	5	6	1	255	27.9	9.91	
A0A2Y4ZTN4	Xaa-Pro dipeptidase OS=Shigella flexneri OX=623 GN=pepQ PE=3 SV=1	8.637	10	4	6	4	443	50.2	9.78	
D2AFY9	NADH-quinone oxidoreductase subunit C/D OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=nuoC PE=3 SV=1	13.178	10	6	6	6	600	68.7	8.57	
F5P004	L-asparaginase 2 OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3757 PE=3 SV=1	19.564	24	4	5	4	235	25.2	14.98	
A0A2A2XR75	EIIAB-Man OS=Shigella flexneri OX=623 GN=manX_2 PE=4 SV=1	18.713	22	5	5	5	323	35	13.77	
I6CJU2	Phosphate acetyltransferase OS=Shigella flexneri K-315 OX=766150 GN=pta PE=3 SV=1	12.361	6	4	5	4	709	76.5	12.78	
A0A1S9K1A0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	9.038	13	5	5	5	419	47	11.79	
A0A2S8DCH8	Cysteine synthase OS=Shigella dysenteriae OX=622 GN=cysK PE=3 SV=1	11.685	21	5	5	5	323	34.5	11.77	

Q0SYD1	L-lactate dehydrogenase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lldD PE=3 SV=1	10.512	15	5	5	5	5	396	42.7	9.59
A0A2S8DUB6	Glycerol-3-phosphate acyltransferase (Fragment) OS=Shigella boydii OX=621 GN=C5K23_08135 PE=3 SV=1	7.718	6	4	5	4	4	667	75.5	8.66
D2AHG2	Autonomous glycy radical cofactor OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfiD PE=3 SV=1	8.934	34	4	5	4	4	127	14.2	8.25
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	8.169	17	5	5	5	5	329	36.5	8.24
A0A3T2UWB2	ATP synthase gamma chain OS=Shigella flexneri OX=623 GN=atpG PE=3 SV=1	6.803	12	4	5	4	4	287	31.6	6.23
D2A917	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	11.166	19	4	4	4	4	124	13.7	11.13
Q7AK95	Chloramphenicol acetyltransferase OS=Shigella flexneri 2b OX=1288825 GN=cat PE=3 SV=1	10.779	4	1	4	1	1	219	25.6	10.88
Q0T1P7	Enolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=eno PE=3 SV=1	11.812	10	4	4	4	4	431	45.7	10.28
A0A6N3RAY3	50S ribosomal protein L15 OS=Shigella flexneri 1485-80 OX=766155 GN=rplO PE=3 SV=1	10.85	29	3	4	3	3	126	13.1	9.1
I6CBL5	ATP synthase F0, B subunit OS=Shigella flexneri K-315 OX=766150 GN=atpF PE=3 SV=1	12.59	39	4	4	4	4	127	14	8.5
Q0T1R3	Chaperone protein ClpB OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=clpB PE=3 SV=1	8.684	5	4	4	4	4	857	95.6	8.05
Q0T6B3	Aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=aspC PE=3 SV=1	4.784	8	3	4	3	3	396	43.6	6.8
A0A2Y4Y9J3	Pyruvate kinase OS=Shigella flexneri OX=623 GN=pykF PE=3 SV=1	8.317	7	4	4	4	4	542	58.7	4.37
A0A2S8DKT5	NADH-quinone oxidoreductase OS=Shigella boydii OX=621 GN=nuoG PE=3 SV=1	7.887	3	3	4	3	3	908	100.3	3.96
D2ABG4	30S ribosomal protein S1 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsA PE=3 SV=1	5.515	4	2	4	2	2	557	61.1	3.94
A0A2S4MYJ5	Glycerophosphodiester phosphodiesterase OS=Shigella flexneri OX=623 GN=glpQ PE=4 SV=1	7.28	9	4	4	4	4	358	40.9	3.78
P0A6P4	Elongation factor Ts OS=Shigella flexneri OX=623 GN=tsf PE=3 SV=2	5.279	9	3	4	3	3	283	30.4	3.72
A0A6N3RKA3	Succinate dehydrogenase iron-sulfur subunit OS=Shigella flexneri 1485-80 OX=766155 GN=sdhB PE=3 SV=1	11.253	15	3	3	3	3	238	26.8	8.39
A0A6N3QME5	50S ribosomal protein L1 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rplA PE=3 SV=1	8.611	15	3	3	3	3	234	24.7	8.15
F5P3A0	Elongation factor G OS=Shigella flexneri K-227 OX=766147 GN=fusA PE=3 SV=1	10.756	5	3	3	3	3	704	77.6	7.85
A0A2S8DTV4	Phosphoenolpyruvate synthase OS=Shigella boydii OX=621 GN=ppsA PE=3 SV=1	5.663	4	3	3	3	3	792	87.4	6.22
Q0T2K3	NADH-quinone oxidoreductase subunit F OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nuoF PE=3 SV=1	4.639	5	2	3	2	2	445	49.4	6.03
A0A6N3REQ7	Nickel-dependent hydrogenase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3604 PE=3 SV=1	6.614	6	3	3	3	3	567	62.5	5.96
A0A658XXS4	Outer membrane protein assembly factor YaeT OS=Shigella flexneri OX=623 GN=yaeT PE=4 SV=1	8.898	5	3	3	3	3	810	90.5	5.54
Q0T2Y9	Periplasmic beta-glucosidase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=bgIX PE=3 SV=1	6.621	4	3	3	3	3	755	82.4	5.3
A0A1Q8M4D3	30S ribosomal protein S18 OS=Shigella boydii OX=621 GN=rpsR PE=3 SV=1	11.116	35	3	3	3	3	75	9	5.26
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	7.262	9	3	3	3	3	376	41	5.16
A0A658YV21	Auxiliary transport protein, membrane fusion protein (MFP) family OS=Shigella flexneri OX=623 GN=macA_2 PE=4 SV=1	4.562	6	2	3	2	2	355	38.7	4.61
A0A2S4MYF8	Outer membrane protein C OS=Shigella flexneri OX=623 GN=ompC PE=1 SV=1	7	9	3	3	3	3	373	41.4	4.54
F5NY87	Phosphoenolpyruvate-protein phosphotransferase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3048 PE=3 SV=1	6.165	6	3	3	3	3	585	64.5	4.43
F5NRH8	Efflux pump membrane transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0715 PE=3 SV=1	7.07	4	3	3	3	3	1049	113.6	4.31
A0A6N3R4Q2	50S ribosomal protein L6 OS=Shigella flexneri 1485-80 OX=766155 GN=rplF PE=3 SV=1	5.035	19	3	3	3	3	177	18.9	4.08
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	5.435	24	2	3	2	2	129	13.8	3.97
A0A6N3RKR4	Iron-sulfur cluster binding protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0392 PE=4 SV=1	4.671	6	3	3	3	3	475	53	3.73
F5NRX2	Serine-type D-Ala-D-Ala carboxypeptidase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0860 PE=3 SV=1	5.127	9	3	3	3	3	403	44.4	3.65
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	2.655	4	2	3	2	2	477	50.7	2.15
D2ACF0	Fatty acid oxidation complex subunit alpha OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=fadB PE=3 SV=1	3.362	4	3	3	3	3	729	79.6	1.82
D2A6V5	Chain length determinant protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_2324 PE=4 SV=1	6.707	6	2	2	2	2	337	37.9	6.27
A0A6L6V006	Ribose-phosphate pyrophosphokinase OS=Shigella flexneri OX=623 GN=prs PE=3 SV=1	6.141	8	2	2	2	2	315	34.2	4.97
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	3.979	9	2	2	2	2	206	23.4	4.83
A0A1S9KGJ3	Peptidyl-prolyl cis-trans isomerase OS=Shigella dysenteriae OX=622 GN=fkpA PE=3 SV=1	4.25	8	2	2	2	2	270	28.9	4.77
A0A2S8D3U0	Catabolite activator protein OS=Shigella dysenteriae OX=622 GN=crp PE=4 SV=1	5.208	10	2	2	2	2	210	23.7	4.65
A0A2S8DRB8	Glucose-6-phosphate 1-dehydrogenase OS=Shigella boydii OX=621 GN=zwf PE=3 SV=1	4.157	4	2	2	2	2	491	55.7	4.4
A0A2S8DV96	50S ribosomal protein L5 OS=Shigella boydii OX=621 GN=rplE PE=3 SV=1	5.233	18	2	2	2	2	179	20.3	4.27
A0A1Q8N7Q2	50S ribosomal protein L11 OS=Shigella dysenteriae OX=622 GN=rplK PE=3 SV=1	4.428	17	2	2	2	2	142	14.9	4.27
A0A6N3RBT1	GTP-binding protein TypA/BipA OS=Shigella flexneri 1485-80 OX=766155 GN=typA PE=4 SV=1	4.875	4	2	2	2	2	607	67.4	4.21
Q6R2C3	Isocitrate dehydrogenase [NADP] (Fragment) OS=Shigella flexneri OX=623 GN=icd PE=3 SV=1	2.837	5	2	2	2	2	389	42.8	4.2
A0A3T2UX48	Outer membrane protein F OS=Shigella flexneri OX=623 GN=ompF PE=3 SV=1	4.869	6	2	2	2	2	362	39.3	4.12
F5P3D3	50S ribosomal protein L17 OS=Shigella flexneri K-227 OX=766147 GN=rplQ PE=3 SV=1	3.884	15	2	2	2	2	127	14.4	4.06
A0A0H2VYI3	Nucleoside permease OS=Shigella flexneri OX=623 GN=nupC PE=3 SV=1	4.736	5	2	2	2	2	400	43.5	3.99

A0A4P7TKC9	NADP-dependent oxaloacetate-decarboxylating malate dehydrogenase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_003280 PE=3 SV=1	3.172	3	2	2	2	759	82.3	3.99
A0A2S8DNR9	Methionine--tRNA ligase OS=Shigella boydii OX=621 GN=metG PE=3 SV=1	3.423	3	2	2	2	677	76.2	3.89
A0A6L6V6S8	Mobilization protein OS=Shigella flexneri OX=623 GN=GLO03_22460 PE=4 SV=1	2.743	13	2	2	2	176	19.9	3.75
F5P300	Glucose-1-phosphate adenyltransferase OS=Shigella flexneri K-227 OX=766147 GN=glcC PE=3 SV=1	3.362	5	2	2	2	431	48.7	3.54
Q0T6W4	Oxoglutarate dehydrogenase (succinyl-transferring) OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=sucA PE=3 SV=1	2.902	2	2	2	2	933	105	3.54
A0A2S8DUL5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase OS=Shigella boydii OX=621 GN=dapD PE=3 SV=1	5.034	8	2	2	2	274	29.9	2.61
I6C9X6	30S ribosomal protein S14 OS=Shigella flexneri K-315 OX=766150 GN=rpsN PE=3 SV=1	4.297	24	2	2	2	96	11	2.4
Q0SYE6	2-amino-3-ketobutyrate coenzyme A ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=klb PE=3 SV=1	3.656	6	2	2	2	398	43.2	2.32
Q0T2G2	3-oxoacyl-[acyl-carrier-protein] synthase I OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fabB PE=3 SV=1	2.788	6	2	2	2	370	39	2.19
D2AEV8	Putative NADPH-dependent glutamate synthase beta chain-like oxidoreductase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yeiT PE=4 SV=1	3.228	6	2	2	2	412	44.4	2
I6C9X3	50S ribosomal protein L14 OS=Shigella flexneri K-315 OX=766150 GN=rplN PE=3 SV=1	2.196	14	2	2	2	117	12.8	1.62
Q0T0X5	Fructose-bisphosphate aldolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fba PE=3 SV=1	2.085	4	2	2	2	387	42.7	1.61
A0A383JRI2	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=hflB PE=3 SV=1	1.826	2	2	2	2	647	71	0
D2AE64	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4630 PE=3 SV=1	2.07	9	1	1	1	141	15.2	3.31
I6CE16	Isocitrate OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3962 PE=4 SV=1	4.275	6	1	1	1	237	26	3.24
I6CI36	Cytoskeleton protein RodZ OS=Shigella flexneri K-315 OX=766150 GN=rodZ PE=3 SV=1	4.107	4	1	1	1	337	36.2	3.11
F5NRH9	Periplasmic linker protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0716 PE=3 SV=1	3.157	4	1	1	1	361	38.4	3.09
A0A6N3QGJ9	UPF0301 protein YqgE OS=Shigella flexneri CDC 796-83 OX=945360 GN=yqgE PE=3 SV=1	3.785	6	1	1	1	187	20.7	2.95
I6C9W2	30S ribosomal protein S10 OS=Shigella flexneri K-315 OX=766150 GN=rpsJ PE=3 SV=1	4.309	15	1	1	1	103	11.7	2.83
I6CBX0	50S ribosomal protein L28 OS=Shigella flexneri K-315 OX=766150 GN=rpmB PE=3 SV=1	2.303	15	1	1	1	66	7.7	2.82
A0A4P7TJ80	Glycine dehydrogenase (decarboxylating) OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=gcvP PE=3 SV=1	4.232	2	1	1	1	957	104.3	2.82
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	3.127	15	1	1	1	78	8.3	2.76
I6CE82	Fumarate hydratase class I OS=Shigella flexneri K-315 OX=766150 GN=fumB PE=3 SV=1	2.426	2	1	1	1	548	60.1	2.74
A0A1Q8M8J0	50S ribosomal protein L7/L12 OS=Shigella boydii OX=621 GN=rplL PE=3 SV=1	3.357	10	1	1	1	121	12.3	2.71
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=lgkc PE=1 SV=1	3.29	6	1	1	1	219	24.2	2.65
I6CG83	Phosphoglycerate kinase OS=Shigella flexneri K-315 OX=766150 GN=pgk PE=3 SV=1	2.632	4	1	1	1	382	40.5	2.64
I6CAV2	Cell division protein FtsX OS=Shigella flexneri K-315 OX=766150 GN=ftsX PE=3 SV=1	2.571	3	1	1	1	352	38.5	2.62
A0A2S4MTA8	Lactate utilization protein C OS=Shigella flexneri OX=623 GN=ykgG PE=4 SV=1	2.04	4	1	1	1	231	25.2	2.58
A0A2S8DJU5	Polyribonucleotide nucleotidyltransferase OS=Shigella boydii OX=621 GN=pnp PE=3 SV=1	3.239	2	1	1	1	711	77.1	2.57
A0A383JWV6	Membrane protein insertase YidC OS=Shigella flexneri OX=623 GN=yidC PE=3 SV=1	3.689	2	1	1	1	548	61.5	2.51
I6D070	Endopeptidase La OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0989 PE=3 SV=1	3.122	2	1	1	1	586	65.9	2.48
I6CI73	Elongation factor 4 OS=Shigella flexneri K-315 OX=766150 GN=lepA PE=3 SV=1	2.356	2	1	1	1	599	66.6	2.41
A0A6N3RIF3	Enoyl-[acyl-carrier-protein] reductase [NADH] OS=Shigella flexneri 1485-80 OX=766155 GN=fabI PE=3 SV=1	1.394	4	1	1	1	262	27.8	2.37
I6CG61	Aminomethyltransferase OS=Shigella flexneri K-315 OX=766150 GN=gcvT PE=3 SV=1	2.581	3	1	1	1	347	38.2	2.37
A0A379Z1S8	Protein HflK OS=Shigella flexneri OX=623 GN=hflK PE=3 SV=1	3.239	3	1	1	1	419	45.6	2.27
Q0SYU1	ATP synthase subunit delta OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=atpH PE=3 SV=1	1.599	6	1	1	1	177	19.3	2.26
A0A658Y5D6	Arginyl-tRNA synthetase OS=Shigella flexneri OX=623 GN=argS PE=3 SV=1	3.144	2	1	1	1	577	64.7	2.25
A0A6N3RFR1	6-phosphogluconate dehydrogenase, decarboxylating OS=Shigella flexneri 1485-80 OX=766155 GN=gnd PE=3 SV=1	2.696	3	1	1	1	468	51.5	2.24
A0A2S8DUZ3	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=Shigella boydii OX=621 GN=accA PE=3 SV=1	3.261	4	1	1	1	319	35.2	2.18
A0A2Y4Y1C7	Fumarate hydratase class I OS=Shigella flexneri OX=623 GN=fumA PE=3 SV=1	1.928	2	1	1	1	548	60.3	2.18
A0A2S8DWS3	Uroporphyrinogen-III C-methyltransferase OS=Shigella boydii OX=621 GN=C5K23_02405 PE=4 SV=1	2.427	2	1	1	1	411	44.4	2.15
A0A6N3QFB8	Anaerobic dimethyl sulfoxide reductase chain A OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04605 PE=3 SV=1	3.019	2	1	1	1	705	78.7	2.11
Q0T4A8	Possible enzyme OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1686 PE=4 SV=1	1.351	3	1	1	1	418	42.9	2.06
F5NUC5	Tail-specific protease OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1725 PE=3 SV=1	1.539	2	1	1	1	680	76.5	2.05
I6D3Q3	Succinate--CoA ligase [ADP-forming] subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=sucD PE=3 SV=1	1.518	3	1	1	1	289	29.8	2.02
A0A6N3QQN1	Trehalosemaltose-specific PTS system components IIBC OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02485 PE=4 SV=1	2.214	2	1	1	1	473	51.2	2.02
A0A6N3R842	Adenylosuccinate synthetase OS=Shigella flexneri 1485-80 OX=766155 GN=purA PE=3 SV=1	1.222	2	1	1	1	432	47.3	1.98

A0A2S8D523	Anaerobic glycerol-3-phosphate dehydrogenase subunit B OS=Shigella dysenteriae OX=622 GN=glpB PE=3 SV=1	1.384	2	1	1	1	1	419	45.3	1.98
Q83IY3	Bifunctional protein GlmU OS=Shigella flexneri OX=623 GN=glmU PE=3 SV=1	1.513	2	1	1	1	1	456	49.2	1.97
A0A380B3G3	Asparagine--tRNA ligase OS=Shigella flexneri OX=623 GN=asnC_2 PE=3 SV=1	1.171	2	1	1	1	1	466	52.6	1.96
A0A2Y4Y099	Glycerol-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=glpD PE=3 SV=1	2.393	2	1	1	1	1	501	56.7	1.92
A0A2Y4XRM3	CTP synthase OS=Shigella flexneri OX=623 GN=pyrG PE=3 SV=1	1.147	2	1	1	1	1	545	60.4	1.92
A0A6N3R6K7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4998 PE=3 SV=1	2.354	3	1	1	1	1	402	42.7	1.85
A0A4P7TM74	30S ribosomal protein S21 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsU PE=3 SV=1	1.292	11	1	1	1	1	71	8.5	1.84
Q83MF6	Cell division protein FtsZ OS=Shigella flexneri OX=623 GN=ftsZ PE=3 SV=2	0.935	3	1	1	1	1	383	40.1	1.8
I6D6H9	Transaldolase OS=Shigella flexneri K-315 OX=766150 GN=tal PE=3 SV=1	1.676	4	1	1	1	1	294	32.8	1.79
I6D405	PhoH-like protein OS=Shigella flexneri K-315 OX=766150 GN=ybeZ PE=3 SV=1	1.005	3	1	1	1	1	346	39	1.73
D2ABV5	ECA polysaccharide chain length modulation protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=wzzE PE=3 SV=1	2.115	4	1	1	1	1	349	39.6	1.65
Q0T0X8	Putative actin OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yggE PE=4 SV=1	0.978	3	1	1	1	1	235	25.4	1.64
A0A380B6P9	PTS N-acetylglucosamine EIICBA component OS=Shigella flexneri OX=623 GN=nagE PE=4 SV=1	1.264	2	1	1	1	1	648	68.3	1.64
A0A6H1JH06	30S ribosomal protein S5 OS=Shigella flexneri OX=623 GN=rpsE PE=3 SV=1	1.05	4	1	1	1	1	167	17.6	1.63
Q0SZY4	50S ribosomal protein L4 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpID PE=3 SV=1	0.976	3	1	1	1	1	201	22.1	1.63
A0A2Y5A6X9	30S ribosomal protein S9 OS=Shigella flexneri OX=623 GN=rpsI PE=3 SV=1	1.592	14	1	1	1	1	85	9.7	0
Q0T8H0	30S ribosomal protein S20 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsT PE=3 SV=1	0.932	10	1	1	1	1	87	9.7	0
A0A6D2W4F9	Chaperone protein Skp OS=Shigella flexneri OX=623 GN=skp PE=3 SV=1	1.284	7	1	1	1	1	161	17.7	0
A0A4P7TR58	NAD(P)/FAD-dependent oxidoreductase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_010675 PE=4 SV=1	1.379	5	1	1	1	1	434	47.3	0
A0A2S8DA55	SH3 domain-containing protein OS=Shigella dysenteriae OX=622 GN=ygiM PE=4 SV=1	0.903	4	1	1	1	1	206	23.1	0
A0A1S9JMU3	Cell shape-determining protein MreB OS=Shigella boydii OX=621 GN=mreB PE=3 SV=1	1.696	4	1	1	1	1	347	36.9	0
D2AGX5	Cysteine desulfurase IscS OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=iscS PE=3 SV=1	2.83	3	1	1	1	1	412	46	0
Q2EUU0	AccD (Fragment) OS=Shigella flexneri OX=623 GN=accD PE=3 SV=1	1.243	3	1	1	1	1	296	32.6	0
A0A2S8DKX6	Insulinase family protein OS=Shigella boydii OX=621 GN=yhjJ PE=3 SV=1	1.533	3	1	1	1	1	498	55.4	0
I6CH97	AMP nucleosidase OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3709 PE=4 SV=1	1.833	2	1	1	1	1	444	49.9	0
A0A658Y285	Protein ydgA OS=Shigella flexneri OX=623 GN=ydgA PE=4 SV=1	1.019	2	1	1	1	1	502	54.6	0
A0A379YVF8	Cell division protein FtsA OS=Shigella flexneri OX=623 GN=ftsA PE=3 SV=1	1.765	2	1	1	1	1	420	45.3	0
Q9AJV8	UshA, probable periplasmic UDP-sugar hydrolase OS=Shigella flexneri OX=623 GN=ushA PE=3 SV=1	1.6	2	1	1	1	1	550	61.2	0
I6CDA5	ATP-dependent protease ATPase subunit HslU OS=Shigella flexneri K-315 OX=766150 GN=hslU PE=2 SV=1	2.244	2	1	1	1	1	443	49.6	0
A0A2X2I0N0	Cell division protein DamX OS=Shigella flexneri OX=623 GN=damX PE=3 SV=1	1.042	2	1	1	1	1	428	46.1	0
A0A2Y4XYI3	Esterase FrsA OS=Shigella flexneri OX=623 GN=frsA PE=3 SV=1	2.015	2	1	1	1	1	414	47	0
A0A2S8DW51	Malate synthase OS=Shigella boydii OX=621 GN=aceB PE=3 SV=1	1.759	2	1	1	1	1	533	60.2	0
A0A3U1D5H5	Alpha-D-glucose phosphate-specific phosphoglucomutase OS=Shigella flexneri OX=623 GN=pgm PE=3 SV=1	1.328	2	1	1	1	1	546	58.3	0
D2AHW6	Peptidoglycan D,D-transpeptidase FtsI OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=ftsI PE=3 SV=1	2.196	2	1	1	1	1	588	63.9	0
A0A379Z0K8	Anaerobic ribonucleoside-triphosphate reductase OS=Shigella flexneri OX=623 GN=nrdD PE=4 SV=1	2.152	2	1	1	1	1	712	80	0
A0A380AET5	SWIM zinc finger OS=Shigella flexneri OX=623 GN=DTY77_20095 PE=4 SV=1	0.931	2	1	1	1	1	667	73.5	0
A0A658YRM6	Peptidase, M48 family OS=Shigella flexneri OX=623 GN=SAMEA3710514_00010 PE=4 SV=1	1.191	2	1	1	1	1	487	53.9	0
I6CB04	Alpha-1,4 glucan phosphorylase OS=Shigella flexneri K-315 OX=766150 GN=malP PE=3 SV=1	0.882	1	1	1	1	1	797	90.5	0

**Appx. D.10: Full protein identification from band 10 of Figure 5.2b.**

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score Sequest HT
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=rfc PE=3 SV=1	29.434	18	9	60	9	382	43.7	122.72
I6C8N1	Modulator of FtsH protease HflC OS=Shigella flexneri K-315 OX=766150 GN=hflC PE=3 SV=1	64.199	60	20	34	20	334	37.6	83.66
P0A9B5	Glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=gapA PE=3 SV=2	64.462	50	14	28	1	331	35.5	68.53
Q0T240	Outer membrane protein assembly factor BamC OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nlpB PE=3 SV=1	59.209	43	11	25	11	344	36.8	69.44
A0A2A2XR75	EIIAB-Man OS=Shigella flexneri OX=623 GN=manX_2 PE=4 SV=1	54.406	32	13	18	13	323	35	56.12
Q83RX6	Dihydroorotate dehydrogenase (quinone) OS=Shigella flexneri OX=623 GN=pyrD PE=3 SV=4	38.516	44	13	16	13	336	36.7	36.67
A0A2S8DUZ3	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=Shigella boydii OX=621 GN=accA PE=3 SV=1	21.357	22	7	12	7	319	35.2	33.31
I6CVK9	Phenylalanine--tRNA ligase alpha subunit OS=Shigella flexneri K-315 OX=766150 GN=pheS PE=3 SV=1	25.808	26	7	11	7	327	36.8	28.36
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	26.461	19	11	11	11	513	55.2	27.72
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	20.288	25	7	10	7	329	36.5	30.08
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	25.702	19	9	10	9	474	49.3	29.65
Q0T4H3	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1619 PE=4 SV=1	20.505	31	9	10	9	314	33.9	21.02
Q0SYD1	L-lactate dehydrogenase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lldD PE=3 SV=1	28.111	28	9	9	9	396	42.7	25.99
A0A2S4MYF8	Outer membrane protein C OS=Shigella flexneri OX=623 GN=ompC PE=1 SV=1	19.112	17	7	9	7	373	41.4	21.54
I6CHF3	MFS transporter, sugar porter family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3768 PE=3 SV=1	16.604	9	6	9	6	472	51.7	21.19
Q0SZX8	Elongation factor Tu 1 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tufI PE=3 SV=1	18.605	18	7	9	7	394	43.3	20.3
A0A2S8D5N6	Aspartate carbamoyltransferase OS=Shigella dysenteriae OX=622 GN=pyrB PE=3 SV=1	14.797	20	6	8	6	311	34.4	21.03
Q0T6G6	UPF0194 membrane protein YbhG OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ybhG PE=3 SV=1	15.796	23	6	8	6	331	36.3	19.95
A0A2S4MYK1	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta OS=Shigella flexneri OX=623 GN=accD PE=3 SV=1	15.794	37	8	8	8	304	33.4	19.42
A0A2S8DCC8	Delta-aminolevulinic acid dehydratase OS=Shigella dysenteriae OX=622 GN=C5K18_12345 PE=3 SV=1	14.018	29	7	7	7	324	35.6	18.36
A0A2Y5B4B8	Malate dehydrogenase OS=Shigella flexneri OX=623 GN=mdh PE=3 SV=1	16.719	29	7	7	7	312	32.3	16.06
A0A6N3QLJ7	Magnesium transport protein CorA OS=Shigella flexneri CDC 796-83 OX=945360 GN=corA PE=3 SV=1	11.329	14	4	7	4	316	36.6	14.65
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	13.69	15	6	7	6	376	41	14.48
A0A379ZSJ2	Alcohol dehydrogenase OS=Shigella flexneri OX=623 GN=yqhD PE=4 SV=1	15.726	17	5	6	5	387	42.1	18.54
D2AHG2	Autonomous glycyl radical cofactor OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfiD PE=3 SV=1	13.764	28	4	6	4	127	14.2	15.51
I6CBL5	ATP synthase F0, B subunit OS=Shigella flexneri K-315 OX=766150 GN=atpF PE=3 SV=1	14.369	32	6	6	6	127	14	11.8
A0A3T2UWB2	ATP synthase gamma chain OS=Shigella flexneri OX=623 GN=atpG PE=3 SV=1	14.782	22	6	6	6	287	31.6	10.54
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsC PE=3 SV=1	12.634	26	6	6	6	224	24.8	10.2
D2A917	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	10.454	27	6	6	6	124	13.7	5.84
A0A4P7TR77	Maltoporin OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=lamb PE=2 SV=1	17.751	19	4	5	4	446	50	16.51
A0A6N3R6K7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4998 PE=3 SV=1	12.136	8	5	5	5	402	42.7	14.66
A0A2S8DDU2	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OS=Shigella dysenteriae OX=622 GN=murG PE=3 SV=1	11.506	20	5	5	5	355	37.8	13.59
I6D6H9	Transaldolase OS=Shigella flexneri K-315 OX=766150 GN=tal PE=3 SV=1	10.522	17	5	5	5	294	32.8	12.75
A0A6N3QME5	50S ribosomal protein L1 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rplA PE=3 SV=1	7.554	20	4	5	4	234	24.7	11.75
A0A4P7TR58	NAD(P)/FAD-dependent oxidoreductase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_010675 PE=4 SV=1	15.801	17	5	5	5	434	47.3	10.77
I6D072	Outer membrane protein A OS=Shigella flexneri K-315 OX=766150 GN=ompA PE=3 SV=1	10.536	16	5	5	5	344	37	10.62
A0A6N3RB60	50S ribosomal protein L2 OS=Shigella flexneri 1485-80 OX=766155 GN=rplB PE=3 SV=1	7.727	17	4	5	4	273	29.8	9.64
Q7AK95	Chloramphenicol acetyltransferase OS=Shigella flexneri 2b OX=1288825 GN=cat PE=3 SV=1	8.378	7	2	5	2	219	25.6	8.93
A0A2S8DKM8	Lipoprotein-releasing ABC transporter permease subunit LolC OS=Shigella boydii OX=621 GN=lolC PE=3 SV=1	12.242	13	4	4	4	399	43.3	11.54
Q0T001	50S ribosomal protein L15 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rplO PE=3 SV=1	11.074	33	4	4	4	144	15	11.1
I6D3P2	Tol-Pal system protein TolR OS=Shigella flexneri K-315 OX=766150 GN=tolR PE=3 SV=1	6.865	17	2	4	2	142	15.4	9.09
A0A1Q8M994	ADP-L-glycero-D-manno-heptose-6-epimerase OS=Shigella boydii OX=621 GN=rfaD PE=3 SV=1	9.562	13	4	4	4	310	34.9	8.86

A0A3T2UY84	Cyclopropane-fatty-acyl-phospholipid synthase OS=Shigella flexneri OX=623 GN=DK174_15880 PE=3 SV=1	9.93	8	3	4	3	382	43.9	7.6
A0A658YV21	Auxiliary transport protein, membrane fusion protein (MFP) family OS=Shigella flexneri OX=623 GN=macA_2 PE=4 SV=1	7.179	11	4	4	4	355	38.7	7.18
A0A380B4C1	Aminotransferase OS=Shigella flexneri OX=623 GN=aspC PE=3 SV=1	7.652	12	4	4	4	396	43.6	6.92
A0A6A9TRT7	ATP synthase subunit beta OS=Shigella flexneri OX=623 GN=atpD PE=3 SV=1	7.831	8	4	4	4	460	50.3	6.56
A0A6N3R4Q2	50S ribosomal protein L6 OS=Shigella flexneri 1485-80 OX=766155 GN=rpI F PE=3 SV=1	7.246	28	4	4	4	177	18.9	5.13
D2AA83	Outer membrane protein induced after carbon starvation OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=slp PE=4 SV=1	11.721	16	3	3	3	243	27.4	10.61
F5NZV9	Mechanosensitive ion channel family protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3712 PE=3 SV=1	7.104	9	2	3	2	286	30.9	9.9
A0A1S9JMU3	Cell shape-determining protein MreB OS=Shigella boydii OX=621 GN=mreB PE=3 SV=1	6.514	11	3	3	3	347	36.9	9.28
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	7.872	8	3	3	3	477	50.7	8.98
A0A6H1JH06	30S ribosomal protein S5 OS=Shigella flexneri OX=623 GN=rpsE PE=3 SV=1	9.806	30	3	3	3	167	17.6	8.88
Q0SYE6	2-amino-3-ketobutyrate coenzyme A ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=klb PE=3 SV=1	10.281	10	3	3	3	398	43.2	8.23
I6C9W2	30S ribosomal protein S10 OS=Shigella flexneri K-315 OX=766150 GN=rpsJ PE=3 SV=1	6.28	24	2	3	2	103	11.7	8.11
F5NZ29	Serine transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3429 PE=4 SV=1	9.371	6	3	3	3	429	46.9	7.77
F5NT24	Lipoprotein releasing system, transmembrane protein LolE OS=Shigella flexneri K-227 OX=766147 GN=lolE PE=3 SV=1	6.179	6	2	3	2	414	45.3	7.67
I6CAV2	Cell division protein FtsX OS=Shigella flexneri K-315 OX=766150 GN=ftsX PE=3 SV=1	7.001	12	3	3	3	352	38.5	7.34
Q0T5R2	Spermidine/putrescine import ATP-binding protein PotA OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=potA PE=3 SV=1	6.165	6	2	3	2	378	43.1	7.29
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	5.004	10	3	3	3	206	23.4	7.04
A0A383JXY5	NAD dependent epimerase/dehydratase family OS=Shigella flexneri OX=623 GN=DOD76_09530 PE=4 SV=1	6.812	9	3	3	3	337	38	6.97
A0A379Z9W6	Heme biosynthesis protein HemY OS=Shigella flexneri OX=623 GN=hemY PE=4 SV=1	4.603	7	3	3	3	398	45.2	6.29
Q0SZA6	ATP-dependent 6-phosphofructokinase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pfkA PE=3 SV=1	4.995	8	3	3	3	340	37.1	6
I6D3P8	Cytochrome d ubiquinol oxidase subunit 1 OS=Shigella flexneri K-315 OX=766150 GN=cydA PE=3 SV=1	6.115	7	3	3	3	500	55.6	5.96
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	4.911	25	3	3	3	129	13.8	4.58
Q0T0X5	Fructose-bisphosphate aldolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fba PE=3 SV=1	3.133	9	2	3	2	387	42.7	4.49
Q0T5C6	Putative protease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=sohB PE=3 SV=1	4.155	8	3	3	3	296	33.4	4.07
A0A2S8DI33	Sec translocon accessory complex subunit YajC OS=Shigella boydii OX=621 GN=yajC PE=3 SV=1	4.997	26	3	3	3	110	11.9	2.41
F5P2C5	L-threonine 3-dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=tdh PE=3 SV=1	4.061	11	3	3	3	341	37.3	2.34
A0A6N3QLC2	Universal stress protein G OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02439 PE=3 SV=1	7.674	23	2	2	2	142	15.9	7.13
P69785	PTS system glucose-specific EIIA component OS=Shigella flexneri OX=623 GN=crr PE=3 SV=2	8.734	20	2	2	2	169	18.2	6.36
F5P2D1	Rhodanese-like domain protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4672 PE=4 SV=1	8.87	16	2	2	2	143	15.6	6.14
Q83PC3	50S ribosomal protein L11 OS=Shigella flexneri OX=623 GN=rpL PE=3 SV=3	3.771	17	2	2	2	142	14.9	6.09
A0A2S8DA55	SH3 domain-containing protein OS=Shigella dysenteriae OX=622 GN=ygiM PE=4 SV=1	4.849	12	2	2	2	206	23.1	5.97
F5P3L0	Fumarate reductase iron-sulfur subunit OS=Shigella flexneri K-227 OX=766147 GN=SFK227_5105 PE=3 SV=1	5.603	5	1	2	1	244	27	5.94
D2A780	Galactose-proton symporter OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=galP PE=3 SV=1	2.793	4	2	2	2	464	51	5.86
A0A6N3RB16	Protein translocase subunit SecY OS=Shigella flexneri 1485-80 OX=766155 GN=prlA PE=3 SV=1	4.334	3	1	2	1	443	48.5	5.74
A0A2Y4XVQ2	Outer membrane protein assembly complex subunit YfgL OS=Shigella flexneri OX=623 GN=yfgL PE=4 SV=1	6.476	15	2	2	2	184	20.1	5.69
A0A4P7TTH9	30S ribosomal protein S2 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsB PE=3 SV=1	5.243	10	2	2	2	241	26.8	5.63
A0A6L6V6S8	Mobilization protein OS=Shigella flexneri OX=623 GN=GLO03_22460 PE=4 SV=1	4.013	13	2	2	2	176	19.9	5.46
A0A6N3RAZ3	Cell division protein FtsN OS=Shigella flexneri 1485-80 OX=766155 GN=ftsN PE=3 SV=1	3.751	8	2	2	2	284	31.8	5.43
F5P004	L-asparaginase 2 OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3757 PE=3 SV=1	4.245	12	2	2	2	235	25.2	5.38
D2AE64	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4630 PE=3 SV=1	4.298	9	1	2	1	141	15.2	5.28
A0A6N3RR48	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0546 PE=3 SV=1	4.594	8	2	2	2	259	28.2	5.27
A0A4V1CUA5	50S ribosomal protein L28 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpmB PE=3 SV=1	2.698	26	2	2	2	78	9	5.2
A0A1Q8M8J0	50S ribosomal protein L7/L12 OS=Shigella boydii OX=621 GN=rpL PE=3 SV=1	2.227	10	1	2	1	121	12.3	5.17
Q7UCQ9	Lipopolysaccharide assembly protein B OS=Shigella flexneri OX=623 GN=yciM PE=3 SV=1	4.388	5	2	2	2	389	44.4	5.09
A0A2S8DV96	50S ribosomal protein L5 OS=Shigella boydii OX=621 GN=rpI E PE=3 SV=1	5.978	18	2	2	2	179	20.3	4.99
A0A4P7TNY5	Alkyl hydroperoxide reductase C OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=ahpC PE=3 SV=1	3.623	11	2	2	2	187	20.7	4.89
A0A4P7TQF0	50S ribosomal protein L9 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpL PE=3 SV=1	4.577	15	2	2	2	149	15.8	4.81
A0A383JRI2	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=hflB PE=3 SV=1	3.585	3	2	2	2	647	71	4.76
A0A0H2VYI3	Nucleoside permease OS=Shigella flexneri OX=623 GN=nupC PE=3 SV=1	3.331	5	2	2	2	400	43.5	4.68

A0A658Y5Z7	L-1,2-propanediol oxidoreductase OS=Shigella flexneri OX=623 GN=fucO PE=4 SV=1	4.502	7	2	2	2	382	40.4	4.61
A0A383JU60	Chorismate synthase OS=Shigella flexneri OX=623 GN=aroC PE=3 SV=1	5.212	7	2	2	2	361	39.1	4.55
A0A658Z505	Transporter, small conductance mechanosensitive ion channel (MscS) family OS=Shigella flexneri OX=623 GN=mscM PE=3 SV=1	3.796	6	2	2	2	415	46.6	4.55
F5NQ21	Cell division protein FtsQ OS=Shigella flexneri K-227 OX=766147 GN=ftsQ PE=3 SV=1	5.059	9	2	2	2	276	31.4	4.45
F5P3D3	50S ribosomal protein L17 OS=Shigella flexneri K-227 OX=766147 GN=rplQ PE=3 SV=1	2.61	15	2	2	2	127	14.4	4.33
A0A6N3QNZ8	Inorganic pyrophosphatase OS=Shigella flexneri CDC 796-83 OX=945360 GN=ppa PE=3 SV=1	2.825	10	2	2	2	176	19.7	4.29
A0A0H2V319	Putative amino acid/amine transport protein OS=Shigella flexneri OX=623 GN=yifK PE=4 SV=1	2.284	5	2	2	2	417	45.3	4.22
A0A658YZ45	Glutamine ABC transporter ATP-binding protein OS=Shigella flexneri OX=623 GN=glnQ PE=3 SV=1	3.32	9	2	2	2	240	26.6	4.04
D2AHT6	Co-chaperone protein DjIA OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=djIA PE=3 SV=1	2.741	6	2	2	2	271	30.5	3.99
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=Igkc PE=1 SV=1	2.652	6	1	2	1	219	24.2	3.7
A0A658Z1Y3	D-lactate dehydrogenase OS=Shigella flexneri OX=623 GN=idhA PE=3 SV=1	4.464	7	2	2	2	329	36.5	3.48
F5NPS2	Lipoprotein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0205 PE=3 SV=1	2.582	7	2	2	2	271	29.4	3.27
A0A6N3RLA6	Dihydroorotase OS=Shigella flexneri 1485-80 OX=766155 GN=pyrC PE=3 SV=1	3.356	8	2	2	2	324	35.9	2.58
A0A6N3RKB3	KefA OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0517 PE=3 SV=1	2.033	1	2	2	2	1135	128.5	2.15
A0A3T2UX48	Outer membrane protein F OS=Shigella flexneri OX=623 GN=ompF PE=3 SV=1	2.461	5	2	2	2	362	39.3	1.9
Q0SZY4	50S ribosomal protein L4 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rplD PE=3 SV=1	2.834	11	2	2	2	201	22.1	1.79
A0A6N3RKA3	Succinate dehydrogenase iron-sulfur subunit OS=Shigella flexneri 1485-80 OX=766155 GN=sdhB PE=3 SV=1	2.024	7	2	2	2	238	26.8	0
F5P0W4	Uroporphyrinogen decarboxylase OS=Shigella flexneri K-227 OX=766147 GN=heme PE=3 SV=1	2.31	5	2	2	2	354	39.2	0
A0A3T2USL9	Putative basic amino acid antiporter YfcO OS=Shigella flexneri OX=623 GN=DK174_04975 PE=4 SV=1	2.185	5	2	2	2	506	54.7	0
D2AGV7	TPR_21 domain-containing protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfgM PE=3 SV=1	5.695	13	1	1	1	206	22.1	5.61
A0A6D2W4F9	Chaperone protein Skp OS=Shigella flexneri OX=623 GN=skp PE=3 SV=1	4.474	9	1	1	1	161	17.7	4.35
F5NXM7	NADH-quinone oxidoreductase subunit L OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2904 PE=3 SV=1	2.355	2	1	1	1	611	66.1	3.51
A0A2Y4XMF0	Sec-independent protein translocase protein TatA OS=Shigella flexneri OX=623 GN=tatA PE=3 SV=1	3.419	15	1	1	1	89	9.7	3.43
I6CG83	Phosphoglycerate kinase OS=Shigella flexneri K-315 OX=766150 GN=pgk PE=3 SV=1	3.287	4	1	1	1	382	40.5	3.43
A0A6N3R6Z9	Lipopolysaccharide export system permease protein LptF OS=Shigella flexneri 1485-80 OX=766155 GN=yjgP PE=3 SV=1	3.767	3	1	1	1	335	36.6	3.38
I6CJU1	Acetate kinase OS=Shigella flexneri K-315 OX=766150 GN=ackA PE=3 SV=1	4.906	6	1	1	1	400	43.3	3.3
A0A6N3RRG2	Outer membrane lipoprotein RcsF OS=Shigella flexneri 1485-80 OX=766155 GN=rscF PE=3 SV=1	3.76	12	1	1	1	117	12.4	3.25
A0A2S8DCH8	Cysteine synthase OS=Shigella dysenteriae OX=622 GN=cysK PE=3 SV=1	4.378	4	1	1	1	323	34.5	3.22
F5NX93	EIII-Fru OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2769 PE=4 SV=1	2.816	3	1	1	1	376	39.7	3.15
A0A2Y4XZJ9	Ferrocyclase OS=Shigella flexneri OX=623 GN=hemH PE=3 SV=1	2.354	7	1	1	1	320	35.9	3.07
A0A6N3QGJ9	UPF0301 protein YggE OS=Shigella flexneri CDC 796-83 OX=945360 GN=yggE PE=3 SV=1	3.85	6	1	1	1	187	20.7	3.02
A0A2S4N174	50S ribosomal protein L22 OS=Shigella flexneri OX=623 GN=rplV PE=3 SV=1	2.615	13	1	1	1	110	12.2	2.96
I6CPN2	Ferritin OS=Shigella flexneri K-315 OX=766150 GN=ftnA PE=3 SV=1	3.716	7	1	1	1	165	19.4	2.93
A0A6L6UVT0	Lipid A biosynthesis lauroyltransferase OS=Shigella flexneri OX=623 GN=lpXL PE=3 SV=1	2.871	4	1	1	1	306	35.4	2.91
A0A6N3QEL1	50S ribosomal protein L19 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rplS PE=3 SV=1	2.719	14	1	1	1	115	13.1	2.9
Q83MF6	Cell division protein FtsZ OS=Shigella flexneri OX=623 GN=ftsZ PE=3 SV=2	4.85	3	1	1	1	383	40.1	2.89
A0A1Q8NKE7	Phosphoheptose isomerase OS=Shigella dysenteriae OX=622 GN=gmhA PE=3 SV=1	2.809	5	1	1	1	192	20.8	2.86
I6C9X6	30S ribosomal protein S14 OS=Shigella flexneri K-315 OX=766150 GN=rpsN PE=3 SV=1	1.866	10	1	1	1	96	11	2.77
A0A2S8DA81	Succinate--CoA ligase [ADP-forming] subunit beta OS=Shigella dysenteriae OX=622 GN=sucC PE=3 SV=1	2.64	4	1	1	1	388	41.4	2.76
A0A2Y5A6X9	30S ribosomal protein S9 OS=Shigella flexneri OX=623 GN=rpsI PE=3 SV=1	2.435	14	1	1	1	85	9.7	2.71
A0A237F1C5	Arginine ABC transporter ATP-binding protein ArtP OS=Shigella boydii OX=621 GN=artP PE=3 SV=1	1.478	4	1	1	1	242	27	2.7
A0A2Y4XUR6	Fructose-bisphosphate aldolase OS=Shigella flexneri OX=623 GN=fbaB PE=4 SV=1	1.976	13	1	1	1	79	8.3	2.67
I6CMC4	Lysine-specific permease OS=Shigella flexneri K-315 OX=766150 GN=lysP PE=4 SV=1	1.906	2	1	1	1	489	53.5	2.61
Q0SY57	ATP-dependent protease subunit HslV OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hslV PE=3 SV=1	1.634	6	1	1	1	176	19.1	2.6
I6D602	Protein-export membrane protein SecF OS=Shigella flexneri K-315 OX=766150 GN=secF PE=3 SV=1	1.456	6	1	1	1	304	33.2	2.57
A0A6N3RQ35	DNA protection during starvation protein OS=Shigella flexneri 1485-80 OX=766155 GN=dps PE=3 SV=1	2.978	11	1	1	1	167	18.7	2.56
A0A2S4MTA8	Lactate utilization protein C OS=Shigella flexneri OX=623 GN=ykgG PE=4 SV=1	2.8	4	1	1	1	231	25.2	2.56
A0A2S8DD79	Threonine/serine transporter TdcC OS=Shigella dysenteriae OX=622 GN=tdcC PE=3 SV=1	2.294	3	1	1	1	443	48.9	2.56
I6CFZ0	50S ribosomal protein L27 OS=Shigella flexneri K-315 OX=766150 GN=rpmA PE=3 SV=1	2.263	16	1	1	1	85	9.1	2.55
A0A658Z1V1	UPF0283 membrane protein YcjF OS=Shigella flexneri OX=623 GN=yjfF PE=3 SV=1	3.895	4	1	1	1	353	39.4	2.53
A0A2S8DET8	Galactose-1-phosphate uridylyltransferase OS=Shigella dysenteriae OX=622 GN=C5K18_07390 PE=3 SV=1	1.671	3	1	1	1	348	39.6	2.53



A0A2Y4XSH5	Periplasmic protein of mal regulon OS=Shigella flexneri OX=623 GN=malM PE=4 SV=1	1.437	3	1	1	1	306	31.9	2.52
A0A3T2UTJ3	N-acetylglucosamine-6-phosphate deacetylase OS=Shigella flexneri OX=623 GN=DK174_06800 PE=3 SV=1	1.643	3	1	1	1	382	41	2.5
A0A6N3QQN1	Trehalosealtose-specific PTS system components IIBC OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02485 PE=4 SV=1	1.843	2	1	1	1	473	51.2	2.5
A0A1Q8MQJ7	Protein RecA OS=Shigella boydii OX=621 GN=recA PE=3 SV=1	1.572	3	1	1	1	353	38	2.47
Q0T239	4-hydroxy-tetrahydrodipicolinate synthase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dapA PE=3 SV=1	1.9	4	1	1	1	292	31.3	2.42
A0A380B6A5	BAX inhibitor (BI)-1/YccA family protein OS=Shigella flexneri OX=623 GN=ybhL_2 PE=3 SV=1	1.777	3	1	1	1	234	25.9	2.41
A0A6N3RL91	L-arabinose isomerase OS=Shigella flexneri 1485-80 OX=766155 GN=araA PE=3 SV=1	2.113	2	1	1	1	500	56.1	2.41
A0A383JYQ3	Aspartate carbamoyltransferase regulatory chain OS=Shigella flexneri OX=623 GN=pyrI PE=3 SV=1	1.594	17	1	1	1	153	17.1	2.4
D2A8T7	Cytochrome d ubiquinol oxidase subunit III OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yhcB PE=4 SV=1	1.42	8	1	1	1	134	15.2	2.39
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	2.787	15	1	1	1	78	8.3	2.38
A0A1S9KGI3	Peptidyl-prolyl cis-trans isomerase OS=Shigella dysenteriae OX=622 GN=fkpA PE=3 SV=1	1.24	4	1	1	1	270	28.9	2.38
F5P3C9	30S ribosomal protein S13 OS=Shigella flexneri K-227 OX=766147 GN=rpsM PE=3 SV=1	1.91	8	1	1	1	118	13.1	2.37
A0A658Z233	Inner membrane protein yjgQ OS=Shigella flexneri OX=623 GN=yjgQ PE=3 SV=1	3.165	4	1	1	1	360	39.6	2.36
A0A6N3QQ51	Maltose regulon modulator MalY OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01515 PE=4 SV=1	2.622	3	1	1	1	390	43.6	2.35
A0A2S8DX13	Aromatic amino acid transporter AroP OS=Shigella boydii OX=621 GN=C5K23_02025 PE=4 SV=1	1.291	2	1	1	1	457	49.7	2.34
A0A2S8DFP5	Cell division protein DedD OS=Shigella dysenteriae OX=622 GN=dedD PE=3 SV=1	2.908	5	1	1	1	220	22.9	2.31
A0A1Q8M4D3	30S ribosomal protein S18 OS=Shigella boydii OX=621 GN=rpsR PE=3 SV=1	1.813	24	1	1	1	75	9	2.3
A0A2Y5Q6A1	YmiA family putative membrane protein OS=Shigella flexneri OX=623 GN=ymiA PE=4 SV=1	1.141	21	1	1	1	42	4.9	2.3
A0A6N3QU25	Thiol peroxidase OS=Shigella flexneri CDC 796-83 OX=945360 GN=tpx PE=3 SV=1	3.385	14	1	1	1	168	17.8	2.28
F5NV08	Tyrosine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=tyrS PE=3 SV=1	1.62	3	1	1	1	424	47.6	2.28
F5P2M7	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4769 PE=4 SV=1	3.67	6	1	1	1	236	25.6	2.26
A0A4P7TM74	30S ribosomal protein S21 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsU PE=3 SV=1	1.888	11	1	1	1	71	8.5	2.21
A0A379Z1S8	Protein HflK OS=Shigella flexneri OX=623 GN=hflK PE=3 SV=1	2.687	3	1	1	1	419	45.6	2.21
F5P0M6	10 kDa chaperonin OS=Shigella flexneri K-227 OX=766147 GN=groS PE=3 SV=1	1.234	14	1	1	1	97	10.4	2.2
A0A383JXW9	UPF0145 protein YbjQ OS=Shigella flexneri OX=623 GN=ybjQ PE=3 SV=1	2.391	11	1	1	1	107	11.4	2.2
A0A1W2MD82	Protease HtpX OS=Shigella flexneri OX=623 GN=htpX PE=3 SV=1	3.114	4	1	1	1	293	31.9	2.2
D2ADR2	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_1449 PE=4 SV=1	1.065	20	1	1	1	54	6.2	2.13
Q0T2K3	NADH-quinone oxidoreductase subunit F OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nuoF PE=3 SV=1	3.271	3	1	1	1	445	49.4	2.12
I6D5T1	Gamma-glutamyl phosphate reductase OS=Shigella flexneri K-315 OX=766150 GN=proA PE=3 SV=1	1.475	3	1	1	1	417	44.5	2.11
I6CFU5	Peptidase Do OS=Shigella flexneri K-315 OX=766150 GN=degS PE=3 SV=1	3.131	3	1	1	1	355	37.6	2.06
A0A658Y8X2	Outer membrane protein IcsA OS=Shigella flexneri OX=623 GN=icsA_2 PE=4 SV=1	1.371	1	1	1	1	1092	114.9	2.05
A0A6N3R6J7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4987 PE=3 SV=1	1.405	2	1	1	1	446	47.8	1.96
I6CXD2	UTP--glucose-1-phosphate uridylyltransferase OS=Shigella flexneri K-315 OX=766150 GN=galU PE=3 SV=1	2.795	4	1	1	1	302	33	1.95
D2A8B5	Cytoplasmic membrane protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yrbD PE=4 SV=1	1.223	7	1	1	1	183	19.6	1.91
F5NVB6	Glutamate/gamma-aminobutyrate antiporter OS=Shigella flexneri K-227 OX=766147 GN=gadC PE=3 SV=1	1.703	2	1	1	1	511	55.1	1.91
A0A2D0WWE7	dTDP-glucose 4,6-dehydratase OS=Shigella flexneri x Salmonella enterica subsp. enterica serovar Typhi OX=1985423 GN=rfbB PE=3 SV=1	1.587	3	1	1	1	361	40.6	1.9
A0A3U1D8Q6	C4-dicarboxylic acid transporter DauA OS=Shigella flexneri OX=623 GN=dauA PE=3 SV=1	1.936	2	1	1	1	559	59.4	1.88
F5NVZ8	Outer membrane porin protein LC OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2311 PE=3 SV=1	2.086	3	1	1	1	360	39.7	1.85
A0A2S8E8N3	Superoxide dismutase OS=Shigella dysenteriae OX=622 GN=sodB PE=3 SV=1	1.494	6	1	1	1	193	21.3	1.8
Q5INY3	Clp protease ATP-binding subunit (Fragment) OS=Shigella flexneri OX=623 GN=clpX PE=4 SV=1	2.462	6	1	1	1	203	21.7	1.72
A0A2S8D3U0	Catabolite activator protein OS=Shigella dysenteriae OX=622 GN=crp PE=4 SV=1	1.55	5	1	1	1	210	23.7	1.72
I6CB41	Tryptophan--tRNA ligase OS=Shigella flexneri K-315 OX=766150 GN=trpS PE=3 SV=1	1.48	4	1	1	1	319	35.9	1.7
A0A1S9KIA0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	1.2	2	1	1	1	419	47	1.7
A0A4P7TTF4	Methionine import ATP-binding protein MetN OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=metN PE=3 SV=1	1.343	2	1	1	1	343	37.8	1.66
A0A6N3QTS6	Uncharacterized protein OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00635 PE=4 SV=1	1.818	16	1	1	1	50	5.7	0
A0A6D2X1F2	30S ribosomal protein S19 OS=Shigella flexneri OX=623 GN=rpsS PE=3 SV=1	2.006	12	1	1	1	92	10.4	0
P64603	Intermembrane phospholipid transport system binding protein MlaB OS=Shigella flexneri OX=623 GN=mlaB PE=3 SV=1	1.036	9	1	1	1	97	10.7	0
Q0SZX6	30S ribosomal protein S7 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsG PE=3 SV=1	1.061	9	1	1	1	179	20	0
A0A4P7TQT3	50S ribosomal protein L10 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rplJ PE=3 SV=1	2.313	7	1	1	1	165	17.7	0

P0A6P4	Elongation factor Ts OS=Shigella flexneri OX=623 GN=tsf PE=3 SV=2	1.144	6	1	1	1	283	30.4	0
F5P316	Fe/S biogenesis protein NfuA OS=Shigella flexneri K-227 OX=766147 GN=nfuA PE=3 SV=1	2.35	6	1	1	1	191	21	0
I6C9W3	50S ribosomal protein L3 OS=Shigella flexneri K-315 OX=766150 GN=rplC PE=3 SV=1	0.957	6	1	1	1	209	22.2	0
A0A380AGY6	Iron-sulfur cluster carrier protein OS=Shigella flexneri OX=623 GN=minD_1 PE=3 SV=1	1.384	5	1	1	1	369	39.9	0
Q0SZX1	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yheO PE=4 SV=1	1.301	5	1	1	1	240	26.8	0
I6CBX6	Lipopolysaccharide heptosyltransferase III, putative OS=Shigella flexneri K-315 OX=766150 GN=SFK315_4420 PE=4 SV=1	1.016	4	1	1	1	254	28	0
A0A6N3RM85	Type IV conjugative transfer system protein TraV OS=Shigella flexneri 1485-80 OX=766155 GN=traV PE=4 SV=1	0.949	4	1	1	1	171	18.4	0
A0A6N3RKN1	Uracil phosphoribosyltransferase OS=Shigella flexneri 1485-80 OX=766155 GN=upp PE=3 SV=1	1.108	4	1	1	1	208	22.5	0
A0A6N3RIF3	Enoyl-[acyl-carrier-protein] reductase [NADH] OS=Shigella flexneri 1485-80 OX=766155 GN=fabI PE=3 SV=1	1.149	4	1	1	1	262	27.8	0
A0A6L6V006	Ribose-phosphate pyrophosphokinase OS=Shigella flexneri OX=623 GN=prs PE=3 SV=1	1.66	4	1	1	1	315	34.2	0
A0A3T2UQA1	Glutamate-1-semialdehyde 2,1-aminomutase OS=Shigella flexneri OX=623 GN=hemL PE=3 SV=1	1.288	3	1	1	1	426	45.3	0
A0A6N3QM36	Protein QmcA OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01963 PE=3 SV=1	0.92	3	1	1	1	305	33.7	0
I6D405	PhoH-like protein OS=Shigella flexneri K-315 OX=766150 GN=ybeZ PE=3 SV=1	1	3	1	1	1	346	39	0
Q0SX09	Phosphoserine aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=serC PE=3 SV=1	1.657	3	1	1	1	350	38.6	0
A0A6G5YYG3	Lipoprotein-releasing system ATP-binding protein LolD OS=Shigella flexneri OX=623 GN=lolD PE=3 SV=1	1.141	3	1	1	1	233	25.5	0
A0A2S8DUL5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase OS=Shigella boydii OX=621 GN=dapD PE=3 SV=1	2.158	3	1	1	1	274	29.9	0
A0A2S8D6C3	Proline-specific permease ProY OS=Shigella dysenteriae OX=622 GN=C5K18_23740 PE=4 SV=1	1.323	3	1	1	1	457	50.1	0
A0A2S8D993	H(+)/Cl(-) exchange transporter ClcA OS=Shigella dysenteriae OX=622 GN=clcA PE=3 SV=1	1.775	3	1	1	1	473	50.4	0
F5NQP3	Molybdenum import ATP-binding protein ModC OS=Shigella flexneri K-227 OX=766147 GN=modC PE=3 SV=1	0.926	3	1	1	1	352	39	0
A0A3T2UTL7	UDP-glucose 4-epimerase OS=Shigella flexneri OX=623 GN=galE PE=3 SV=1	0.938	2	1	1	1	338	37.2	0
A0A6N3RLZ1	Cytochrome d ubiquinol oxidase, subunit II OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0759 PE=3 SV=1	1.36	2	1	1	1	379	42.4	0
A0A4P7TRM2	Lipopolysaccharide N-acetylglucosaminyltransferase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_019395 PE=4 SV=1	1.209	2	1	1	1	380	43.5	0
Q0T0J7	DNA primase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dnaG PE=3 SV=1	1.014	2	1	1	1	581	65.6	0
A0A2S8E636	Acetyltransferase component of pyruvate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=aceF PE=3 SV=1	1.36	1	1	1	1	630	66.1	0
F5NT19	Transcription-repair-coupling factor OS=Shigella flexneri K-227 OX=766147 GN=mfd PE=3 SV=1	1.08	1	1	1	1	1148	129.9	0

**Appx. D.11: Full protein identification from band 11 of Figure 5.2b.**

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score	Sequest HT
A0A3U1DEW5	Formate C-acetyltransferase OS=Shigella flexneri OX=623 GN=pfIB PE=3 SV=1	148.42	37	30	57	30	760	85.3	145.5	
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=rfc PE=3 SV=1	35.985	18	9	57	9	382	43.7	103.38	
A0A4P7TP61	Mechanosensitive channel MscK OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=mscK PE=3 SV=1	164.975	37	33	56	33	1120	127.2	163.27	
Q0SXC2	Fumarate reductase flavoprotein subunit OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=frdA PE=3 SV=1	113.979	46	24	45	24	602	65.9	109.82	
A0A383JRI2	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=hfIB PE=3 SV=1	86.229	34	24	38	24	647	71	90.23	
A0A2S8D4S3	Aldehyde-alcohol dehydrogenase OS=Shigella dysenteriae OX=622 GN=C5K18_27725 PE=3 SV=1	114.966	33	25	34	25	891	96.1	88.48	
A0A1Q8MQG3	Chaperone protein DnaK OS=Shigella boydii OX=621 GN=dnaK PE=2 SV=1	114.827	51	29	32	29	638	69.1	85.87	
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	83.557	47	19	31	19	513	55.2	92.23	
A0A380B9F2	Peptidylprolyl isomerase OS=Shigella flexneri OX=623 GN=ppiD PE=4 SV=1	112.19	51	23	30	23	623	68.1	88.52	
A0A4P7TR58	NAD(P)/FAD-dependent oxidoreductase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_010675 PE=4 SV=1	94.636	46	16	30	16	434	47.3	77.25	
A0A658XXS4	Outer membrane protein assembly factor YaeT OS=Shigella flexneri OX=623 GN=yaeT PE=4 SV=1	103.1	31	25	30	25	810	90.5	76.72	
A0A4P7TRN0	Pyruvate dehydrogenase E1 component OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=aceE PE=4 SV=1	83.531	28	24	30	24	887	99.6	71.23	
Q0SZX8	Elongation factor Tu 1 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tuf1 PE=3 SV=1	82.639	60	20	30	2	394	43.3	70.85	
Q0T1R3	Chaperone protein ClpB OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=clpB PE=3 SV=1	74.365	28	24	30	24	857	95.6	63.46	
A0A6G5YV19	Elongation factor Tu OS=Shigella flexneri OX=623 GN=tuf PE=3 SV=1	76.64	60	20	28	2	394	43.3	62.67	
A0A6D2XMH9	ATP synthase subunit beta OS=Shigella flexneri OX=623 GN=atpD PE=3 SV=1	105.877	54	19	26	19	460	50.3	83.53	
A0A379Z3L5	Glycerol-3-phosphate acyltransferase OS=Shigella flexneri OX=623 GN=plsB PE=3 SV=1	64.695	27	20	25	20	807	91.4	55.83	
A0A0H2UYV7	Nitrate reductase (quinone) OS=Shigella flexneri OX=623 GN=narG PE=3 SV=1	79.959	17	19	24	19	1247	140.4	63.56	
A0A2Y4Y099	Glycerol-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=glpD PE=3 SV=1	60.008	35	16	23	16	501	56.7	49.86	
P0A852	Trigger factor OS=Shigella flexneri OX=623 GN=tig PE=3 SV=1	53.41	32	15	22	15	432	48.2	54.53	
F5P3A0	Elongation factor G OS=Shigella flexneri K-227 OX=766147 GN=fusA PE=3 SV=1	79.468	32	19	22	19	704	77.6	52.33	
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	68.814	25	13	21	13	474	49.3	58.32	
A0A2S8D6C0	Protein translocase subunit SecD OS=Shigella dysenteriae OX=622 GN=secD PE=3 SV=1	63.41	30	16	20	16	615	66.6	51.59	
A0A6N3R3T7	Aspartate ammonia-lyase OS=Shigella flexneri 1485-80 OX=766155 GN=aspA PE=3 SV=1	59.001	33	15	20	15	478	52.3	50.18	
A0A6N3QN52	Lon protease OS=Shigella flexneri CDC 796-83 OX=945360 GN=lon PE=2 SV=1	52.849	25	19	20	19	799	89	39.35	
A0A379Z256	60 kDa chaperonin OS=Shigella flexneri OX=623 GN=groEL PE=3 SV=1	62.115	34	15	19	15	551	57.5	51.12	
I6CMA1	Quinone-dependent D-lactate dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=dld PE=3 SV=1	51.976	32	15	19	15	571	64.6	41.55	
A0A4P7TME0	Transketolase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tkt PE=3 SV=1	48.157	22	14	18	14	663	72.1	38.95	
I6D6Q1	Peptidoglycan D,D-transpeptidase FtsI OS=Shigella flexneri K-315 OX=766150 GN=ftsI PE=3 SV=1	57.84	29	14	16	14	588	63.9	42.24	
Q83SE6	Chaperone protein HtpG OS=Shigella flexneri OX=623 GN=htpG PE=3 SV=1	50.229	29	15	16	15	624	71.3	38.97	
A0A0H2VVY4	Anaerobic dimethyl sulfoxide reductase subunit A OS=Shigella flexneri OX=623 GN=dmsA PE=3 SV=1	47.603	20	13	15	13	785	87.4	35.66	
D2AD66	DNA-directed RNA polymerase subunit beta OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpoB PE=3 SV=1	31.035	11	14	15	14	1342	150.5	21.18	
F5NRH8	Efflux pump membrane transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0715 PE=3 SV=1	43.306	15	12	14	12	1049	113.6	37.58	
I6CAU4	Cadmium-translocating P-type ATPase OS=Shigella flexneri K-315 OX=766150 GN=cadA PE=3 SV=1	39.034	19	11	14	11	732	76.7	33.39	
A0A2S4MYF8	Outer membrane protein C OS=Shigella flexneri OX=623 GN=ompC PE=1 SV=1	35.645	20	9	14	9	373	41.4	31.82	
I6D6T4	Dihydrolipoyl dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=lpdA PE=3 SV=1	32.037	20	9	13	9	474	50.7	33.43	
A0A2S8E1V7	Protein HflK OS=Shigella dysenteriae OX=622 GN=hflK PE=3 SV=1	34.453	24	8	13	8	419	45.5	29.92	
A0A6N3QD48	Succinate dehydrogenase flavoprotein subunit OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04889 PE=3 SV=1	33.894	13	8	13	8	588	64.4	29.36	
Q83SN2	Protein translocase subunit SecA OS=Shigella flexneri OX=623 GN=secA PE=3 SV=1	34.743	13	13	13	13	901	102	27.94	
A0A658YVY8	L-tryptophan indole-lyase OS=Shigella flexneri OX=623 GN=tnaA PE=3 SV=1	28.446	21	12	13	12	471	52.8	22.09	
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsC PE=3 SV=1	32.353	34	10	13	10	224	24.8	20.25	
A0A658Y1M8	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=gapA PE=3 SV=1	47.71	37	11	12	11	331	35.5	35.83	
A0A658YVYQ4	NAD(P) transhydrogenase subunit alpha OS=Shigella flexneri OX=623 GN=pntA PE=3 SV=1	37.757	18	8	12	8	510	54.5	32.63	

A0A3T2UWB2	ATP synthase gamma chain OS=Shigella flexneri OX=623 GN=atpG PE=3 SV=1	38.112	38	12	12	12	287	31.6	28.2
A0A658YV63	Bifunctional acyl-[acyl carrier protein] synthetase/2-acylglycerophosphoethanolamine acyltransferase OS=Shigella flexneri OX=623 GN=aas PE=4 SV=1	30.403	16	12	12	12	719	80.6	24.99
D2ABG4	30S ribosomal protein S1 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsA PE=3 SV=1	22.968	16	9	12	9	557	61.1	21.07
A0A2Y5C0F7	Putative uroporphyrinogen III C-methyltransferase OS=Shigella flexneri OX=623 GN=hemX PE=4 SV=1	41.745	32	10	11	10	401	43.6	34.05
A0A2S8D9R8	Penicillin-binding protein 1B OS=Shigella dysenteriae OX=622 GN=mrcB PE=3 SV=1	36.164	15	10	11	10	840	93.8	27.07
I6CS25	Protein ydgA OS=Shigella flexneri K-315 OX=766150 GN=ydgA PE=4 SV=1	32.151	26	10	11	10	502	54.7	26.92
A0A2S8E636	Acetyltransferase component of pyruvate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=aceF PE=3 SV=1	33.477	18	10	11	10	630	66.1	26.62
F5NUC5	Tail-specific protease OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1725 PE=3 SV=1	24.696	17	10	11	10	680	76.5	21.29
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	30.964	21	8	10	8	477	50.7	25.74
A0A6N3RB60	50S ribosomal protein L2 OS=Shigella flexneri 1485-80 OX=766155 GN=rplB PE=3 SV=1	22.124	26	6	10	2	273	29.8	25.1
A0A3T2V040	Formate dehydrogenase-N subunit alpha OS=Shigella flexneri OX=623 GN=fdnG PE=3 SV=1	30.954	11	10	10	10	1016	112.5	23.17
Q0SYD1	L-lactate dehydrogenase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lldD PE=3 SV=1	30.998	30	9	10	9	396	42.7	22.72
Q0T4H3	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SfV_1619 PE=4 SV=1	26.764	28	9	10	9	314	33.9	20.72
A0A1S9KGJ3	Peptidyl-prolyl cis-trans isomerase OS=Shigella dysenteriae OX=622 GN=fkpA PE=3 SV=1	21.523	35	8	10	8	270	28.9	19.8
A0A2A2XR75	EIIAB-Man OS=Shigella flexneri OX=623 GN=manX_2 PE=4 SV=1	46.547	30	9	9	9	323	35	30.52
A0A2S8DUZ3	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=Shigella boydii OX=621 GN=accA PE=3 SV=1	33.954	24	7	9	7	319	35.2	27.74
A0A6N3RCV1	Alanine--tRNA ligase OS=Shigella flexneri 1485-80 OX=766155 GN=alaS PE=3 SV=1	36.553	12	9	9	9	876	96	26.74
A0A2Y4Y9J3	Pyruvate kinase OS=Shigella flexneri OX=623 GN=pykF PE=3 SV=1	33.716	17	9	9	9	542	58.7	24.41
I6CBL5	ATP synthase F0, B subunit OS=Shigella flexneri K-315 OX=766150 GN=atpF PE=3 SV=1	27.321	62	8	9	8	127	14	24.1
Q0T1P7	Enolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=eno PE=3 SV=1	28.252	19	8	9	8	431	45.7	24.03
I6CI36	Cytoskeleton protein RodZ OS=Shigella flexneri K-315 OX=766150 GN=rodZ PE=3 SV=1	26.164	22	7	9	7	337	36.2	23.58
F5NPX4	LPS-assembly protein LptD OS=Shigella flexneri K-227 OX=766147 GN=lptD PE=3 SV=1	28.885	15	9	9	9	753	86.3	22.95
A0A379Z9W6	Heme biosynthesis protein HemY OS=Shigella flexneri OX=623 GN=hemY PE=4 SV=1	20.068	19	9	9	9	398	45.2	21.55
Q0T8D5	L-arabinose isomerase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=araA PE=3 SV=1	22.187	16	8	9	8	500	56	20.21
Q2EUU0	AccD (Fragment) OS=Shigella flexneri OX=623 GN=accD PE=3 SV=1	24.742	26	7	9	7	296	32.6	18.62
F5NRX2	Serine-type D-Ala-D-Ala carboxypeptidase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0860 PE=3 SV=1	22.919	20	7	9	7	403	44.4	17.12
F5NUR0	Threonine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=thrS PE=3 SV=1	24.517	11	9	9	9	642	74	14.87
F5NY87	Phosphoenolpyruvate-protein phosphotransferase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3048 PE=3 SV=1	35.511	16	8	8	8	585	64.5	25
D2AHG2	Autonomous glycol radical cofactor OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfiD PE=3 SV=1	26.7	33	5	8	5	127	14.2	24.82
I6CS12	NAD(P) transhydrogenase subunit beta OS=Shigella flexneri K-315 OX=766150 GN=pntB PE=3 SV=1	36.063	16	5	8	5	462	48.7	23.95
A0A2S8DDU2	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OS=Shigella dysenteriae OX=622 GN=murG PE=3 SV=1	24.307	22	6	8	6	355	37.8	22.38
A0A2Y4JWQ5	MbeB-like, N-term conserved region OS=Shigella flexneri OX=623 GN=AMEA3710514_05138 PE=4 SV=1	25.409	32	6	8	6	176	19.9	20.81
I6D3P8	Cytochrome d ubiquinol oxidase subunit 1 OS=Shigella flexneri K-315 OX=766150 GN=cydA PE=3 SV=1	26.736	17	8	8	8	500	55.6	20.66
A0A2X2I0N0	Cell division protein DamX OS=Shigella flexneri OX=623 GN=damX PE=3 SV=1	34.111	21	6	8	6	428	46.1	20.63
A0A380B6V8	Copper exporting ATPase OS=Shigella flexneri OX=623 GN=copA PE=3 SV=1	19.961	8	6	8	6	834	87.9	20.6
A0A658Y983	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degQ PE=3 SV=1	28.571	22	8	8	8	455	47.2	20.21
I6C9W6	50S ribosomal protein L2 OS=Shigella flexneri K-315 OX=766150 GN=rplB PE=3 SV=1	15.964	22	5	8	1	255	27.9	19.61
F5P0F1	Diguanylate cyclase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3908 PE=4 SV=1	23.8	11	6	8	6	602	68.4	19.57
Q7BCK4	Outer membrane protein IcsA autotransporter OS=Shigella flexneri OX=623 GN=icsA PE=1 SV=1	24.161	8	8	8	8	1102	116.2	18.26
I6CJU2	Phosphate acetyltransferase OS=Shigella flexneri K-315 OX=766150 GN=pta PE=3 SV=1	21.814	11	8	8	6	709	76.5	17.8
A0A2S8DJU5	Polyribonucleotide nucleotidyltransferase OS=Shigella boydii OX=621 GN=pnp PE=3 SV=1	20.278	13	8	8	8	711	77.1	17.09
D2AFY9	NADH-quinone oxidoreductase subunit C/D OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=nuoC PE=3 SV=1	21.924	12	7	8	7	600	68.7	16.09
D2A8B5	Cytoplasmic membrane protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yrbD PE=4 SV=1	24.87	43	6	8	6	183	19.6	15.98
A0A6N3REQ7	Nickel-dependent hydrogenase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3604 PE=3 SV=1	12.841	9	5	8	5	567	62.5	14.73
A0A383JWV6	Membrane protein insertase YidC OS=Shigella flexneri OX=623 GN=yidC PE=3 SV=1	17.103	10	4	8	4	548	61.5	14.54
F5NX93	EIII-Fru OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2769 PE=4 SV=1	19.442	22	7	8	7	376	39.7	14.43
A0A1S9KIA0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	19.799	24	8	8	8	419	47	13.47
A0A380B6P9	PTS N-acetylglucosamine EIICBA component OS=Shigella flexneri OX=623 GN=nagE PE=4 SV=1	21.242	11	8	8	8	648	68.3	12.62
F5P070	Translation initiation factor IF-2 OS=Shigella flexneri K-227 OX=766147 GN=infB PE=3 SV=1	17.069	9	8	8	8	890	97.3	12.57

A0A2S8DKT5	NADH-quinone oxidoreductase OS=Shigella boydii OX=621 GN=nuoG PE=3 SV=1	28.986	11	7	7	7	908	100.3	19.3
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	20.737	21	6	7	6	329	36.5	18.38
A0A4V1CUD1	Histidine kinase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=cpxA PE=4 SV=1	17.944	14	7	7	7	457	51.6	17.69
A0A4P7TRQ9	Ribulokinase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=araB PE=3 SV=1	25.683	17	7	7	7	566	61.1	16.64
A0A6N3QN50	Cysteine synthase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02637 PE=3 SV=1	23.391	29	6	7	6	323	34.5	16.3
A0A4P7TSA3	Dihydropolyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=odhB PE=3 SV=1	24.955	19	7	7	1	405	44	15.19
A0A1S9JMU3	Cell shape-determining protein MreB OS=Shigella boydii OX=621 GN=mreB PE=3 SV=1	21.244	20	6	7	6	347	36.9	13.82
P0A6P4	Elongation factor Ts OS=Shigella flexneri OX=623 GN=tsf PE=3 SV=2	18.295	25	7	7	7	283	30.4	13.71
A0A3T2UUK6	Energy-dependent translational throttle protein EttA OS=Shigella flexneri OX=623 GN=yjjK PE=3 SV=1	15.479	10	5	7	5	555	62.4	13.51
A0A2S8D9Q6	Dihydropolyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=sucB PE=3 SV=1	20.025	17	7	7	1	405	44.1	12.63
I6CAV2	Cell division protein FtsX OS=Shigella flexneri K-315 OX=766150 GN=ftsX PE=3 SV=1	21.696	19	6	7	6	352	38.5	12.36
D2ABQ4	Transposon Tn7 transposition protein tnsC OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4148 PE=4 SV=1	2.735	4	1	7	1	555	63	0
Q0T240	Outer membrane protein assembly factor BamC OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nlpB PE=3 SV=1	21.694	21	5	6	5	344	36.8	17.87
A0A6N3QJ73	Fructose-specific PTS system IIBC component OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03096 PE=4 SV=1	26.263	12	5	6	5	563	57.5	16.36
I6D072	Outer membrane protein A OS=Shigella flexneri K-315 OX=766150 GN=ompA PE=3 SV=1	20.417	19	6	6	6	344	37	15.34
A0A6N3RBI6	Protein translocase subunit SecY OS=Shigella flexneri 1485-80 OX=766155 GN=prlA PE=3 SV=1	17.451	11	5	6	5	443	48.5	15.16
Q7AK95	Chloramphenicol acetyltransferase OS=Shigella flexneri 2b OX=1288825 GN=cat PE=3 SV=1	16.538	7	2	6	2	219	25.6	14.42
A0A658YX10	Acriflavine resistance protein A OS=Shigella flexneri OX=623 GN=acrA PE=3 SV=1	19.783	22	6	6	6	397	42.1	14.26
I6D632	ATP-dependent Clp protease ATP-binding subunit ClpX OS=Shigella flexneri K-315 OX=766150 GN=clpX PE=3 SV=1	16.798	13	5	6	5	424	46.4	12.91
I6D059	Dihydroorotate dehydrogenase (quinone) OS=Shigella flexneri K-315 OX=766150 GN=pyrD PE=3 SV=1	21.474	21	6	6	6	336	36.8	12.73
A0A2S8DEF0	Chaperone SurA OS=Shigella dysenteriae OX=622 GN=surA PE=3 SV=1	15.172	9	4	6	4	428	47.3	11.91
Q0T6G6	UPF0194 membrane protein YbhG OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ybhG PE=3 SV=1	15.091	18	6	6	6	331	36.3	11.9
I6CG83	Phosphoglycerate kinase OS=Shigella flexneri K-315 OX=766150 GN=pgk PE=3 SV=1	21.54	21	6	6	6	382	40.5	11.55
Q0T8G8	Isoleucine-tRNA ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ileS PE=3 SV=1	13.369	6	6	6	6	922	102.5	11.47
D2AAJ1	2,3-bisphosphoglycerate-independent phosphoglycerate mutase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=gpmM PE=3 SV=1	14.793	9	5	6	5	548	60.1	10.87
A0A3T2UZJ5	Protease 4 OS=Shigella flexneri OX=623 GN=DK174_18290 PE=3 SV=1	11.719	7	5	6	5	618	67.2	10.86
A0A3U1D5H5	Alpha-D-glucose phosphate-specific phosphoglucomutase OS=Shigella flexneri OX=623 GN=pgm PE=3 SV=1	9.996	10	5	6	5	546	58.3	10.68
Q0T6W4	Oxoglutarate dehydrogenase (succinyl-transferring) OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=sucA PE=3 SV=1	13.217	5	5	6	5	933	105	10.59
A0A3T2URE0	Catalase-peroxidase OS=Shigella flexneri OX=623 GN=katG PE=3 SV=1	14.504	10	5	6	5	726	80	10.48
D2ACQ6	Sulfatase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yjjP PE=4 SV=1	15.205	11	6	6	6	577	66.5	10.3
A0A6L6V4U1	Oligopeptidase A OS=Shigella flexneri OX=623 GN=prlC PE=3 SV=1	15.033	9	5	6	5	680	77	9.95
A0A658YVC1	Aconitate hydratase B OS=Shigella flexneri OX=623 GN=acnB PE=3 SV=1	14.153	6	5	6	5	874	94.5	9.7
A0A2S8DKX6	Insulinase family protein OS=Shigella boydii OX=621 GN=yhjJ PE=3 SV=1	17.645	13	5	6	5	498	55.4	8.05
A0A383K220	Valine-tRNA ligase OS=Shigella flexneri OX=623 GN=valS PE=3 SV=1	14.329	6	5	6	5	951	108.1	7.93
A0A658YT81	Penicillin-binding protein 2 OS=Shigella flexneri OX=623 GN=mrdA PE=4 SV=1	11.389	7	5	6	5	633	70.8	6.54
A0A3T3S5U3	Formate dehydrogenase-N subunit alpha OS=Shigella flexneri OX=623 GN=fdnG PE=3 SV=1	9.611	4	5	6	5	1015	112.9	4.74
A0A4P7TJ80	Glycine dehydrogenase (decarboxylating) OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=gcvP PE=3 SV=1	19.443	6	5	5	5	957	104.3	14.99
A0A6N3R6K7	Aerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4998 PE=3 SV=1	16.942	8	5	5	5	402	42.7	14.93
A0A6N3RAZ3	Cell division protein FtsN OS=Shigella flexneri 1485-80 OX=766155 GN=ftsN PE=3 SV=1	16.304	19	5	5	5	284	31.8	14.41
A0A2S8DV96	50S ribosomal protein L5 OS=Shigella boydii OX=621 GN=rplE PE=3 SV=1	18.911	33	5	5	5	179	20.3	14.18
A0A2Y4ZTN4	Xaa-Pro dipeptidase OS=Shigella flexneri OX=623 GN=pepQ PE=3 SV=1	14.643	12	5	5	5	443	50.2	13.73
I6D6H9	Transaldolase OS=Shigella flexneri K-315 OX=766150 GN=tal PE=3 SV=1	13.219	17	5	5	5	294	32.8	12.02
A0A2S8DNR9	Methionine-tRNA ligase OS=Shigella boydii OX=621 GN=metG PE=3 SV=1	16.226	8	5	5	5	677	76.2	11.89
A0A6N3R4Q2	50S ribosomal protein L6 OS=Shigella flexneri 1485-80 OX=766155 GN=rplF PE=3 SV=1	9.492	22	3	5	3	177	18.9	11.88
A0A4P7TNY5	Alkyl hydroperoxide reductase C OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=ahpC PE=3 SV=1	14.257	24	4	5	4	187	20.7	11.85
A0A2S8D3U0	Catabolite activator protein OS=Shigella dysenteriae OX=622 GN=crp PE=4 SV=1	12.954	23	5	5	5	210	23.7	11.74

Q7UB84	Glycerol kinase OS=Shigella flexneri OX=623 GN=glpK PE=3 SV=2	15.119	11	5	5	5	502	56.2	11.68
Q0T2G2	3-oxoacyl-[acyl-carrier-protein] synthase I OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fabB PE=3 SV=1	13.689	13	5	5	5	370	39	11.55
A0A383JTR9	Cardiolipin synthase A OS=Shigella flexneri OX=623 GN=cIs PE=3 SV=1	14.833	11	4	5	4	486	54.8	11.45
I6CDA5	ATP-dependent protease ATPase subunit HslU OS=Shigella flexneri K-315 OX=766150 GN=hslU PE=2 SV=1	12.676	10	5	5	5	443	49.6	11.2
D2ADL5	Respiratory nitrate reductase I beta chain OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=narH PE=4 SV=1	13.398	10	5	5	5	512	58	10.21
A0A0H2VYI3	Nucleoside permease OS=Shigella flexneri OX=623 GN=nupC PE=3 SV=1	11.239	11	5	5	5	400	43.5	9.42
A0A2Y4XRM3	CTP synthase OS=Shigella flexneri OX=623 GN=pyrG PE=3 SV=1	10.252	8	5	5	5	545	60.4	8.36
I6CBQ2	DNA gyrase subunit B OS=Shigella flexneri K-315 OX=766150 GN=gyrB PE=3 SV=1	10.473	5	4	5	4	804	89.9	8.21
A0A380B3G3	Asparagine--tRNA ligase OS=Shigella flexneri OX=623 GN=asnC_2 PE=3 SV=1	11.327	9	4	5	4	466	52.6	7.62
A0A6L6UZB9	Ribonuclease E OS=Shigella flexneri OX=623 GN=me PE=3 SV=1	8.962	4	5	5	5	1063	118.4	6.96
I6C9W2	30S ribosomal protein S10 OS=Shigella flexneri K-315 OX=766150 GN=rpsJ PE=3 SV=1	10.886	39	4	5	4	103	11.7	6.43
D2A6V6	6-phosphogluconate dehydrogenase, decarboxylating OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=gnd PE=3 SV=1	11.861	12	5	5	5	468	51.3	6.42
D2ADW1	Putative transport protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yegH PE=3 SV=1	6.288	6	4	5	4	549	62.1	5.43
A0A658Z2J6	Aminopeptidase N OS=Shigella flexneri OX=623 GN=pepN PE=3 SV=1	8.386	5	5	5	5	870	98.9	3.87
A0A3T6J5Y5	Involved in lipopolysaccharide biosynthesis OS=Shigella flexneri OX=623 GN=htrL PE=4 SV=1	6.694	13	4	5	4	285	33.5	3.39
I6C8N1	Modulator of FtsH protease HflC OS=Shigella flexneri K-315 OX=766150 GN=hflC PE=3 SV=1	17.628	18	4	4	4	334	37.6	14.1
F5P2D1	Rhodanese-like domain protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4672 PE=4 SV=1	16.444	33	4	4	4	143	15.6	13.63
A0A2S8DI03	Signal recognition particle-docking protein FtsY (Fragment) OS=Shigella boydii OX=621 GN=C5K23_31365 PE=3 SV=1	18.44	13	4	4	4	486	53.2	13.55
F5P2W0	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4852 PE=4 SV=1	14.962	23	3	4	3	185	19.8	12.37
A0A6N3RAY3	50S ribosomal protein L15 OS=Shigella flexneri 1485-80 OX=766155 GN=rplO PE=3 SV=1	15.704	39	4	4	4	126	13.1	12.35
F5P004	L-asparaginase 2 OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3757 PE=3 SV=1	20.772	24	4	4	4	235	25.2	11.86
F5P3L0	Fumarate reductase iron-sulfur subunit OS=Shigella flexneri K-227 OX=766147 GN=SFK227_5105 PE=3 SV=1	14.662	15	3	4	3	244	27	11.75
A0A4P7TQY3	Bifunctional purine biosynthesis protein PurH OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=purH PE=3 SV=1	14.573	9	4	4	4	529	57.3	11.65
D2AGX5	Cysteine desulfurase IscS OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=iscS PE=3 SV=1	11.555	10	4	4	4	412	46	11.58
I6D602	Protein-export membrane protein SecF OS=Shigella flexneri K-315 OX=766150 GN=secF PE=3 SV=1	21.335	17	3	4	3	304	33.2	11.27
A0A658Y016	Glucan biosynthesis protein G OS=Shigella flexneri OX=623 GN=mDoG PE=3 SV=1	14.898	11	4	4	4	500	56.6	10.81
Q7UCQ9	Lipopolysaccharide assembly protein B OS=Shigella flexneri OX=623 GN=yCiM PE=3 SV=1	10.538	8	3	4	3	389	44.4	10.58
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	12.173	12	4	4	4	376	41	10.19
A0A658Y003	DNA-directed RNA polymerase subunit OS=Shigella flexneri OX=623 GN=rpoC PE=3 SV=1	12.202	3	4	4	4	1407	155.1	10.13
A0A6D2W4F9	Chaperone protein Skp OS=Shigella flexneri OX=623 GN=skp PE=3 SV=1	13.708	17	4	4	4	161	17.7	10.1
D2A917	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	14.236	19	4	4	4	124	13.7	10.05
Q0T8M9	Serine--tRNA ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=serS PE=3 SV=1	9.161	9	4	4	4	430	48.4	9.8
A0A2S8DA55	SH3 domain-containing protein OS=Shigella dysenteriae OX=622 GN=ygiM PE=4 SV=1	11.041	18	4	4	4	206	23.1	9.65
A0A658XY46	Glucose dehydrogenase OS=Shigella flexneri OX=623 GN=gcd PE=3 SV=1	18.196	7	4	4	4	796	86.7	9.5
I6CVL0	Phenylalanine--tRNA ligase beta subunit OS=Shigella flexneri K-315 OX=766150 GN=pheT PE=3 SV=1	13.708	6	4	4	4	795	87.3	9.42
F5NZF4	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3556 PE=3 SV=1	6.815	5	3	4	3	493	53.7	9.24
A0A6N3RBT1	GTP-binding protein TypA/BipA OS=Shigella flexneri 1485-80 OX=766155 GN=typA PE=4 SV=1	13.39	8	4	4	4	607	67.4	8.97
I6C8V5	Fructose-1,6-bisphosphatase class 1 OS=Shigella flexneri K-315 OX=766150 GN=fbp PE=3 SV=1	11.313	12	3	4	3	332	36.8	8.94
F5NPS2	Lipoprotein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0205 PE=3 SV=1	10.951	21	4	4	4	271	29.4	8.9
A0A2Y4XSH5	Periplasmic protein of mal regulon OS=Shigella flexneri OX=623 GN=malM PE=4 SV=1	9.691	12	4	4	4	306	31.9	8.57
A0A4P7TTH9	30S ribosomal protein S2 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsB PE=3 SV=1	16.269	17	4	4	4	241	26.8	8.51
A0A2S8DUL5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase OS=Shigella boydii OX=621 GN=dapD PE=3 SV=1	13.857	15	4	4	4	274	29.9	8.37
A0A3T2UY84	Cyclopropane-fatty-acyl-phospholipid synthase OS=Shigella flexneri OX=623 GN=DK174_15880 PE=3 SV=1	10.772	11	4	4	4	382	43.9	8.36
A0A2S8DCH3	Lipid ABC transporter permease/ATP-binding protein (Fragment) OS=Shigella dysenteriae OX=622 GN=C5K18_12120 PE=4 SV=1	8.869	8	4	4	4	573	63.4	7.74
I6CB04	Alpha-1,4 glucan phosphorylase OS=Shigella flexneri K-315 OX=766150 GN=malP PE=3 SV=1	11.063	5	3	4	3	797	90.5	7.57
A0A2Y5B4B8	Malate dehydrogenase OS=Shigella flexneri OX=623 GN=mdh PE=3 SV=1	9.379	16	4	4	4	312	32.3	7.54
D2ADZ6	Glucose-6-phosphate isomerase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=pgi PE=3 SV=1	14.439	9	4	4	4	549	61.5	7.4
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	7.26	14	3	4	3	206	23.4	7.35
I6CX73	Cell division inhibitor MinD OS=Shigella flexneri K-315 OX=766150 GN=minD PE=3 SV=1	9.18	14	4	4	4	235	26.3	7.35
A0A3T2UYA0	Carbamoyl-phosphate synthase large chain OS=Shigella flexneri OX=623 GN=carB PE=3 SV=1	9.41	4	4	4	4	1073	117.8	7.33

F5P0R0	Maltodextrin-binding protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4033 PE=3 SV=1	8.736	11	4	4	4	396	43.4	7.16
A0A6N3QQC4	Probable malate:quinone oxidoreductase OS=Shigella flexneri CDC 796-83 OX=945360 GN=mqo PE=3 SV=1	8.83	6	3	4	3	548	60.2	6.93
A0A2S8DTV4	Phosphoenolpyruvate synthase OS=Shigella boydii OX=621 GN=ppsA PE=3 SV=1	8.436	5	4	4	4	792	87.4	6.81
F5P455	Carbon starvation protein A OS=Shigella flexneri K-227 OX=766147 GN=SFK227_5301 PE=3 SV=1	6.619	4	3	4	3	716	77.3	6.79
A0A6N3QJK5	AsmA protein OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04099 PE=4 SV=1	10.96	6	4	4	4	617	68.9	6.3
I6D070	Endopeptidase La OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0989 PE=3 SV=1	11.472	9	4	4	4	586	65.9	6.25
A0A2S8DKK2	Ribonucleoside-diphosphate reductase OS=Shigella boydii OX=621 GN=nrda PE=3 SV=1	8.996	5	4	4	4	761	85.7	6.23
A0A6N3QQ54	Histidine kinase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01033 PE=4 SV=1	10.149	6	3	4	3	450	50.3	6.22
A0A4P7TRM2	Lipopolysaccharide N-acetylglucosaminyltransferase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_019395 PE=4 SV=1	7.159	7	3	4	3	380	43.5	6.17
D2AHT6	Co-chaperone protein DjlA OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=djlA PE=3 SV=1	4.725	8	3	4	3	271	30.5	6.14
A0A658Z505	Transporter, small conductance mechanosensitive ion channel (MscS) family OS=Shigella flexneri OX=623 GN=mscM PE=3 SV=1	7.362	9	4	4	4	415	46.6	5.69
A0A4P7TSC3	3-octaprenyl-4-hydroxybenzoate carboxy-lyase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=ubiD PE=3 SV=1	11.98	9	4	4	4	497	55.6	5.27
A0A2S8DAH1	Histidine biosynthesis bifunctional protein HisB OS=Shigella dysenteriae OX=622 GN=hisB PE=3 SV=1	5.705	8	3	4	3	355	40.2	4.64
A0A1S9KGS8	Phosphatidylserine decarboxylase proenzyme OS=Shigella dysenteriae OX=622 GN=psd PE=3 SV=1	9.042	17	4	4	4	322	35.9	4.48
A0A2A2XF62	Cell division ATP-binding protein FtsE OS=Shigella flexneri OX=623 GN=ftsE PE=3 SV=1	6.366	11	3	4	3	222	24.4	4.22
A0A6N3R9B1	PTS system, trehalose-specific IIBC component OS=Shigella flexneri 1485-80 OX=766155 GN=treP PE=4 SV=1	6.802	7	3	4	3	472	51.1	4.18
A0A6N3RKR4	Iron-sulfur cluster binding protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0392 PE=4 SV=1	6.613	8	4	4	4	475	53	4.16
A0A658Z2S5	GMP synthase OS=Shigella flexneri OX=623 GN=guaA PE=4 SV=1	9.621	5	3	4	3	525	58.7	4.07
F5P2J3	C4-dicarboxylate transport protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4734 PE=4 SV=1	7.934	11	4	4	4	395	41.7	2.43
A0A3T2UZZ4	Glucans biosynthesis glucosyltransferase H OS=Shigella flexneri OX=623 GN=mdoH PE=3 SV=1	5.568	4	4	4	4	847	96.9	2
Q0T7Q8	Aminoacyl-histidine dipeptidase (Peptidase D) OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pepD PE=4 SV=1	7.802	10	4	4	4	485	52.9	1.8
A0A6N3RGK2	FAD linked oxidase domain protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_1959 PE=4 SV=1	7.613	3	4	4	4	1018	113.2	1.69
Q0T239	4-hydroxy-tetrahydrodipicolinate synthase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dapA PE=3 SV=1	12.769	15	3	3	3	292	31.3	10.51
D2AGV7	TPR_21 domain-containing protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfgM PE=3 SV=1	19.032	24	3	3	3	206	22.1	9.88
A0A6N3QME5	50S ribosomal protein L1 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rplA PE=3 SV=1	11.712	15	3	3	3	234	24.7	9.75
A0A6H1JG84	Phosphoenolpyruvate carboxylase OS=Shigella flexneri OX=623 GN=ppc PE=3 SV=1	13.344	5	3	3	3	883	99	9.2
I6CHF3	MFS transporter, sugar porter family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3768 PE=3 SV=1	11.551	5	2	3	2	472	51.7	9.08
A0A1Q8MQJ7	Protein RecA OS=Shigella boydii OX=621 GN=recA PE=3 SV=1	10.381	10	3	3	3	353	38	8.99
F5NZV9	Mechanosensitive ion channel family protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3712 PE=3 SV=1	10.625	9	2	3	2	286	30.9	8.87
A0A6N3RAZ0	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4958 PE=3 SV=1	10.738	7	3	3	3	428	47.7	8.81
A0A6L6V006	Ribose-phosphate pyrophosphokinase OS=Shigella flexneri OX=623 GN=prs PE=3 SV=1	9.691	10	3	3	3	315	34.2	8.48
F5P2M7	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4769 PE=4 SV=1	10.637	10	3	3	3	236	25.6	8.45
P69785	PTS system glucose-specific EIIA component OS=Shigella flexneri OX=623 GN=crr PE=3 SV=2	11.538	20	2	3	2	169	18.2	8.18
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	14.073	25	3	3	3	129	13.8	8.08
A0A658YV21	Auxiliary transport protein, membrane fusion protein (MFP) family OS=Shigella flexneri OX=623 GN=macA_2 PE=4 SV=1	7.801	11	3	3	3	355	38.7	7.77
A0A379ZSJ2	Alcohol dehydrogenase OS=Shigella flexneri OX=623 GN=yqhD PE=4 SV=1	9.817	7	3	3	3	387	42.1	7.58
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=Igkc PE=1 SV=1	6.3	9	2	3	2	219	24.2	7.45
I6CH97	AMP nucleosidase OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3709 PE=4 SV=1	10.459	7	3	3	3	444	49.9	7.41
Q0T6B3	Aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=aspC PE=3 SV=1	8.156	8	3	3	3	396	43.6	6.74
A0A2S8DI33	Sec translocon accessory complex subunit YajC OS=Shigella boydii OX=621 GN=yajC PE=3 SV=1	6.549	26	3	3	3	110	11.9	6.6
F5NST7	Paraquat-inducible protein B OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1183 PE=4 SV=1	7.544	6	3	3	3	527	58.3	6.59
F5P3W0	UDP-N-acetylmuramate-L-alanyl-gamma-D-glutamyl-meso-2,6-diaminoheptandioate ligase OS=Shigella flexneri K-227 OX=766147 GN=mpl PE=3 SV=1	5.149	4	2	3	2	457	49.9	6.45
A0A2Y4XYI3	Esterase FrsA OS=Shigella flexneri OX=623 GN=frsA PE=3 SV=1	7.976	7	3	3	3	414	47	6.34
A0A380AZK5	D-amino acid dehydrogenase OS=Shigella flexneri OX=623 GN=dadA PE=3 SV=1	10.149	9	3	3	3	432	47.6	6.12
I6CI73	Elongation factor 4 OS=Shigella flexneri K-315 OX=766150 GN=lepA PE=3 SV=1	5.585	4	3	3	3	599	66.6	6.09
A0A2Y4XMF0	Sec-independent protein translocase protein TatA OS=Shigella flexneri OX=623 GN=tatA PE=3 SV=1	10.334	37	3	3	3	89	9.7	5.99
A0A2S8DJM8	Na+/H+ antiporter OS=Shigella boydii OX=621 GN=C5K23_27965 PE=3 SV=1	8.672	8	3	3	3	517	56.9	5.7
A0A383JXY5	NAD dependent epimerase/dehydratase family OS=Shigella flexneri OX=623 GN=DOD76_09530 PE=4 SV=1	8.818	9	3	3	3	337	38	5.4
Q83MF6	Cell division protein FtsZ OS=Shigella flexneri OX=623 GN=ftsZ PE=3 SV=2	7.494	9	3	3	3	383	40.1	5.32

A0A4P7TP07	Tol-Pal system protein TolB OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tolB PE=3 SV=1	8.726	8	3	3	3	430	45.9	5.16
A0A2S8DR37	L-arabinose-binding periplasmic protein OS=Shigella boydii OX=621 GN=araF PE=3 SV=1	5.291	9	3	3	3	329	35.6	5.03
A0A2Y5I422	Diguanylate cyclase OS=Shigella flexneri OX=623 GN=yfgF_2 PE=4 SV=1	7.768	7	3	3	3	505	57.7	4.97
Q0T5P3	Sensor histidine protein kinase/phosphatase PhoQ OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=phoQ PE=4 SV=1	3.931	4	2	3	2	486	55.3	4.96
Q83IY3	Bifunctional protein GlmU OS=Shigella flexneri OX=623 GN=glmU PE=3 SV=1	8.151	7	3	3	3	456	49.2	4.85
A0A6H1JH06	30S ribosomal protein S5 OS=Shigella flexneri OX=623 GN=rpsE PE=3 SV=1	4.239	19	2	3	2	167	17.6	4.83
A0A2Y4YUV3	Peptidyl-prolyl cis-trans isomerase OS=Shigella flexneri OX=623 GN=fkIB PE=3 SV=1	8.782	18	3	3	3	206	22.2	4.82
A0A2S8DCL9	Adenylosuccinate lyase OS=Shigella dysenteriae OX=622 GN=purB PE=3 SV=1	5.094	4	2	3	2	456	51.6	4.64
Q0T2K3	NADH-quinone oxidoreductase subunit F OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nuoF PE=3 SV=1	6.248	5	2	3	2	445	49.4	4.61
Q0SXI3	Magnesium-transporting ATPase, P-type I OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=mgta PE=3 SV=1	8.82	4	3	3	3	898	99.4	4.49
A0A2S8DWW7	Lysine--tRNA ligase OS=Shigella boydii OX=621 GN=lysS PE=3 SV=1	7.778	6	3	3	3	505	57.6	4.38
A0A6N3RN87	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ OS=Shigella flexneri 1485-80 OX=766155 GN=fabZ PE=3 SV=1	5.386	17	3	3	3	151	17	4.33
A0A383JTM9	Periplasmic glutamine-binding protein permease OS=Shigella flexneri OX=623 GN=fliY PE=3 SV=1	6.058	10	3	3	3	285	31	4.3
D2A8B1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=murA PE=3 SV=1	5.004	5	2	3	2	419	44.8	3.98
A0A6H1JLX0	AsmA2 domain-containing protein OS=Shigella flexneri OX=623 GN=FOC24_08420 PE=4 SV=1	5.368	3	2	3	2	1266	139	3.95
A0A6N3QSE0	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00807 PE=3 SV=1	5.135	6	2	3	2	401	41.7	3.93
A0A2S8DRB8	Glucose-6-phosphate 1-dehydrogenase OS=Shigella boydii OX=621 GN=zwf PE=3 SV=1	5.357	6	3	3	3	491	55.7	3.92
F5P316	Fe/S biogenesis protein NfuA OS=Shigella flexneri K-227 OX=766147 GN=nfuA PE=3 SV=1	4.82	17	3	3	3	191	21	3.91
I6D6C2	Peptidyl-prolyl cis-trans isomerase OS=Shigella flexneri K-315 OX=766150 GN=ppiB PE=3 SV=1	7.599	11	2	3	2	164	18.2	3.82
A0A6N3R842	Adenylosuccinate synthetase OS=Shigella flexneri 1485-80 OX=766155 GN=purA PE=3 SV=1	5.708	6	3	3	3	432	47.3	3.54
A0A3T2V110	Aspartate--tRNA ligase OS=Shigella flexneri OX=623 GN=aspS PE=3 SV=1	6.315	4	3	3	3	590	65.8	3.54
I6CI74	Signal peptidase I OS=Shigella flexneri K-315 OX=766150 GN=lepB PE=3 SV=1	8.387	10	3	3	3	324	36	3.44
I6CBM2	Glutamine--fructose-6-phosphate aminotransferase [isomerizing] OS=Shigella flexneri K-315 OX=766150 GN=glmS PE=3 SV=1	5.152	4	3	3	3	609	66.9	3.35
A0A658Z233	Inner membrane protein yjgQ OS=Shigella flexneri OX=623 GN=yjgQ PE=3 SV=1	7.368	7	3	3	3	360	39.6	3.17
A0A2S8DD45	Isocitrate dehydrogenase [NADP] OS=Shigella dysenteriae OX=622 GN=icd PE=3 SV=1	5.394	7	3	3	3	416	45.8	2.96
F5NPR9	Proline--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=proS PE=3 SV=1	8.143	3	3	3	3	572	63.6	2.87
A0A2S8D364	Phosphate acetyltransferase (Fragment) OS=Shigella dysenteriae OX=622 GN=C5K18_11165 PE=4 SV=1	5.864	40	3	3	1	117	12.8	2.55
A0A658Z1V1	UPF0283 membrane protein YcjF OS=Shigella flexneri OX=623 GN=ycjF PE=3 SV=1	7.273	9	3	3	3	353	39.4	2.52
A0A2S8D5N6	Aspartate carbamoyltransferase OS=Shigella dysenteriae OX=622 GN=pyrB PE=3 SV=1	6.311	11	3	3	3	311	34.4	2.51
A0A3T3S2E7	Pyruvate kinase OS=Shigella flexneri OX=623 GN=pyk PE=3 SV=1	6.302	6	3	3	3	480	51.3	2.31
A0A3T2UTU6	Sensor protein OS=Shigella flexneri OX=623 GN=DK174_07305 PE=4 SV=1	3.548	3	2	3	2	598	67.1	2.21
A0A2S8E9Y4	Acid-resistance membrane protein OS=Shigella dysenteriae OX=622 GN=hdeD_2 PE=4 SV=1	4.529	7	3	3	3	190	20.9	1.78
A0A6N3R6Z9	Lipopolysaccharide export system permease protein LptF OS=Shigella flexneri 1485-80 OX=766155 GN=yjgP PE=3 SV=1	6.849	7	2	2	2	335	36.6	6.71
I6CMG4	Inner membrane protein YejM OS=Shigella flexneri K-315 OX=766150 GN=yejM PE=4 SV=1	8.084	4	2	2	2	586	67.2	6.32
A0A3T3S7B2	ParB/RepB/Spo0J family partition protein OS=Shigella flexneri OX=623 GN=sopB_2 PE=3 SV=1	7.314	7	2	2	2	326	36.8	6.18
A0A2S8DD51	Cell division protein ZipA OS=Shigella dysenteriae OX=622 GN=zipA PE=3 SV=1	5.753	3	1	2	1	328	36.5	6.03
Q83PC3	50S ribosomal protein L11 OS=Shigella flexneri OX=623 GN=rplK PE=3 SV=3	6.741	17	2	2	2	142	14.9	5.96
D2A8I7	Cytochrome d ubiquinol oxidase subunit III OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yhcB PE=4 SV=1	5.786	16	2	2	2	134	15.2	5.85
A0A379Z3I1	(Fe-S)-binding protein OS=Shigella flexneri OX=623 GN=ykgE PE=4 SV=1	7.701	10	2	2	2	239	26.1	5.63
Q0T4A8	Possible enzyme OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SfV_1686 PE=4 SV=1	6.634	6	2	2	2	418	42.9	5.42
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	5.839	24	2	2	2	78	8.3	5.28
A0A6N3RKA3	Succinate dehydrogenase iron-sulfur subunit OS=Shigella flexneri 1485-80 OX=766155 GN=sdhB PE=3 SV=1	8.218	10	2	2	2	238	26.8	5.28
F5NZ29	Serine transporter OS=Shigella flexneri K-227 OX=766147 GN=SfK227_3429 PE=4 SV=1	6.441	5	2	2	2	429	46.9	5.27
F5NXM7	NADH-quinone oxidoreductase subunit L OS=Shigella flexneri K-227 OX=766147 GN=SfK227_2904 PE=3 SV=1	4.081	2	1	2	1	611	66.1	5.16
I6CFU5	Peptidase Do OS=Shigella flexneri K-315 OX=766150 GN=degS PE=3 SV=1	7.401	10	2	2	2	355	37.6	5.09
I6CG61	Aminomethyltransferase OS=Shigella flexneri K-315 OX=766150 GN=gcvT PE=3 SV=1	7.261	7	2	2	2	347	38.2	5.08
D2AE64	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SfXv_4630 PE=3 SV=1	5.022	13	2	2	2	141	15.2	5.02
A0A658XZH7	Oxidoreductase OS=Shigella flexneri OX=623 GN=NCTC8524_01188 PE=4 SV=1	4.813	3	2	2	2	804	89.3	4.84
Q0T5C6	Putative protease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=sohB PE=3 SV=1	6.666	8	2	2	2	296	33.4	4.82
A0A2S4MTA8	Lactate utilization protein C OS=Shigella flexneri OX=623 GN=ykgG PE=4 SV=1	4.146	8	2	2	2	231	25.2	4.77



I6CBy6	Lipopolysaccharide heptosyltransferase II OS=Shigella flexneri K-315 OX=766150 GN=rfaF PE=4 SV=1	4.139	5	2	2	2	348	39	4.75
QOSZ45	Thiol:disulfide interchange protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dsbA PE=3 SV=1	3.914	8	2	2	2	208	23.1	4.7
A0A6N3RQG4	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydD OS=Shigella flexneri 1485-80 OX=766155 GN=cydD PE=4 SV=1	3.941	3	2	2	2	588	65	4.61
A0A6G5Z373	Sensor protein QseC OS=Shigella flexneri OX=623 GN=qseC PE=4 SV=1	5.58	4	2	2	2	449	50.3	4.51
A0A6N3RIF3	Enoyl-[acyl-carrier-protein] reductase [NADH] OS=Shigella flexneri 1485-80 OX=766155 GN=fabI PE=3 SV=1	5.742	8	2	2	2	262	27.8	4.49
QOSZA6	ATP-dependent 6-phosphofructokinase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pfkA PE=3 SV=1	4.051	5	2	2	2	340	37.1	4.44
A0A2Y4X2P5	Proline/glycine betaine transporter OS=Shigella flexneri OX=623 GN=proP_1 PE=4 SV=1	4.473	4	2	2	2	366	40.2	4.36
A0A2S8DXT0	High frequency lysogenization protein HflD homolog OS=Shigella boydii OX=621 GN=hflD PE=3 SV=1	3.067	9	2	2	2	213	22.9	4.33
I6CCX4	Sec-independent protein translocase protein TatB OS=Shigella flexneri K-315 OX=766150 GN=tatB PE=3 SV=1	4.591	13	2	2	2	171	18.5	4.29
I6CJU1	Acetate kinase OS=Shigella flexneri K-315 OX=766150 GN=ackA PE=3 SV=1	9.792	8	2	2	2	400	43.3	4.23
A0A380B6A5	BAX inhibitor (BI)-1/YccA family protein OS=Shigella flexneri OX=623 GN=ybhL_2 PE=3 SV=1	2.83	6	2	2	2	234	25.9	4.22
I6CXD2	UTP--glucose-1-phosphate uridylyltransferase OS=Shigella flexneri K-315 OX=766150 GN=galU PE=3 SV=1	6.863	8	2	2	2	302	33	4.13
A0A4P7TKN2	O13/O129/O135 family O-antigen flippase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=wzx PE=4 SV=1	6.324	5	2	2	2	418	46.3	3.78
A0A2S8DD79	Threonine/serine transporter TdcC OS=Shigella dysenteriae OX=622 GN=tdcC PE=3 SV=1	4.046	7	2	2	2	443	48.9	3.64
QOSZA9	Triosephosphate isomerase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tpiA PE=3 SV=1	6.856	16	2	2	2	255	27	3.26
A0A6N3QLC2	Universal stress protein G OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02439 PE=3 SV=1	3.821	10	1	2	1	142	15.9	3.26
A0A3Y2RM52	Phosphoglucosamine mutase OS=Shigella flexneri OX=623 GN=glmM PE=3 SV=1	5.121	5	2	2	2	445	47.5	3.25
A0A2S8D3C6	S-ribosylhomocysteine lyase OS=Shigella dysenteriae OX=622 GN=luxS PE=3 SV=1	6.487	14	2	2	2	171	19.4	3.05
Q0T5R2	Spermidine/putrescine import ATP-binding protein PotA OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=potA PE=3 SV=1	3.997	4	2	2	2	378	43.1	2.9
A0A3U1DEI9	Electron transport complex subunit RxC (Fragment) OS=Shigella flexneri OX=623 GN=DOD76_18490 PE=3 SV=1	5.204	4	2	2	2	565	61.3	2.87
A0A2Y4XZJ9	Ferrocyclase OS=Shigella flexneri OX=623 GN=hemH PE=3 SV=1	4.757	9	2	2	2	320	35.9	2.74
A0A3T2UU59	Metalloprotease PmbA OS=Shigella flexneri OX=623 GN=DK174_07880 PE=3 SV=1	5.915	5	2	2	2	450	48.4	2.74
F5NRS1	Oxygen-insensitive NAD(P)H nitroreductase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0809 PE=3 SV=1	5.449	10	2	2	2	217	23.8	2.69
A0A4V1CTY5	ATP-dependent Clp protease ATP-binding subunit ClpA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=clpA PE=3 SV=1	4.881	3	2	2	2	758	84.2	2.66
I6C9X6	30S ribosomal protein S14 OS=Shigella flexneri K-315 OX=766150 GN=rpsN PE=3 SV=1	4.313	19	2	2	2	96	11	2.61
A0A2S8DFP5	Cell division protein DedD OS=Shigella dysenteriae OX=622 GN=dedD PE=3 SV=1	5.716	10	2	2	2	220	22.9	2.61
A0A2S8DPI0	RNA polymerase sigma factor OS=Shigella boydii OX=621 GN=rpoE PE=3 SV=1	3.661	9	2	2	2	191	21.7	2.61
A0A3T2URR1	TDP-N-acetylglucosamine:lipid II N-acetylglucosaminyltransferase OS=Shigella flexneri OX=623 GN=wecF PE=3 SV=1	6.541	6	2	2	2	359	40.5	2.6
I6D3P2	Tol-Pal system protein TolR OS=Shigella flexneri K-315 OX=766150 GN=tolR PE=3 SV=1	7.132	17	2	2	2	142	15.4	2.49
Q83Q93	Biosynthetic arginine decarboxylase OS=Shigella flexneri OX=623 GN=speA PE=3 SV=2	3.387	2	2	2	2	662	74.3	2.42
A0A380AZ64	Phosphoenolpyruvate--glycerone phosphotransferase OS=Shigella flexneri OX=623 GN=ycgC PE=3 SV=1	5.189	5	2	2	2	472	51.4	2.41
Q0T2Q0	DNA gyrase subunit A OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=gyrA PE=3 SV=1	2.573	1	1	2	1	875	96.9	2.38
A0A6N3QPX8	Prophage P4 integrase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03306 PE=4 SV=1	1.785	2	1	2	1	422	48.2	2.28
I6CI71	Sigma-E factor regulatory protein rseB OS=Shigella flexneri K-315 OX=766150 GN=rseB PE=3 SV=1	3.556	4	1	2	1	318	35.7	2.26
A0A2S8DE90	Long-chain-fatty-acid--CoA ligase FadD OS=Shigella dysenteriae OX=622 GN=C5K18_08425 PE=4 SV=1	4.916	3	2	2	2	561	62.2	2.23
A0A4P7TQT3	50S ribosomal protein L10 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpLJ PE=3 SV=1	4.637	12	2	2	2	165	17.7	2.2
Q0T0X5	Fructose-bisphosphate aldolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fba PE=3 SV=1	4.718	2	1	2	1	387	42.7	2.19
A0A6G5Z350	Serine-type D-Ala-D-Ala carboxypeptidase OS=Shigella flexneri OX=623 GN=dacB PE=3 SV=1	3.653	4	2	2	2	477	51.8	2.14
A0A6A9TXP0	Lipid A biosynthesis (KDO)2-(Lauroyl)-lipid IVA acyltransferase (Fragment) OS=Shigella flexneri OX=623 GN=GLN84_25205 PE=4 SV=1	3.241	9	2	2	2	194	22.6	2.12
A0A3T2UQ06	Penicillin-binding protein activator LpoA OS=Shigella flexneri OX=623 GN=lpoA PE=3 SV=1	4.507	3	2	2	2	675	72.6	2.11
A0A6N3RRT0	Probable peptidoglycan glycosyltransferase FtsW OS=Shigella flexneri 1485-80 OX=766155 GN=ftsW PE=3 SV=1	2	3	1	2	1	372	41.2	2.05
A0A658YZ45	Glutamine ABC transporter ATP-binding protein OS=Shigella flexneri OX=623 GN=glnQ PE=3 SV=1	3.986	7	2	2	2	240	26.6	2.02
F5P2C5	L-threonine 3-dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=tdh PE=3 SV=1	3.94	8	2	2	2	341	37.3	2
A0A6G5Z3B5	Biotin carboxylase OS=Shigella flexneri OX=623 GN=accC PE=4 SV=1	3.98	4	2	2	2	449	49.3	1.92
Q83Q07	30S ribosomal protein S9 OS=Shigella flexneri OX=623 GN=rpsI PE=3 SV=3	2.692	15	2	2	2	130	14.9	1.84
D2AJ35	Delta-aminolevulinic acid dehydratase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=hemB PE=3 SV=1	2.708	3	1	2	1	335	36.9	1.81
A0A380A2S8	Histidine kinase OS=Shigella flexneri OX=623 GN=yfhK PE=4 SV=1	3.837	5	2	2	2	460	51.4	1.8

A0A6N3QJ05	Macrolide-specific efflux protein MacA OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04622 PE=3 SV=1	1.274	3	1	2	1	371	40.6	1.77
F5NQ21	Cell division protein FtsQ OS=Shigella flexneri K-227 OX=766147 GN=ftsQ PE=3 SV=1	2.911	9	2	2	2	276	31.4	1.74
A0A379ZX42	Glutamate-cysteine ligase OS=Shigella flexneri OX=623 GN=gshA PE=3 SV=1	3.791	3	2	2	2	518	58.3	1.73
I6D6Z7	Zinc metalloprotease OS=Shigella flexneri K-315 OX=766150 GN=rseP PE=3 SV=1	2.528	4	2	2	2	450	49.1	1.71
A0A2S8DBS7	Glycosyltransferase family I protein OS=Shigella dysenteriae OX=622 GN=mshA PE=4 SV=1	2.429	5	2	2	2	362	40.6	1.64
A0A6G5Z3B1	Lipopolysaccharide export system ATP-binding protein LptB OS=Shigella flexneri OX=623 GN=lptB PE=3 SV=1	2.692	6	2	2	2	241	26.8	1.63
I6D6W3	Poly(A) polymerase I OS=Shigella flexneri K-315 OX=766150 GN=pcnB PE=3 SV=1	2.903	4	2	2	2	454	52.5	1.63
I6C193	Serine hydroxymethyltransferase OS=Shigella flexneri K-315 OX=766150 GN=glyA PE=3 SV=1	3.444	4	2	2	2	417	45.3	1.62
I6C9W8	50S ribosomal protein L22 OS=Shigella flexneri K-315 OX=766150 GN=rplV PE=3 SV=1	2.767	19	2	2	2	110	12.2	0
F5P3C9	30S ribosomal protein S13 OS=Shigella flexneri K-227 OX=766147 GN=rpsM PE=3 SV=1	2.497	16	2	2	2	118	13.1	0
I6C9X3	50S ribosomal protein L14 OS=Shigella flexneri K-315 OX=766150 GN=rplN PE=3 SV=1	2.477	14	2	2	2	117	12.8	0
I6CXR3	Universal stress protein E OS=Shigella flexneri K-315 OX=766150 GN=uspE PE=4 SV=1	5.247	9	2	2	2	314	35.5	0
A0A6N3RN78	Uridylate kinase OS=Shigella flexneri 1485-80 OX=766155 GN=pyrH PE=3 SV=1	3.521	7	2	2	2	241	26	0
I6CVK9	Phenylalanine-tRNA ligase alpha subunit OS=Shigella flexneri K-315 OX=766150 GN=pheS PE=3 SV=1	2.517	5	2	2	2	327	36.8	0
A0A6N3RLZ1	Cytochrome d ubiquinol oxidase, subunit II OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0759 PE=3 SV=1	2.581	4	2	2	2	379	42.4	0
A0A6N3QP90	Glucans biosynthesis protein D OS=Shigella flexneri CDC 796-83 OX=945360 GN=mdoD PE=3 SV=1	2.661	3	2	2	2	539	61.3	0
A0A6G5YYG3	Lipoprotein-releasing system ATP-binding protein LolD OS=Shigella flexneri OX=623 GN=lolD PE=3 SV=1	1.159	3	1	2	1	233	25.5	0
A0A6N3RJH6	Sulfatase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_1839 PE=3 SV=1	2.56	3	2	2	2	539	60.7	0
Q83J75	Putative ATP-binding component of a transport system OS=Shigella flexneri OX=623 GN=yhiH PE=4 SV=1	2.901	2	2	2	2	911	100.6	0
A0A383JXU2	Alkaline phosphatase isozyme conversion aminopeptidase OS=Shigella flexneri OX=623 GN=iap PE=4 SV=1	1.516	2	1	2	1	345	37.9	0
F5NVB6	Glutamate/gamma-aminobutyrate antiporter OS=Shigella flexneri K-227 OX=766147 GN=gadC PE=3 SV=1	1.706	2	1	2	1	511	55.1	0
Q5IP18	Heat shock protein (Fragrant) OS=Shigella flexneri OX=623 GN=grpE PE=3 SV=1	6.484	9	1	1	1	158	17.6	4.11
A0A6L6UVT0	Lipid A biosynthesis lauroyltransferase OS=Shigella flexneri OX=623 GN=lpXL PE=3 SV=1	5.175	4	1	1	1	306	35.4	4.03
D2A780	Galactose-proton symporter OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=galP PE=3 SV=1	2.769	2	1	1	1	464	51	3.7
QOSY71	Glutamate decarboxylase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=gadA PE=3 SV=1	4.644	4	1	1	1	466	52.7	3.6
A0A2S8DCN0	Lipoprotein-releasing ABC transporter permease subunit LolE OS=Shigella dysenteriae OX=622 GN=C5K18_10460 PE=3 SV=1	3.285	2	1	1	1	414	45.3	3.37
F5NV08	Tyrosine-tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=tyrS PE=3 SV=1	4.124	3	1	1	1	424	47.6	3.24
A0A1Q8M8J0	50S ribosomal protein L7/L12 OS=Shigella boydii OX=621 GN=rplL PE=3 SV=1	3.284	10	1	1	1	121	12.3	3.18
A0A6N3R6J7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4987 PE=3 SV=1	3.368	2	1	1	1	446	47.8	3.07
A0A383JXW9	UPF0145 protein YbjQ OS=Shigella flexneri OX=623 GN=ybjQ PE=3 SV=1	4.331	11	1	1	1	107	11.4	3
A0A0H2VZX0	Thiol:disulfide interchange protein DsbD OS=Shigella flexneri OX=623 GN=dsbD PE=3 SV=1	5.525	2	1	1	1	565	61.8	2.99
A0A6N3RRG2	Outer membrane lipoprotein RcsF OS=Shigella flexneri 1485-80 OX=766155 GN=rscF PE=3 SV=1	5.008	12	1	1	1	117	12.4	2.97
A0A2Y4XP64	Oligopeptide ABC transporter periplasmic oligopeptide-binding protein OS=Shigella flexneri OX=623 GN=oppA PE=4 SV=1	3.449	3	1	1	1	442	50.4	2.97
A0A237F1C5	Arginine ABC transporter ATP-binding protein ArtP OS=Shigella boydii OX=621 GN=artP PE=3 SV=1	2.909	4	1	1	1	242	27	2.94
A0A4P7TQF0	50S ribosomal protein L9 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rplI PE=3 SV=1	3.078	7	1	1	1	149	15.8	2.91
A0A6N3QGJ9	UPF0301 protein YggE OS=Shigella flexneri CDC 796-83 OX=945360 GN=yggE PE=3 SV=1	3.84	6	1	1	1	187	20.7	2.91
A0A1Q8NKE7	Phosphoheptose isomerase OS=Shigella dysenteriae OX=622 GN=gmhA PE=3 SV=1	3.17	5	1	1	1	192	20.8	2.86
A0A3T2UX48	Outer membrane protein F OS=Shigella flexneri OX=623 GN=ompF PE=3 SV=1	3.249	3	1	1	1	362	39.3	2.84
QOSZX1	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yheO PE=4 SV=1	4.198	5	1	1	1	240	26.8	2.8
I6CBX0	50S ribosomal protein L28 OS=Shigella flexneri K-315 OX=766150 GN=rpmB PE=3 SV=1	2.466	15	1	1	1	66	7.7	2.79
I6CE82	Fumarate hydratase class I OS=Shigella flexneri K-315 OX=766150 GN=fumB PE=3 SV=1	3.071	2	1	1	1	548	60.1	2.76
I6CGI0	Bifunctional glutathionylspermidine synthetase/amidase OS=Shigella flexneri K-315 OX=766150 GN=gsp PE=3 SV=1	3.736	2	1	1	1	619	70.5	2.75
A0A3T2UV43	Fumarate reductase subunit D OS=Shigella flexneri OX=623 GN=frdD PE=3 SV=1	3.341	9	1	1	1	119	13.1	2.73
A0A6N3QIM1	Polysaccharide deacetylase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04901 PE=4 SV=1	4.393	4	1	1	1	280	32.9	2.69
I6CFV1	Putative stringent starvation protein A OS=Shigella flexneri K-315 OX=766150 GN=sspA PE=3 SV=1	4.012	7	1	1	1	212	24.3	2.66
I6CMC4	Lysine-specific permease OS=Shigella flexneri K-315 OX=766150 GN=lysP PE=4 SV=1	3.227	2	1	1	1	489	53.5	2.66
A0A2S8DU80	Agmatinase OS=Shigella boydii OX=621 GN=speB PE=3 SV=1	2.687	3	1	1	1	306	33.5	2.65
P0A8A1	Probable transcriptional regulatory protein YebC OS=Shigella flexneri OX=623 GN=yebC PE=3 SV=1	4.501	4	1	1	1	246	26.4	2.62
A0A3T2UY26	Beta-glucosidase BglX OS=Shigella flexneri OX=623 GN=DK174_15745 PE=3 SV=1	4.021	2	1	1	1	765	83.5	2.62
A0A6N3RR48	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0546 PE=3 SV=1	2.712	4	1	1	1	259	28.2	2.6
F5P090	50S ribosomal protein L21 OS=Shigella flexneri K-227 OX=766147 GN=rplU PE=3 SV=1	3.204	12	1	1	1	103	11.5	2.58

A0A2S8DKM1	Putrescine-binding periplasmic protein OS=Shigella boydii OX=621 GN=potD PE=3 SV=1	3.891	3	1	1	1	1	348	38.9	2.58
I6CAS1	Phosphate transporter OS=Shigella flexneri K-315 OX=766150 GN=pitA PE=3 SV=1	3.009	2	1	1	1	1	499	53.4	2.58
A0A6N3QN40	Glutamate--tRNA ligase OS=Shigella flexneri CDC 796-83 OX=945360 GN=glx PE=3 SV=1	2.99	2	1	1	1	1	471	53.8	2.52
A0A6N3RI00	Alpha-D-glucosyl-1-phosphate uridylyltransferase OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_2337 PE=3 SV=1	3.03	6	1	1	1	1	198	22	2.51
A0A1Q8M4D3	30S ribosomal protein S18 OS=Shigella boydii OX=621 GN=rpsR PE=3 SV=1	5.614	24	1	1	1	1	75	9	2.49
A0A4V1CUG6	Transcription termination/antitermination protein NusA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=nusA PE=3 SV=1	2.546	2	1	1	1	1	495	54.8	2.46
A0A658YSH2	Dehydrogenase OS=Shigella flexneri OX=623 GN=yrfF PE=3 SV=1	2.642	1	1	1	1	1	711	79.5	2.46
F5NQG6	Glutamine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=glnS PE=3 SV=1	3.412	2	1	1	1	1	554	63.4	2.45
D2AG45	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfcZ PE=3 SV=1	3.654	13	1	1	1	1	102	11.3	2.44
F5P2B3	Lipopolysaccharide core biosynthesis protein rfaG OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4654 PE=4 SV=1	3	3	1	1	1	1	374	42.5	2.43
Q0T0X8	Putative actin OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yggE PE=4 SV=1	1.191	3	1	1	1	1	235	25.4	2.43
Q83J96	Phosphoenolpyruvate carboxykinase (ATP) OS=Shigella flexneri OX=623 GN=pckA PE=3 SV=4	2.682	2	1	1	1	1	540	59.6	2.41
D2AD60	Protein translocase subunit SecE OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=secE PE=3 SV=1	2.442	7	1	1	1	1	127	13.6	2.39
A0A658YTG8	Putative F0F1-type ATP synthase, subunit b OS=Shigella flexneri OX=623 GN=SAMEA3710514_00636 PE=4 SV=1	1.799	6	1	1	1	1	160	18.9	2.39
A0A2Y5BFL6	Uncharacterized protein OS=Shigella flexneri OX=623 GN=SAMEA3710404_02763 PE=4 SV=1	1.65	18	1	1	1	1	141	15.8	2.37
P59107	Exoribonuclease 2 OS=Shigella flexneri OX=623 GN=rmb PE=3 SV=1	2.647	2	1	1	1	1	644	72.4	2.35
Q0T2S7	Cytochrome c-type biogenesis protein CcmE OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ccmE PE=3 SV=1	3.699	8	1	1	1	1	159	17.7	2.34
Q0SY57	ATP-dependent protease subunit HslV OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hslV PE=3 SV=1	2.966	6	1	1	1	1	176	19.1	2.34
A0A6N3QNZ8	Inorganic pyrophosphatase OS=Shigella flexneri CDC 796-83 OX=945360 GN=ppa PE=3 SV=1	2.062	5	1	1	1	1	176	19.7	2.34
A0A1Q8M6D1	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase OS=Shigella boydii OX=621 GN=gpmA PE=3 SV=1	4.851	5	1	1	1	1	250	28.5	2.34
A0A1W2MD82	Protease HtpX OS=Shigella flexneri OX=623 GN=htpX PE=3 SV=1	2.705	4	1	1	1	1	293	31.9	2.34
A0A0H2VZP5	RNA polymerase sigma factor RpoD OS=Shigella flexneri OX=623 GN=rpoD PE=3 SV=1	2.676	2	1	1	1	1	608	69.6	2.31
A0A2S8DUK8	Methionine aminopeptidase OS=Shigella boydii OX=621 GN=map PE=3 SV=1	2.132	4	1	1	1	1	264	29.3	2.28
A0A6N3RC82	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3360 PE=4 SV=1	2.377	8	1	1	1	1	117	13.8	2.27
A0A2S8DN14	RNA-binding transcriptional accessory protein (Fragment) OS=Shigella boydii OX=621 GN=C5K23_19395 PE=4 SV=1	3.039	4	1	1	1	1	304	34.4	2.25
I6CFX7	Lipopolysaccharide export system protein LptC OS=Shigella flexneri K-315 OX=766150 GN=lptC PE=3 SV=1	1.969	4	1	1	1	1	191	21.7	2.25
A0A379ZPY0	Aerobic respiration control sensor protein OS=Shigella flexneri OX=623 GN=arcB PE=4 SV=1	2.941	1	1	1	1	1	778	87.9	2.24
A0A383JQI3	3-oxoacyl-[acyl-carrier-protein] reductase OS=Shigella flexneri OX=623 GN=fabG_1 PE=3 SV=1	1.725	5	1	1	1	1	244	25.5	2.22
I6CFZ0	50S ribosomal protein L27 OS=Shigella flexneri K-315 OX=766150 GN=rpmA PE=3 SV=1	2.721	16	1	1	1	1	85	9.1	2.21
F5P300	Glucose-1-phosphate adenyltransferase OS=Shigella flexneri K-227 OX=766147 GN=glgC PE=3 SV=1	1.885	2	1	1	1	1	431	48.7	2.21
P0A7Z3	Ribose-5-phosphate isomerase A OS=Shigella flexneri OX=623 GN=rpiA PE=3 SV=1	2.517	5	1	1	1	1	219	22.8	2.2
I6D405	PhoH-like protein OS=Shigella flexneri K-315 OX=766150 GN=ybeZ PE=3 SV=1	2.97	3	1	1	1	1	346	39	2.2
A0A2S8DRX8	Sulfate ABC transporter substrate-binding protein OS=Shigella boydii OX=621 GN=cysP PE=3 SV=1	1.886	3	1	1	1	1	338	37.7	2.19
F5P3E9	3'(2'),5'-bisphosphate nucleotidase CysQ OS=Shigella flexneri K-227 OX=766147 GN=cysQ PE=3 SV=1	2.508	4	1	1	1	1	230	25.4	2.18
A0A3U1D8Q6	C4-dicarboxylic acid transporter DauA OS=Shigella flexneri OX=623 GN=dauA PE=3 SV=1	2.057	2	1	1	1	1	559	59.4	2.17
A0A6N3RAZ2	Trk system potassium uptake protein TrkA OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4799 PE=4 SV=1	2.107	3	1	1	1	1	458	50.3	2.15
A0A4P7TM74	30S ribosomal protein S21 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsU PE=3 SV=1	2.03	11	1	1	1	1	71	8.5	2.13
F5NQR5	Tol-Pal system protein TolQ OS=Shigella flexneri K-227 OX=766147 GN=tolQ PE=3 SV=1	2.381	7	1	1	1	1	210	23.3	2.12
A0A2Y4YFW7	Adenylate kinase OS=Shigella flexneri OX=623 GN=adk PE=3 SV=1	2.108	5	1	1	1	1	234	25.8	2.11
A0A658YXY5	Aminopeptidase B OS=Shigella flexneri OX=623 GN=pepB PE=3 SV=1	2.488	3	1	1	1	1	427	46.2	2.11
F5NVZ8	Outer membrane porin protein LC OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2311 PE=3 SV=1	3.807	3	1	1	1	1	360	39.7	2.09
A0A2Y5A0I3	Repressor protein for FtsI OS=Shigella flexneri OX=623 GN=sufI PE=4 SV=1	3.704	4	1	1	1	1	370	40.8	2.05
A0A2S8DH97	Pyridoxine/pyridoxamine 5'-phosphate oxidase OS=Shigella dysenteriae OX=622 GN=pdxH PE=3 SV=1	2.016	4	1	1	1	1	218	25.6	2.04
I6CI31	GTPase Der OS=Shigella flexneri K-315 OX=766150 GN=der PE=3 SV=1	1.914	2	1	1	1	1	490	55	2.03
I6CBX6	Lipopolysaccharide heptosyltransferase III, putative OS=Shigella flexneri K-315 OX=766150 GN=SFK315_4420 PE=4 SV=1	2.342	4	1	1	1	1	254	28	2.02
A0A379ZAE3	Dihydroxy-acid dehydratase OS=Shigella flexneri OX=623 GN=ilvD PE=3 SV=1	0.985	2	1	1	1	1	616	65.5	2
A0A2S8DA81	Succinate--CoA ligase [ADP-forming] subunit beta OS=Shigella dysenteriae OX=622 GN=sucC PE=3 SV=1	1.597	2	1	1	1	1	388	41.4	2
A0A2S8DMY7	1-deoxy-D-xylulose-5-phosphate synthase (Fragment) OS=Shigella boydii OX=621 GN=dxs PE=3 SV=1	2.499	3	1	1	1	1	295	32.1	1.98
A0A2S8DHD8	AI-2E family transporter OS=Shigella dysenteriae OX=622 GN=C5K18_00760 PE=3 SV=1	1.635	2	1	1	1	1	370	39.9	1.96
F5NY55	Phosphoribosylformylglycinamide synthase OS=Shigella flexneri K-227 OX=766147 GN=purL PE=3 SV=1	2.956	1	1	1	1	1	1294	141.2	1.94

I6CB18	Transcriptional regulatory protein ompR OS=Shigella flexneri K-315 OX=766150 GN=ompR PE=4 SV=1	3.049	4	1	1	1	239	27.4	1.93
I6D6Z3	Ribosome-recycling factor OS=Shigella flexneri K-315 OX=766150 GN=frr PE=3 SV=1	1.772	4	1	1	1	185	20.6	1.91
A0A2S8E8N3	Superoxide dismutase OS=Shigella dysenteriae OX=622 GN=sodB PE=3 SV=1	2.834	6	1	1	1	193	21.3	1.9
A0A4P7TP15	Cu(+)/Ag(+) efflux RND transporter permease subunit CusA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=cusA PE=3 SV=1	1.835	1	1	1	1	1059	115.8	1.9
A0A6N3RFX8	N-acetylmuramoyl-L-alanine amidase OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3202 PE=4 SV=1	1.385	2	1	1	1	417	45.6	1.88
Q0SYE6	2-amino-3-ketobutyrate coenzyme A ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=kbl PE=3 SV=1	1.65	3	1	1	1	398	43.2	1.85
A0A658YRM6	Peptidase, M48 family OS=Shigella flexneri OX=623 GN=SAMEA3710514_00010 PE=4 SV=1	2.389	2	1	1	1	487	53.9	1.85
A0A379ZL4	DNA mismatch repair protein MutS OS=Shigella flexneri OX=623 GN=mutS PE=3 SV=1	1.673	1	1	1	1	853	95.2	1.83
Q0SZ40	Protoporphyrin oxidase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hemG PE=3 SV=1	2.337	4	1	1	1	181	21.2	1.8
I6CB41	Tryptophan-tRNA ligase OS=Shigella flexneri K-315 OX=766150 GN=trpS PE=3 SV=1	1.592	2	1	1	1	319	35.9	1.8
I6CD34	Undecaprenyl-phosphate alpha-N-acetylglucosaminyl 1-phosphate transferase OS=Shigella flexneri K-315 OX=766150 GN=wecA PE=3 SV=1	1.486	2	1	1	1	367	40.9	1.78
A0A2Y4Y1C7	Fumarate hydratase class I OS=Shigella flexneri OX=623 GN=fumA PE=3 SV=1	2.054	2	1	1	1	548	60.3	1.78
A0A4P7TVF5	Cyclic di-GMP-binding protein OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=bcsB PE=3 SV=1	2.85	1	1	1	1	779	86.1	1.78
A0A379ZH24	Glycine-tRNA ligase beta subunit OS=Shigella flexneri OX=623 GN=glyS PE=3 SV=1	1.754	1	1	1	1	689	76.7	1.77
Q7UBI8	Bifunctional protein HldE OS=Shigella flexneri OX=623 GN=hldE PE=3 SV=2	1.574	2	1	1	1	477	51.1	1.76
Q83PW3	Fe(2+) transporter FeoB OS=Shigella flexneri OX=623 GN=f eoB PE=3 SV=4	1.747	2	1	1	1	773	84.4	1.75
A0A6N3QV54	YciO family OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00295 PE=4 SV=1	1.539	5	1	1	1	218	24.5	1.73
A0A379ZBB8	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG OS=Shigella flexneri OX=623 GN=gidA PE=3 SV=1	1.377	2	1	1	1	629	69.5	1.72
A0A2Y4XVQ2	Outer membrane protein assembly complex subunit YfgL OS=Shigella flexneri OX=623 GN=yfgL PE=4 SV=1	2.574	7	1	1	1	184	20.1	1.69
Q0SZY4	50S ribosomal protein L4 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rplD PE=3 SV=1	1.495	3	1	1	1	201	22.1	1.69
A0A2S8DSZ9	Putative glucose-6-phosphate 1-epimerase OS=Shigella boydii OX=621 GN=yeaD PE=3 SV=1	1.514	3	1	1	1	294	32.6	1.69
A0A237FIF1	UPF0234 protein YajQ OS=Shigella boydii OX=621 GN=yajQ PE=3 SV=1	1.671	7	1	1	1	163	18.3	1.67
A0A372UQA1	Glutamate-1-semialdehyde 2,1-aminomutase OS=Shigella flexneri OX=623 GN=hemL PE=3 SV=1	2.348	3	1	1	1	426	45.3	1.66
F5P3D3	50S ribosomal protein L17 OS=Shigella flexneri K-227 OX=766147 GN=rplQ PE=3 SV=1	2.152	6	1	1	1	127	14.4	1.65
A0A6N3QL16	Putative secreted protein OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03831 PE=4 SV=1	1.101	29	1	1	1	126	12.9	0
A0A2Y4XXM5	Uncharacterized protein OS=Shigella flexneri OX=623 GN=SAMEA3710404_03077 PE=4 SV=1	1.115	25	1	1	1	87	9	0
A0A2Y4Y6A2	Uncharacterized protein OS=Shigella flexneri OX=623 GN=SAMEA3710404_04396 PE=4 SV=1	1.317	19	1	1	1	96	10.9	0
A0A2S8DPE3	Replication protein (Fragment) OS=Shigella boydii OX=621 GN=C5K23_18305 PE=3 SV=1	1.191	17	1	1	1	117	13.3	0
F5P0M6	10 kDa chaperonin OS=Shigella flexneri K-227 OX=766147 GN=groS PE=3 SV=1	1.608	14	1	1	1	97	10.4	0
I6CAR9	Universal stress protein OS=Shigella flexneri K-315 OX=766150 GN=uspA PE=3 SV=1	2.066	14	1	1	1	144	16.1	0
A0A2S8DDL0	DUF2732 domain-containing protein OS=Shigella dysenteriae OX=622 GN=C5K18_08875 PE=4 SV=1	0.924	12	1	1	1	74	8.3	0
A0A6D2X1F2	30S ribosomal protein S19 OS=Shigella flexneri OX=623 GN=rpsS PE=3 SV=1	1.639	12	1	1	1	92	10.4	0
A0A2Y4Y2T5	2,5-diketo-D-gluconate reductase A OS=Shigella flexneri OX=623 GN=dkgA PE=4 SV=1	2.188	8	1	1	1	275	31	0
A0A3Y2RTA3	Translation initiation factor IF-3 OS=Shigella flexneri OX=623 GN=infC PE=3 SV=1	0.928	8	1	1	1	180	20.6	0
I6CGK0	Uncharacterized protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3457 PE=4 SV=1	0.983	8	1	1	1	191	19.8	0
A0A6N3RJ42	Glutaredoxin OS=Shigella flexneri 1485-80 OX=766155 GN=ydhD PE=3 SV=1	1.244	7	1	1	1	115	12.9	0
I6C8L3	Fumarate reductase subunit C OS=Shigella flexneri K-315 OX=766150 GN=frcC PE=3 SV=1	1.5	7	1	1	1	120	13.6	0
A0A2Y5B179	DNA-binding transcriptional repressor PurR OS=Shigella flexneri OX=623 GN=purR_2 PE=4 SV=1	1.896	6	1	1	1	174	19.5	0
A0A2Y4XX41	Uncharacterized protein OS=Shigella flexneri OX=623 GN=SAMEA3710404_02870 PE=4 SV=1	1.466	6	1	1	1	118	13.8	0
Q0SZX6	30S ribosomal protein S7 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsG PE=3 SV=1	2.086	6	1	1	1	179	20	0
A0A4P7TME5	Osmotic shock tolerance protein YggT OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=yggT PE=3 SV=1	1.574	5	1	1	1	188	21.1	0
A0A4P7TS42	Elongation factor P OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=efp PE=3 SV=1	1.558	5	1	1	1	188	20.6	0
A0A2S8DMQ5	MobA/MobL family protein (Fragment) OS=Shigella boydii OX=621 GN=C5K23_21360 PE=4 SV=1	1.039	5	1	1	1	117	13.9	0
A0A6N3RQ35	DNA protection during starvation protein OS=Shigella flexneri 1485-80 OX=766155 GN=dps PE=3 SV=1	1.891	5	1	1	1	167	18.7	0
A0A6N3QQV1	Lipoyl synthase OS=Shigella flexneri CDC 796-83 OX=945360 GN=lipA PE=3 SV=1	1.567	5	1	1	1	234	26.3	0
I6D2B3	Amino ABC transporter, permease, 3-TM region, His/Glu/Gln/Arg/opine family domain protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0742 PE=3 SV=1	0.96	5	1	1	1	219	24.3	0
A0A2S8D5U3	Phosphatase OS=Shigella dysenteriae OX=622 GN=yihX PE=4 SV=1	1.023	5	1	1	1	199	22.7	0
I6CY67	UPF0227 protein YcfP OS=Shigella flexneri K-315 OX=766150 GN=yfp PE=3 SV=1	1.635	4	1	1	1	180	21.2	0

A0A1S9JGL5	Cytidylate kinase OS=Shigella boydii OX=621 GN=cmk PE=3 SV=1	1.754	4	1	1	1	227	24.7	0
D2ACL4	Superoxide dismutase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=sodA PE=3 SV=1	1.292	4	1	1	1	206	23.1	0
D2AEJ0	RNA chaperone ProQ OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=proQ PE=3 SV=1	1.948	4	1	1	1	232	25.8	0
A0A2S8DVS8	Carbonic anhydrase OS=Shigella boydii OX=621 GN=can PE=3 SV=1	1.275	4	1	1	1	220	25.1	0
A0A3T3SOR7	Glutamine ABC transporter periplasmic protein OS=Shigella flexneri OX=623 GN=glmH PE=3 SV=1	1.31	4	1	1	1	248	27.2	0
Q0T3Y0	Fumarate and nitrate reduction Regulatory protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fnr PE=4 SV=1	2.954	4	1	1	1	250	27.9	0
Q0SYU1	ATP synthase subunit delta OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=atpH PE=3 SV=1	1.095	4	1	1	1	177	19.3	0
A0A6N3RKN1	Uracil phosphoribosyltransferase OS=Shigella flexneri 1485-80 OX=766155 GN=upp PE=3 SV=1	1.51	4	1	1	1	208	22.5	0
I6D702	Acyl-[acyl-carrier-protein]-UDP-N-acetylglucosamine O-acyltransferase OS=Shigella flexneri K-315 OX=766150 GN=lpxA PE=3 SV=1	1.327	4	1	1	1	262	28	0
I6CGI6	1-acyl-sn-glycerol-3-phosphate acyltransferase OS=Shigella flexneri K-315 OX=766150 GN=plsC PE=3 SV=1	1.212	3	1	1	1	211	23.4	0
I6CDH9	DNA polymerase I OS=Shigella flexneri K-315 OX=766150 GN=polA PE=3 SV=1	1.057	3	1	1	1	605	68.1	0
Q5IPA5	Aerobic respiratory control protein (Fragment) OS=Shigella flexneri OX=623 GN=arcA PE=4 SV=1	1.136	3	1	1	1	182	21	0
A0A3T2USI5	Glucokinase OS=Shigella flexneri OX=623 GN=glk PE=3 SV=1	1.23	3	1	1	1	321	34.7	0
A0A4P7TSW8	Pirin family protein OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_023325 PE=3 SV=1	1.018	3	1	1	1	233	25.9	0
A0A658YX65	Phosphopentomutase OS=Shigella flexneri OX=623 GN=deoB PE=3 SV=1	1.295	3	1	1	1	407	44.4	0
I6D6S9	Protein AmpE OS=Shigella flexneri K-315 OX=766150 GN=ampE PE=4 SV=1	1.106	3	1	1	1	276	31.2	0
A0A6N3RE59	S-adenosylmethionine synthase OS=Shigella flexneri 1485-80 OX=766155 GN=metK PE=3 SV=1	1.783	3	1	1	1	384	41.9	0
D2ABV0	ATP-dependent RNA helicase RhlB OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rhlB PE=3 SV=1	0.92	3	1	1	1	421	47.1	0
A0A2S8DHN0	Ubiquinone/menaquinone biosynthesis C-methyltransferase UbiE OS=Shigella dysenteriae OX=622 GN=ubiE PE=3 SV=1	1.458	3	1	1	1	251	28.1	0
D2A949	Bifunctional protein FoLD OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=foLD PE=3 SV=1	1.109	3	1	1	1	288	30.9	0
A0A6N3RFW5	Penicillin-binding protein 7 OS=Shigella flexneri 1485-80 OX=766155 GN=pbpG PE=3 SV=1	1.088	3	1	1	1	293	32.5	0
A0A2S8DET8	Galactose-1-phosphate uridylyltransferase OS=Shigella dysenteriae OX=622 GN=C5K18_07390 PE=3 SV=1	1.555	3	1	1	1	348	39.6	0
A0A1Q8M994	ADP-L-glycero-D-manno-heptose-6-epimerase OS=Shigella boydii OX=621 GN=rfad PE=3 SV=1	1.961	3	1	1	1	310	34.9	0
P0A6A2	Probable protein kinase UbiB OS=Shigella flexneri OX=623 GN=ubiB PE=3 SV=1	1.074	3	1	1	1	546	63.2	0
A0A658Y1F9	Mannitol dehydrogenase family protein OS=Shigella flexneri OX=623 GN=por PE=4 SV=1	0.938	2	1	1	1	486	53.6	0
A0A6N3REG1	NADH-quinone oxidoreductase subunit H OS=Shigella flexneri 1485-80 OX=766155 GN=nuoH PE=3 SV=1	1.276	2	1	1	1	325	36.2	0
F5NRC2	Curved DNA-binding protein OS=Shigella flexneri K-227 OX=766147 GN=cbpA PE=3 SV=1	1.12	2	1	1	1	306	34.4	0
A0A2S8DL49	NADH-quinone oxidoreductase subunit N OS=Shigella boydii OX=621 GN=nuoN PE=3 SV=1	1.848	2	1	1	1	485	52	0
A0A6G5Z0C8	Inosine-5'-monophosphate dehydrogenase OS=Shigella flexneri OX=623 GN=guaB PE=3 SV=1	1.236	2	1	1	1	488	52	0
A0A6N3QIL7	NADP-dependent malic enzyme OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02679 PE=3 SV=1	1.269	2	1	1	1	279	30.5	0
I6D448	Alkyl hydroperoxide reductase subunit F OS=Shigella flexneri K-315 OX=766150 GN=ahpF PE=3 SV=1	1.021	2	1	1	1	521	56.1	0
A0A383JSV3	Cysteine--tRNA ligase OS=Shigella flexneri OX=623 GN=cysS PE=3 SV=1	1.798	2	1	1	1	461	52.2	0
A0A0H2UZ50	Asparagine synthase (glutamine-hydrolyzing) OS=Shigella flexneri OX=623 GN=asnB PE=3 SV=1	0.948	2	1	1	1	515	58.4	0
Q0T8P0	ATP-binding component of cytochrome-related transport OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=cydC PE=4 SV=1	1.298	2	1	1	1	573	62.9	0
A0A3Y2RP03	Lipopolysaccharide 3-alpha-galactosyltransferase (Fragment) OS=Shigella flexneri OX=623 GN=DTY77_07815 PE=4 SV=1	1.031	2	1	1	1	318	37.2	0
Q0T6Q2	Leucine--tRNA ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=leuS PE=3 SV=2	2.445	2	1	1	1	860	97.2	0
I6CVA3	L-serine dehydratase OS=Shigella flexneri K-315 OX=766150 GN=SFK315_1603 PE=3 SV=1	0.976	2	1	1	1	432	46.6	0
A0A658Y4L9	Exopolyphosphatase OS=Shigella flexneri OX=623 GN=ppx PE=3 SV=1	2.115	2	1	1	1	513	58.1	0
A0A380AGY6	Iron-sulfur cluster carrier protein OS=Shigella flexneri OX=623 GN=minD_1 PE=3 SV=1	1.168	2	1	1	1	369	39.9	0
A0A4P7TS84	Probable cytosol aminopeptidase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=pepA PE=3 SV=1	1.399	2	1	1	1	503	54.8	0
Q0T0J7	DNA primase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dnaG PE=3 SV=1	1.201	2	1	1	1	581	65.6	0
A0A2S8D566	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase OS=Shigella dysenteriae OX=622 GN=ubiF PE=3 SV=1	1.039	2	1	1	1	391	42.9	0
A0A658Y5D6	Arginyl-tRNA synthetase OS=Shigella flexneri OX=623 GN=argS PE=3 SV=1	1.296	1	1	1	1	577	64.7	0
A0A6N3RA69	Proton/glutamate-aspartate symporter OS=Shigella flexneri 1485-80 OX=766155 GN=glrT PE=3 SV=1	1.072	1	1	1	1	437	47.1	0
D2ACF0	Fatty acid oxidation complex subunit alpha OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=fadB PE=3 SV=1	1.754	1	1	1	1	729	79.6	0
A0A2S8DE29	Low affinity potassium transport system protein kup OS=Shigella dysenteriae OX=622 GN=trkD PE=3 SV=1	0.946	1	1	1	1	587	65.6	0
F5P339	DD-transpeptidase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4931 PE=3 SV=1	2.142	1	1	1	1	843	92.6	0

### Appx. D.12: Full protein identification from band 12 of Figure 5.2b.

Accession	Description	Sum PEP Score	Coverage [%]	# Peptides	# PSMs	# Unique Peptides	# AAs	MW [kDa]	Score Sequest HT
P37784	O-antigen polymerase OS=Shigella flexneri OX=623 GN=rfc PE=3 SV=1	41.475	18	9	120	9	382	43.7	216.06
A0A658XXS4	Outer membrane protein assembly factor YaeT OS=Shigella flexneri OX=623 GN=yaeT PE=4 SV=1	159.106	39	30	49	30	810	90.5	124.88
A0A3U1DEW5	Formate C-acetyltransferase OS=Shigella flexneri OX=623 GN=pfIB PE=3 SV=1	115.149	33	27	46	27	760	85.3	109.21
A0A383JRI2	ATP-dependent zinc metalloprotease FtsH OS=Shigella flexneri OX=623 GN=hfIB PE=3 SV=1	87.996	32	24	40	24	647	71	91.43
Q0SZX8	Elongation factor Tu 1 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tufI PE=3 SV=1	83.608	53	18	35	18	394	43.3	80.53
Q0SXC2	Fumarate reductase flavoprotein subunit OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=frdA PE=3 SV=1	99.08	41	21	35	21	602	65.9	90.69
A0A380B9F2	Peptidylprolyl isomerase OS=Shigella flexneri OX=623 GN=ppiD PE=4 SV=1	95.369	51	22	32	22	623	68.1	83.17
A0A4P7TR58	NAD(P)/FAD-dependent oxidoreductase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_010675 PE=4 SV=1	95.337	48	17	32	17	434	47.3	84.93
I6CBL7	ATP synthase subunit alpha OS=Shigella flexneri K-315 OX=766150 GN=atpA PE=3 SV=1	73.705	48	20	29	20	513	55.2	73.59
A0A379Z256	60 kDa chaperonin OS=Shigella flexneri OX=623 GN=groEL PE=3 SV=1	73.247	40	20	25	20	551	57.5	63.36
Q7BCK4	Outer membrane protein IcsA autotransporter OS=Shigella flexneri OX=623 GN=icsA PE=1 SV=1	50.379	15	13	25	13	1102	116.2	51.88
A0A1Q8MQG3	Chaperone protein DnaK OS=Shigella boydii OX=621 GN=dnaK PE=2 SV=1	83.53	42	23	24	23	638	69.1	57.84
R4NJV1	Outer membrane protein C (Fragment) OS=Shigella flexneri 3a OX=424717 GN=ompC PE=3 SV=1	57.311	34	13	21	7	351	39.3	43.67
A0A4P7TME0	Transketolase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tkt PE=3 SV=1	58.082	21	16	21	16	663	72.1	41.67
P0A852	Trigger factor OS=Shigella flexneri OX=623 GN=tig PE=3 SV=1	41.362	27	13	19	13	432	48.2	35.68
A0A658Y1M8	NAD-dependent glyceraldehyde-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=gapA PE=3 SV=1	52.14	39	12	18	12	331	35.5	45.5
A0A6N3R3T7	Aspartate ammonia-lyase OS=Shigella flexneri 1485-80 OX=766155 GN=aspA PE=3 SV=1	49.419	33	15	17	15	478	52.3	37.75
A0A2S8DUZ3	Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha OS=Shigella boydii OX=621 GN=accA PE=3 SV=1	40.626	30	10	16	10	319	35.2	34.9
Q83SE6	Chaperone protein HtpG OS=Shigella flexneri OX=623 GN=htpG PE=3 SV=1	51.335	27	15	16	15	624	71.3	38.4
A0A2S8D6C0	Protein translocase subunit SecD OS=Shigella dysenteriae OX=622 GN=secD PE=3 SV=1	43.585	27	15	16	15	615	66.6	31.37
A0A2Y4WVQ3	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degP PE=3 SV=1	67.596	21	11	16	11	474	49.3	53.81
A0A2A2XR75	EIIAB-Man OS=Shigella flexneri OX=623 GN=manX_2 PE=4 SV=1	56.334	31	12	15	12	323	35	43.28
F5NXP4	LPS-assembly protein LptD OS=Shigella flexneri K-227 OX=766147 GN=lptD PE=3 SV=1	53.037	23	13	15	13	753	86.3	29.75
Q0T4H3	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1619 PE=4 SV=1	36.82	35	11	14	11	314	33.9	30.09
D2A904	30S ribosomal protein S3 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsC PE=3 SV=1	31.326	29	8	14	8	224	24.8	22.07
A0A2Y4Y9J3	Pyruvate kinase OS=Shigella flexneri OX=623 GN=pykF PE=3 SV=1	30.524	22	11	13	11	542	58.7	18.01
A0A6N3RAZ3	Cell division protein FtsN OS=Shigella flexneri 1485-80 OX=766155 GN=ftsN PE=3 SV=1	23.142	33	9	12	9	284	31.8	19.09
A0A2S8E1V7	Protein HflK OS=Shigella dysenteriae OX=622 GN=hflK PE=3 SV=1	35.41	23	8	12	8	419	45.5	25.95
A0A379Z3L5	Glycerol-3-phosphate acyltransferase OS=Shigella flexneri OX=623 GN=plsB PE=3 SV=1	17.912	16	12	12	12	807	91.4	9.34
Q83RX6	Dihydroorotate dehydrogenase (quinone) OS=Shigella flexneri OX=623 GN=pyrD PE=3 SV=4	35.451	37	11	11	11	336	36.7	24.56
Q2EUU0	AccD (Fragment) OS=Shigella flexneri OX=623 GN=accD PE=3 SV=1	31.677	29	8	11	8	296	32.6	24.05
Q0T4H5	NAD(P) transhydrogenase subunit beta OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pntB PE=3 SV=1	42.376	18	6	11	6	462	48.7	29.07
A0A2Y4Y099	Glycerol-3-phosphate dehydrogenase OS=Shigella flexneri OX=623 GN=glpD PE=3 SV=1	29.453	16	8	11	8	501	56.7	18.09
D2AFY9	NADH-quinone oxidoreductase subunit C/D OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=nuoC PE=3 SV=1	26.69	16	9	11	9	600	68.7	25.31
A0A0H2VVY4	Anaerobic dimethyl sulfoxide reductase subunit A OS=Shigella flexneri OX=623 GN=dmsA PE=3 SV=1	30.011	15	10	11	10	785	87.4	19
D2ABQ4	Transposon Tn7 transposition protein tnsC OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4148 PE=4 SV=1	2.508	4	1	11	1	555	63	2.01
I6CBL5	ATP synthase F0, B subunit OS=Shigella flexneri K-315 OX=766150 GN=atpF PE=3 SV=1	35.662	69	9	10	9	127	14	28.83
A0A4P7TNY5	Alkyl hydroperoxide reductase C OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=ahpC PE=3 SV=1	33.152	47	8	10	8	187	20.7	22.6
A0A6D2XZS5	DNA-directed RNA polymerase subunit alpha OS=Shigella flexneri OX=623 GN=rpoA PE=3 SV=1	25.984	27	8	10	8	329	36.5	18.91
A0A1S9KGJ3	Peptidyl-prolyl cis-trans isomerase OS=Shigella dysenteriae OX=622 GN=fkpa PE=3 SV=1	25.6	26	6	10	6	270	28.9	20.15
A0A6N3RB60	50S ribosomal protein L2 OS=Shigella flexneri 1485-80 OX=766155 GN=rplB PE=3 SV=1	19.241	26	6	10	6	273	29.8	21.77
I6CG83	Phosphoglycerate kinase OS=Shigella flexneri K-315 OX=766150 GN=pgk PE=3 SV=1	31.809	25	9	10	9	382	40.5	26.49
F5NXX2	Serine-type D-Ala-D-Ala carboxypeptidase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0860 PE=3 SV=1	29.293	22	8	10	8	403	44.4	18.71
F5NT05	EIICB-Glc OS=Shigella flexneri K-227 OX=766147 GN=ptsG PE=4 SV=1	38.709	21	8	10	8	477	50.7	22.71

I6D3P8	Cytochrome d ubiquinol oxidase subunit I OS=Shigella flexneri K-315 OX=766150 GN=cydA PE=3 SV=1	27.657	19	10	10	10	500	55.6	18.18
A0A2S8E636	Acetyltransferase component of pyruvate dehydrogenase complex OS=Shigella dysenteriae OX=622 GN=aceF PE=3 SV=1	36.621	16	9	10	9	630	66.1	26.45
A0A658YYQ4	NAD(P) transhydrogenase subunit alpha OS=Shigella flexneri OX=623 GN=pntA PE=3 SV=1	22.993	15	7	10	7	510	54.5	18.42
A0A2S8D4S3	Aldehyde-alcohol dehydrogenase OS=Shigella dysenteriae OX=622 GN=C5K18_27725 PE=3 SV=1	39.25	12	9	10	9	891	96.1	27.79
A0A6N3RKB3	KefA OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0517 PE=3 SV=1	20.64	9	10	10	10	1135	128.5	20.69
A0A2Y5B4B8	Malate dehydrogenase OS=Shigella flexneri OX=623 GN=mdh PE=3 SV=1	23.004	32	8	9	8	312	32.3	21.09
Q0SYD1	L-lactate dehydrogenase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lldD PE=3 SV=1	40.283	30	9	9	9	396	42.7	28.42
A0A3T2UWB2	ATP synthase gamma chain OS=Shigella flexneri OX=623 GN=atpG PE=3 SV=1	23.773	28	8	9	8	287	31.6	19.6
I6CAV2	Cell division protein FtsX OS=Shigella flexneri K-315 OX=766150 GN=ftsX PE=3 SV=1	24.422	21	7	9	7	352	38.5	17.46
A0A658YVY8	L-tryptophan indole-lyase OS=Shigella flexneri OX=623 GN=tnaA PE=3 SV=1	21.817	20	9	9	9	471	52.8	16.59
A0A379Z9W6	Heme biosynthesis protein HemY OS=Shigella flexneri OX=623 GN=hemY PE=4 SV=1	24.657	19	9	9	9	398	45.2	21.21
A0A6N3RG64	Signal peptidase I OS=Shigella flexneri 1485-80 OX=766155 GN=lepB PE=3 SV=1	27.199	18	7	9	7	321	35.6	15.94
I6CJU2	Phosphate acetyltransferase OS=Shigella flexneri K-315 OX=766150 GN=pta PE=3 SV=1	22.353	10	7	9	7	709	76.5	18.21
A0A6N3QN52	Lon protease OS=Shigella flexneri CDC 796-83 OX=945360 GN=lon PE=2 SV=1	21.788	10	9	9	9	799	89	14.14
A0A6L6V006	Ribose-phosphate pyrophosphokinase OS=Shigella flexneri OX=623 GN=prs PE=3 SV=1	26.37	30	8	8	8	315	34.2	17.52
P0A6P4	Elongation factor Ts OS=Shigella flexneri OX=623 GN=tsf PE=3 SV=2	20.998	25	7	8	7	283	30.4	15.12
A0A1S9JMU3	Cell shape-determining protein MreB OS=Shigella boydii OX=621 GN=mreB PE=3 SV=1	22.18	23	7	8	7	347	36.9	15.55
A0A2S8DDU2	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase OS=Shigella dysenteriae OX=622 GN=murG PE=3 SV=1	24.642	19	6	8	6	355	37.8	20.51
I6CS25	Protein ydgA OS=Shigella flexneri K-315 OX=766150 GN=ydgA PE=4 SV=1	22.652	19	8	8	8	502	54.7	18.01
I6D072	Outer membrane protein A OS=Shigella flexneri K-315 OX=766150 GN=ompA PE=3 SV=1	21.557	19	6	8	6	344	37	17.9
I6CI36	Cytoskeleton protein RodZ OS=Shigella flexneri K-315 OX=766150 GN=rodZ PE=3 SV=1	18.607	18	6	8	6	337	36.2	17.06
I6CMA1	Quinone-dependent D-lactate dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=dld PE=3 SV=1	19.045	12	7	8	7	571	64.6	14.04
Q0T1R3	Chaperone protein ClpB OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=clpB PE=3 SV=1	23.256	11	8	8	8	857	95.6	14.24
Q7AK95	Chloramphenicol acetyltransferase OS=Shigella flexneri 2b OX=1288825 GN=cat PE=3 SV=1	17.222	7	2	8	2	219	25.6	17.94
F5P2D1	Rhodanese-like domain protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4672 PE=4 SV=1	26.845	50	6	7	6	143	15.6	17.32
I6C9W2	30S ribosomal protein S10 OS=Shigella flexneri K-315 OX=766150 GN=rpsJ PE=3 SV=1	14.4	39	5	7	5	103	11.7	13.91
F5P3L0	Fumarate reductase iron-sulfur subunit OS=Shigella flexneri K-227 OX=766147 GN=SFK227_5105 PE=3 SV=1	23.949	28	6	7	6	244	27	17.21
A0A2S8DA55	SH3 domain-containing protein OS=Shigella dysenteriae OX=622 GN=ygiM PE=4 SV=1	20.023	25	5	7	5	206	23.1	15.57
A0A2Y5C0F7	Putative uroporphyrinogen III C-methyltransferase OS=Shigella flexneri OX=623 GN=hemX PE=4 SV=1	23.903	23	7	7	7	401	43.6	18.66
A0A2Y4JWQ5	MbeB-like, N-term conserved region OS=Shigella flexneri OX=623 GN=SAMEA3710514_05138 PE=4 SV=1	17.92	22	5	7	5	176	19.9	17.46
Q0T8M9	Serine--tRNA ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=serS PE=3 SV=1	15.983	18	7	7	7	430	48.4	10.35
A0A379ZSJ2	Alcohol dehydrogenase OS=Shigella flexneri OX=623 GN=yqhD PE=4 SV=1	25.76	17	6	7	6	387	42.1	16.22
A0A4P7TSA3	Dihydrolypoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=odhB PE=3 SV=1	23.297	17	6	7	6	405	44	12.27
A0A2Y4ZTN4	Xaa-Pro dipeptidase OS=Shigella flexneri OX=623 GN=pepQ PE=3 SV=1	16.114	16	7	7	7	443	50.2	12.95
Q0T1P7	Enolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=eno PE=3 SV=1	19.028	15	6	7	6	431	45.7	18.54
I6D6T4	Dihydrolypoyl dehydrogenase OS=Shigella flexneri K-315 OX=766150 GN=lpdA PE=3 SV=1	20.948	15	7	7	7	474	50.7	18.12
A0A4V1CUD1	Histidine kinase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=cpxA PE=4 SV=1	18.814	14	7	7	7	457	51.6	18.01
A0A4P7TRM2	Lipopolysaccharide N-acetylglucosaminyltransferase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=EKN05_019395 PE=4 SV=1	10.674	13	5	7	5	380	43.5	10.48
F5P3A0	Elongation factor G OS=Shigella flexneri K-227 OX=766147 GN=fusA PE=3 SV=1	19.773	11	7	7	7	704	77.6	12.73
A0A4P7TRQ9	Ribulokinase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=araB PE=3 SV=1	25.896	11	6	7	6	566	61.1	17.49
A0A6N3REQ7	Nickel-dependent hydrogenase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3604 PE=3 SV=1	13.443	10	6	7	6	567	62.5	10.63
A0A2S8DJU5	Polyribonucleotide nucleotidyltransferase OS=Shigella boydii OX=621 GN=pnp PE=3 SV=1	13.902	9	6	7	6	711	77.1	10.83
I6CHF3	MFS transporter, sugar porter family protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3768 PE=3 SV=1	17.157	9	4	7	4	472	51.7	16.6
F5NUR0	Threonine--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=thrS PE=3 SV=1	12.633	9	7	7	7	642	74	11.39
A0A383JWV6	Membrane protein insertase YidC OS=Shigella flexneri OX=623 GN=yidC PE=3 SV=1	13.93	8	5	7	5	548	61.5	13.9
D2AHG2	Autonomous glycy radical cofactor OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfiD PE=3 SV=1	19.664	32	4	6	4	127	14.2	17.01
D2A917	30S ribosomal protein S12 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsL PE=3 SV=1	16.967	32	6	6	6	124	13.7	13
A0A2S8DV96	50S ribosomal protein L5 OS=Shigella boydii OX=621 GN=rplE PE=3 SV=1	17.625	29	4	6	4	179	20.3	13.66

Q0T6Q1	LPS-assembly lipoprotein LptE OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=lptE PE=3 SV=1	15.787	27	4	6	4	193	21.4	12.06
Q0T240	Outer membrane protein assembly factor BamC OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nlpB PE=3 SV=1	25.23	25	6	6	6	344	36.8	16.64
I6C8N1	Modulator of FtsH protease HflC OS=Shigella flexneri K-315 OX=766150 GN=hflC PE=3 SV=1	21.504	24	6	6	6	334	37.6	15.76
A0A2S8D3U0	Catabolite activator protein OS=Shigella dysenteriae OX=622 GN=crp PE=4 SV=1	14.346	23	5	6	5	210	23.7	10.21
A0A2S8DUL5	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase OS=Shigella boydii OX=621 GN=dapD PE=3 SV=1	17.759	21	5	6	5	274	29.9	12.96
I6D6H9	Transaldolase OS=Shigella flexneri K-315 OX=766150 GN=tal PE=3 SV=1	17.501	21	6	6	6	294	32.8	15.3
A0A658YX10	Acriflavine resistance protein A OS=Shigella flexneri OX=623 GN=acrA PE=3 SV=1	23.794	20	5	6	5	397	42.1	15.07
A0A6N3RKA3	Succinate dehydrogenase iron-sulfur subunit OS=Shigella flexneri 1485-80 OX=766155 GN=sdhB PE=3 SV=1	13.705	20	5	6	5	238	26.8	7.4
A0A380AZK5	D-amino acid dehydrogenase OS=Shigella flexneri OX=623 GN=dadA PE=3 SV=1	21.417	16	6	6	6	432	47.6	13.9
D2AGX5	Cysteine desulfurase IscS OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=iscS PE=3 SV=1	18.534	15	6	6	6	412	46	15.37
F5NX93	EIII-Fru OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2769 PE=4 SV=1	15.731	14	5	6	5	376	39.7	11.98
A0A6N3RDJ8	Spermidine/putrescine import ATP-binding protein PotA OS=Shigella flexneri 1485-80 OX=766155 GN=potA PE=3 SV=1	12.229	14	5	6	5	378	43	7.83
A0A383JTR9	Cardiolipin synthase A OS=Shigella flexneri OX=623 GN=cls PE=3 SV=1	18.722	13	5	6	5	486	54.8	13.2
A0A3T2UY84	Cyclopropane-fatty-acyl-phospholipid synthase OS=Shigella flexneri OX=623 GN=DK174_15880 PE=3 SV=1	13.101	13	5	6	5	382	43.9	11.45
Q0T2G2	3-oxoacyl-[acyl-carrier-protein] synthase I OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fabB PE=3 SV=1	16.574	13	5	6	5	370	39	14.68
A0A0H2VYI3	Nucleoside permease OS=Shigella flexneri OX=623 GN=nupC PE=3 SV=1	10.98	11	5	6	5	400	43.5	11.58
F5NY87	Phosphoenolpyruvate-protein phosphotransferase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3048 PE=3 SV=1	19.357	11	5	6	5	585	64.5	15.61
I6CDA5	ATP-dependent protease ATPase subunit HslU OS=Shigella flexneri K-315 OX=766150 GN=hslU PE=2 SV=1	12.718	10	5	6	5	443	49.6	11.15
A0A6N3RB16	Protein translocase subunit SecY OS=Shigella flexneri 1485-80 OX=766155 GN=prlA PE=3 SV=1	13.68	10	4	6	4	443	48.5	11.3
D2ABG4	30S ribosomal protein S1 OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpsA PE=3 SV=1	15.737	10	5	6	5	557	61.1	8.32
A0A3U1D5H5	Alpha-D-glucose phosphate-specific phosphoglucomutase OS=Shigella flexneri OX=623 GN=pgm PE=3 SV=1	11.583	9	5	6	5	546	58.3	9.93
A0A3T2URE0	Catalase-peroxidase OS=Shigella flexneri OX=623 GN=katG PE=3 SV=1	15.325	9	5	6	5	726	80	11.11
A0A6N3R6K7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4998 PE=3 SV=1	17.152	8	5	6	5	402	42.7	20.18
I6D3R0	Succinate dehydrogenase flavoprotein subunit OS=Shigella flexneri K-315 OX=766150 GN=sdhA PE=3 SV=1	18.462	7	4	6	4	588	64.4	13.12
A0A658YV63	Bifunctional acyl-[acyl carrier protein] synthetase/2-acylglycerophosphoethanolamine acyltransferase OS=Shigella flexneri OX=623 GN=aas PE=4 SV=1	9.836	7	5	6	5	719	80.6	9.99
A0A4P7TRN0	Pyruvate dehydrogenase E1 component OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=aceE PE=4 SV=1	13.517	6	6	6	6	887	99.6	10.03
A0A6N3R4Q2	50S ribosomal protein L6 OS=Shigella flexneri 1485-80 OX=766155 GN=rplF PE=3 SV=1	12.595	32	5	5	5	177	18.9	6.34
A0A6N3QME5	50S ribosomal protein L1 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rplA PE=3 SV=1	15.895	26	5	5	5	234	24.7	13.65
F5P004	L-asparaginase 2 OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3757 PE=3 SV=1	21.791	24	4	5	4	235	25.2	12.29
I6CX73	Cell division inhibitor MinD OS=Shigella flexneri K-315 OX=766150 GN=minD PE=3 SV=1	13.31	22	5	5	5	235	26.3	9.38
Q0T239	4-hydroxy-tetrahydrodipicolinate synthase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dapA PE=3 SV=1	19.153	22	5	5	5	292	31.3	14.05
A0A2S8DCH8	Cysteine synthase OS=Shigella dysenteriae OX=622 GN=cysK PE=3 SV=1	22.24	21	5	5	5	323	34.5	15.58
D2AE64	Uncharacterized protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_4630 PE=3 SV=1	10.416	21	3	5	3	141	15.2	9.62
A0A2S8DV51	Chaperone protein DnaJ OS=Shigella boydii OX=621 GN=dnaJ PE=3 SV=1	14.975	14	5	5	5	376	41	12.54
A0A658Y983	Periplasmic serine endoprotease DegP-like OS=Shigella flexneri OX=623 GN=degQ PE=3 SV=1	16.267	14	5	5	5	455	47.2	13.27
A0A3T2UX48	Outer membrane protein F OS=Shigella flexneri OX=623 GN=ompF PE=3 SV=1	17.954	12	4	5	4	362	39.3	13.76
A0A658Z505	Transporter, small conductance mechanosensitive ion channel (MscS) family OS=Shigella flexneri OX=623 GN=mscM PE=3 SV=1	10.157	11	5	5	5	415	46.6	9.16
A0A2S8DV74	Glycerol kinase OS=Shigella boydii OX=621 GN=glpK PE=3 SV=1	15.498	11	5	5	5	502	56.1	12.78
F5P2J3	C4-dicarboxylate transport protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4734 PE=4 SV=1	17.442	11	4	5	4	395	41.7	10.61
Q0SZA6	ATP-dependent 6-phosphofructokinase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pfkA PE=3 SV=1	9.68	10	4	5	4	340	37.1	6.39
A0A380B3G3	Asparagine--tRNA ligase OS=Shigella flexneri OX=623 GN=asnC_2 PE=3 SV=1	12.676	10	5	5	5	466	52.6	9.02
I6D070	Endopeptidase La OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0989 PE=3 SV=1	14.699	10	5	5	5	586	65.9	10.97
A0A658Z233	Inner membrane protein yjgQ OS=Shigella flexneri OX=623 GN=yjgQ PE=3 SV=1	8.807	10	4	5	4	360	39.6	7.06
A0A2S8DD45	Isocitrate dehydrogenase [NADP] OS=Shigella dysenteriae OX=622 GN=icd PE=3 SV=1	9.442	9	4	5	4	416	45.8	8.36
A0A383JXY5	NAD dependent epimerase/dehydratase family OS=Shigella flexneri OX=623 GN=DOD76_09530 PE=4 SV=1	9.995	9	3	5	3	337	38	8.29
D2ADL5	Respiratory nitrate reductase I beta chain OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=narH PE=4 SV=1	10.313	9	5	5	5	512	58	8.58
D2AHW6	Peptidoglycan D,D-transpeptidase FtsI OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=ftsI PE=3 SV=1	12.277	8	4	5	4	588	63.9	11.46
A0A6N3QQ54	Histidine kinase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01033 PE=4 SV=1	8.42	8	4	5	4	450	50.3	5.25
Q0T8D5	L-arabinose isomerase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=araA PE=3 SV=1	10.928	8	5	5	5	500	56	11.43
A0A6N3QJ73	Fructose-specific PTS system IIBC component OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03096 PE=4 SV=1	25.268	6	3	5	3	563	57.5	15.91



A0A2S8DPK5	Copper exporting ATPase OS=Shigella boydii OX=621 GN=copA PE=3 SV=1	12.578	6	5	5	5	834	87.9	9.26
A0A2S8DKT5	NADH-quinone oxidoreductase OS=Shigella boydii OX=621 GN=nuoG PE=3 SV=1	16.05	4	4	5	4	908	100.3	12.45
A0A2Y4XMF0	Sec-independent protein translocase protein TatA OS=Shigella flexneri OX=623 GN=tatA PE=3 SV=1	12.326	49	4	4	4	89	9.7	7.67
F5P0M6	10 kDa chaperonin OS=Shigella flexneri K-227 OX=766147 GN=groS PE=3 SV=1	13.596	39	3	4	3	97	10.4	9.88
A0A6N3RAY3	50S ribosomal protein L15 OS=Shigella flexneri 1485-80 OX=766155 GN=rpL15 PE=3 SV=1	10.752	39	4	4	4	126	13.1	6.5
Q0T005	30S ribosomal protein S11 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rpsK PE=3 SV=1	13.442	25	3	4	3	129	13.8	9.11
F5P2W0	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4852 PE=4 SV=1	13.32	23	3	4	3	185	19.8	10.43
I6D6Z3	Ribosome-recycling factor OS=Shigella flexneri K-315 OX=766150 GN=frf PE=3 SV=1	6.422	21	4	4	4	185	20.6	3.92
P69785	PTS system glucose-specific EIIA component OS=Shigella flexneri OX=623 GN=crr PE=3 SV=2	13.06	20	2	4	2	169	18.2	6.69
F5P316	Fe/S biogenesis protein NfuA OS=Shigella flexneri K-227 OX=766147 GN=nfuA PE=3 SV=1	8.364	17	3	4	3	191	21	5.22
I6CFU5	Peptidase Do OS=Shigella flexneri K-315 OX=766150 GN=degS PE=3 SV=1	14.927	17	4	4	4	355	37.6	10.07
A0A2Y4YFW7	Adenylate kinase OS=Shigella flexneri OX=623 GN=adk PE=3 SV=1	7.66	16	4	4	4	234	25.8	8.42
D2A8B5	Cytoplasmic membrane protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yrbD PE=4 SV=1	8.226	16	3	4	3	183	19.6	6.82
A0A2A2XF62	Cell division ATP-binding protein FtsE OS=Shigella flexneri OX=623 GN=ftsE PE=3 SV=1	8.55	14	3	4	3	222	24.4	7.64
A0A4P7TTH9	30S ribosomal protein S2 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsB PE=3 SV=1	10.54	14	3	4	3	241	26.8	5.99
I6CXR3	Universal stress protein E OS=Shigella flexneri K-315 OX=766150 GN=uspE PE=4 SV=1	12.838	13	4	4	4	314	35.5	9.26
F5NZV9	Mechanosensitive ion channel family protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3712 PE=3 SV=1	15.697	13	3	4	3	286	30.9	9.76
F5NRS1	Oxygen-insensitive NAD(P)H nitroreductase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0809 PE=3 SV=1	5.64	13	3	4	3	217	23.8	5.47
A0A658YV21	Auxiliary transport protein, membrane fusion protein (MFP) family OS=Shigella flexneri OX=623 GN=macA_2 PE=4 SV=1	10.513	12	4	4	4	355	38.7	8.79
I6C8V5	Fructose-1,6-bisphosphatase class 1 OS=Shigella flexneri K-315 OX=766150 GN=fbp PE=3 SV=1	8.245	12	3	4	3	332	36.8	6.99
F5P0R0	Maltodextrin-binding protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4033 PE=3 SV=1	13.045	11	4	4	4	396	43.4	6.31
D2AHT6	Co-chaperone protein DjIA OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=djIA PE=3 SV=1	6.94	11	4	4	4	271	30.5	6.3
A0A1S9KIA0	Transcription termination factor Rho OS=Shigella dysenteriae OX=622 GN=rho PE=3 SV=1	12.997	11	4	4	4	419	47	10.58
I6CVK9	Phenylalanine--tRNA ligase alpha subunit OS=Shigella flexneri K-315 OX=766150 GN=pheS PE=3 SV=1	7.005	11	4	4	4	327	36.8	5.24
I6D632	ATP-dependent Clp protease ATP-binding subunit ClpX OS=Shigella flexneri K-315 OX=766150 GN=clpX PE=3 SV=1	13.903	11	4	4	4	424	46.4	10.79
A0A2S8D5N6	Aspartate carbamoyltransferase OS=Shigella dysenteriae OX=622 GN=pyrB PE=3 SV=1	13.127	11	3	4	3	311	34.4	10.77
D2A6V6	6-phosphogluconate dehydrogenase, decarboxylating OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=gnd PE=3 SV=1	11.571	10	4	4	4	468	51.3	8.63
A0A4P7TQY3	Bifunctional purine biosynthesis protein PurH OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=purH PE=3 SV=1	12.779	9	4	4	4	529	57.3	10.69
A0A3T2URR1	TDP-N-acetylglucosamine:lipid II N-acetylglucosaminyltransferase OS=Shigella flexneri OX=623 GN=wecF PE=3 SV=1	12.184	9	3	4	3	359	40.5	7.03
A0A658Z1V1	UPF0283 membrane protein YcjF OS=Shigella flexneri OX=623 GN=ycjF PE=3 SV=1	10.446	9	3	4	3	353	39.4	5.16
Q7UCQ9	Lipopolysaccharide assembly protein B OS=Shigella flexneri OX=623 GN=yciM PE=3 SV=1	10.104	8	3	4	3	389	44.4	8.25
A0A6N3RAZ0	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4958 PE=3 SV=1	9.895	7	3	4	3	428	47.7	7.93
A0A6N3RKR4	Iron-sulfur cluster binding protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0392 PE=4 SV=1	8.195	7	4	4	4	475	53	5.48
A0A2Y4XYI3	Esterase FrsA OS=Shigella flexneri OX=623 GN=frsA PE=3 SV=1	8.395	7	3	4	3	414	47	4.17
A0A380B6P9	PTS N-acetylglucosamine EIICBA component OS=Shigella flexneri OX=623 GN=nagE PE=4 SV=1	9.599	6	4	4	4	648	68.3	6.18
A0A6N3R842	Adenylosuccinate synthetase OS=Shigella flexneri 1485-80 OX=766155 GN=purA PE=3 SV=1	6.901	6	3	4	3	432	47.3	4.1
I6CVF4	Protease 4 OS=Shigella flexneri K-315 OX=766150 GN=sppA PE=3 SV=1	9.809	6	4	4	4	618	67.2	5.11
F5NZF4	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3556 PE=3 SV=1	5.34	5	3	4	3	493	53.7	4.26
A0A3T2UY26	Beta-glucosidase BglX OS=Shigella flexneri OX=623 GN=DK174_15745 PE=3 SV=1	12.128	5	4	4	4	765	83.5	6.58
Q0T0X5	Fructose-bisphosphate aldolase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fba PE=3 SV=1	5.523	4	2	4	2	387	42.7	4.12
A0A2S8DI33	Sec translocon accessory complex subunit YajC OS=Shigella boydii OX=621 GN=yajC PE=3 SV=1	8.448	26	3	3	3	110	11.9	6.76
A0A4P7TM74	30S ribosomal protein S21 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpsU PE=3 SV=1	2.555	25	2	3	2	71	8.5	4.22
D2AGH9	Major outer membrane lipoprotein Lpp OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=lpp PE=3 SV=1	9.193	24	2	3	2	78	8.3	7.37
F5P3C9	30S ribosomal protein S13 OS=Shigella flexneri K-227 OX=766147 GN=rpsM PE=3 SV=1	5.287	23	3	3	3	118	13.1	0
A0A6H1JH06	30S ribosomal protein S5 OS=Shigella flexneri OX=623 GN=rpsE PE=3 SV=1	10.757	22	3	3	3	167	17.6	8.54
A0A1Q8M8J0	50S ribosomal protein L7/L12 OS=Shigella boydii OX=621 GN=rpL7 PE=3 SV=1	7.616	21	3	3	3	121	12.3	7.19
A0A4P7TQT3	50S ribosomal protein L10 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpL10 PE=3 SV=1	8.102	19	3	3	3	165	17.7	7.54
D2AGV7	TPR_21 domain-containing protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yfgM PE=3 SV=1	15.142	18	3	3	3	206	22.1	8.2
A0A2S8D3C6	S-ribosylhomocysteine lyase OS=Shigella dysenteriae OX=622 GN=luxS PE=3 SV=1	9.213	18	3	3	3	171	19.4	4.89
I6D602	Protein-export membrane protein SecF OS=Shigella flexneri K-315 OX=766150 GN=secF PE=3 SV=1	18.831	17	3	3	3	304	33.2	9.98

I6D3P2	Tol-Pal system protein TolR OS=Shigella flexneri K-315 OX=766150 GN=tolR PE=3 SV=1	13.48	17	2	3	2	142	15.4	5.74
A0A1S9KGS8	Phosphatidylserine decarboxylase proenzyme OS=Shigella dysenteriae OX=622 GN=psd PE=3 SV=1	9.359	14	3	3	3	322	35.9	4.54
Q0SYU1	ATP synthase subunit delta OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=atpH PE=3 SV=1	6.554	14	3	3	3	177	19.3	5.11
A0A6N3QLC2	Universal stress protein G OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02439 PE=3 SV=1	5.655	14	2	3	2	142	15.9	3.42
P59132	30S ribosomal protein S4 OS=Shigella flexneri OX=623 GN=rpsD PE=3 SV=1	8.231	14	3	3	3	206	23.4	7.58
A0A6D2W4F9	Chaperone protein Skp OS=Shigella flexneri OX=623 GN=skp PE=3 SV=1	12.647	14	3	3	3	161	17.7	10.03
A0A379Z3I1	(Fe-S)-binding protein OS=Shigella flexneri OX=623 GN=ykgE PE=4 SV=1	8.025	13	3	3	3	239	26.1	3.28
A0A6N3QHQ0	Ferrochelatase OS=Shigella flexneri CDC 796-83 OX=945360 GN=hemH PE=3 SV=1	12.297	13	3	3	3	320	35.9	6.77
F5NPS2	Lipoprotein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0205 PE=3 SV=1	9.297	13	3	3	3	271	29.4	7.35
A0A658YTG8	Putative F0F1-type ATP synthase, subunit b OS=Shigella flexneri OX=623 GN=SAMEA3710514_00636 PE=4 SV=1	4.55	12	2	3	2	160	18.9	4.53
A0A2S4MTA8	Lactate utilization protein C OS=Shigella flexneri OX=623 GN=ykgG PE=4 SV=1	3.612	12	3	3	3	231	25.2	4.53
I6CBY6	Lipopolysaccharide heptosyltransferase II OS=Shigella flexneri K-315 OX=766150 GN=rfaF PE=4 SV=1	6.319	11	3	3	3	348	39	7.1
A0A2Y4XSL4	Peptidyl-prolyl cis-trans isomerase OS=Shigella flexneri OX=623 GN=ppiB PE=3 SV=1	5.811	11	2	3	2	164	18.2	1.82
A0A383JQI3	3-oxoacyl-[acyl-carrier-protein] reductase OS=Shigella flexneri OX=623 GN=fabG_1 PE=3 SV=1	5.928	11	3	3	3	244	25.5	4.53
Q0T6G6	UPF0194 membrane protein YbhG OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ybhG PE=3 SV=1	8.79	11	3	3	3	331	36.3	6.95
A0A1Q8MQJ7	Protein RecA OS=Shigella boydii OX=621 GN=recA PE=3 SV=1	12.906	10	3	3	3	353	38	9.07
A0A2S8DD79	Threonine/serine transporter TdcC OS=Shigella dysenteriae OX=622 GN=tdcC PE=3 SV=1	10.765	9	3	3	3	443	48.9	7.27
A0A383JTM9	Periplasmic glutamine-binding protein permease OS=Shigella flexneri OX=623 GN=fliY PE=3 SV=1	5.875	9	3	3	3	285	31	6.15
A0A2S8DKX6	Insulinase family protein OS=Shigella boydii OX=621 GN=yhjJ PE=3 SV=1	5.563	9	3	3	3	498	55.4	1.78
I6D6X5	Glutamate-1-semialdehyde 2,1-aminomutase OS=Shigella flexneri K-315 OX=766150 GN=hemL PE=3 SV=1	9.366	9	3	3	3	426	45.3	5.87
A2NHM3	If kappa light chain (Fragment) OS=Mus musculus OX=10090 GN=Igkc PE=1 SV=1	6.797	9	2	3	2	219	24.2	5.78
A0A658Z1Y3	D-lactate dehydrogenase OS=Shigella flexneri OX=623 GN=idhA PE=3 SV=1	11.908	9	3	3	3	329	36.5	7.86
A0A2S8DR37	L-arabinose-binding periplasmic protein OS=Shigella boydii OX=621 GN=araF PE=3 SV=1	6.448	9	3	3	3	329	35.6	3.63
Q0SYE6	2-amino-3-ketobutyrate coenzyme A ligase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=klb PE=3 SV=1	8.023	9	3	3	3	398	43.2	6.95
Q83MF6	Cell division protein FtsZ OS=Shigella flexneri OX=623 GN=ftsZ PE=3 SV=2	10.13	9	3	3	3	383	40.1	6.99
A0A2X2I0N0	Cell division protein DamX OS=Shigella flexneri OX=623 GN=damX PE=3 SV=1	13.572	8	3	3	3	428	46.1	8.99
Q0T4A8	Possible enzyme OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_1686 PE=4 SV=1	10.512	8	3	3	3	418	42.9	5.68
A0A380D5J7	Lipid A biosynthesis myristoyltransferase OS=Shigella flexneri OX=623 GN=msbB_2 PE=3 SV=1	6.41	8	3	3	3	324	38	2.34
I6D405	PhoH-like protein OS=Shigella flexneri K-315 OX=766150 GN=ybeZ PE=3 SV=1	5.702	8	3	3	3	346	39	1.91
A0A3Y2RP03	Lipopolysaccharide 3-alpha-galactosyltransferase (Fragment) OS=Shigella flexneri OX=623 GN=DTY77_07815 PE=4 SV=1	5.094	8	3	3	3	318	37.2	1.81
A0A2S8DJM8	Na+/H+ antiporter OS=Shigella boydii OX=621 GN=C5K23_27965 PE=3 SV=1	4.979	8	3	3	3	517	56.9	5.95
A0A2Y4XSH5	Periplasmic protein of mal regulon OS=Shigella flexneri OX=623 GN=malM PE=4 SV=1	5.026	8	2	3	2	306	31.9	6.59
A0A2S8DA81	Succinate--CoA ligase [ADP-forming] subunit beta OS=Shigella dysenteriae OX=622 GN=sucC PE=3 SV=1	8.513	7	3	3	3	388	41.4	6.76
A0A2S8E9Y4	Acid-resistance membrane protein OS=Shigella dysenteriae OX=622 GN=hdeD_2 PE=4 SV=1	4.407	7	3	3	3	190	20.9	3.46
Q83IY3	Bifunctional protein GlmU OS=Shigella flexneri OX=623 GN=glmU PE=3 SV=1	8.898	7	3	3	3	456	49.2	7.96
I6CH97	AMP nucleosidase OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3709 PE=4 SV=1	7.945	7	3	3	3	444	49.9	6.42
A0A6N3R6Z9	Lipopolysaccharide export system permease protein LptF OS=Shigella flexneri 1485-80 OX=766155 GN=yjgP PE=3 SV=1	9.539	7	2	3	2	335	36.6	8.92
I6D6Z7	Zinc metalloprotease OS=Shigella flexneri K-315 OX=766150 GN=rseP PE=3 SV=1	6.278	7	3	3	3	450	49.1	4.57
A0A2S8DBS7	Glycosyltransferase family 1 protein OS=Shigella dysenteriae OX=622 GN=mshA PE=4 SV=1	5.276	7	3	3	3	362	40.6	2.3
I6CGI0	Bifunctional glutathionylspermidine synthetase/amidase OS=Shigella flexneri K-315 OX=766150 GN=gsp PE=3 SV=1	7.119	7	3	3	3	619	70.5	5.01
F5NST7	Paraquat-inducible protein B OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1183 PE=4 SV=1	3.614	6	3	3	3	527	58.3	0
A0A2S8DRB8	Glucose-6-phosphate 1-dehydrogenase OS=Shigella boydii OX=621 GN=zwf PE=3 SV=1	6.301	6	3	3	3	491	55.7	3.86
A0A6N3QSE0	3-oxoacyl-[acyl-carrier-protein] synthase 2 OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00807 PE=3 SV=1	5.232	6	2	3	2	401	41.7	3.92
F5NVZ8	Outer membrane porin protein LC OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2311 PE=3 SV=1	8.614	6	3	3	2	360	39.7	5.24
I6D6W3	Poly(A) polymerase I OS=Shigella flexneri K-315 OX=766150 GN=pcnB PE=3 SV=1	5.697	6	3	3	3	454	52.5	1.95
A0A4P7TME5	Osmotic shock tolerance protein YggT OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=yggT PE=3 SV=1	3.417	5	1	3	1	188	21.1	2.2
A0A2S8DNR9	Methionine--tRNA ligase OS=Shigella boydii OX=621 GN=metG PE=3 SV=1	7.296	5	3	3	3	677	76.2	5.02
F5NZ29	Serine transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3429 PE=4 SV=1	7.708	5	2	3	2	429	46.9	5.51
A0A2S8DCH3	Lipid ABC transporter permease/ATP-binding protein (Fragment) OS=Shigella dysenteriae OX=622 GN=C5K18_12120 PE=4 SV=1	5.812	5	3	3	3	573	63.4	5.14
D2AAJ1	2,3-bisphosphoglycerate-independent phosphoglycerate mutase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=gpmM PE=3 SV=1	4.412	5	2	3	2	548	60.1	4.63

A0A6N3R9B1	PTS system, trehalose-specific IIBC component OS=Shigella flexneri 1485-80 OX=766155 GN=treP PE=4 SV=1	6.373	5	2	3	2	472	51.1	5.86
A0A2S8DEF0	Chaperone SurA OS=Shigella dysenteriae OX=622 GN=surA PE=3 SV=1	6.94	4	2	3	2	428	47.3	4.53
A0A4P7TPX8	Ferrichrome porin FhuA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=fhuA PE=3 SV=1	5.189	4	3	3	3	747	82.1	3.88
A0A6N3RQG4	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydD OS=Shigella flexneri 1485-80 OX=766155 GN=cydD PE=4 SV=1	3.647	4	3	3	3	588	65	1.94
I6CAS1	Phosphate transporter OS=Shigella flexneri K-315 OX=766150 GN=pitA PE=3 SV=1	5.064	4	2	3	2	499	53.4	4.29
F5NUC5	Tail-specific protease OS=Shigella flexneri K-227 OX=766147 GN=SFK227_1725 PE=3 SV=1	8.251	4	3	3	3	680	76.5	7.09
A0A658XZH7	Oxidoreductase OS=Shigella flexneri OX=623 GN=NCTC8524_01188 PE=4 SV=1	6.873	4	3	3	3	804	89.3	5.15
A0A6N3R6J7	Anaerobic C4-dicarboxylate transporter OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_4987 PE=3 SV=1	7.115	4	3	3	3	446	47.8	5.56
I6CMC4	Lysine-specific permease OS=Shigella flexneri K-315 OX=766150 GN=lysP PE=4 SV=1	5.251	4	2	3	2	489	53.5	7.21
A0A2S8DJM4	Translation initiation factor IF-2 OS=Shigella boydii OX=621 GN=infB PE=3 SV=1	5.504	3	3	3	3	890	97.3	2.65
A0A6L6UZB9	Ribonuclease E OS=Shigella flexneri OX=623 GN=rne PE=3 SV=1	6.882	3	3	3	3	1063	118.4	5.83
D2A780	Galactose-proton symporter OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=galP PE=3 SV=1	4.855	2	2	3	2	464	51	3.62
D2AD66	DNA-directed RNA polymerase subunit beta OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=rpoB PE=3 SV=1	6.476	2	2	3	2	1342	150.5	3.92
A0A2Y5A6X9	30S ribosomal protein S9 OS=Shigella flexneri OX=623 GN=rpsI PE=3 SV=1	6.052	26	2	2	2	85	9.7	2.5
A0A4V1CUA5	50S ribosomal protein L28 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rpmB PE=3 SV=1	4.727	26	2	2	2	78	9	5.56
A0A2Y4XXM5	Uncharacterized protein OS=Shigella flexneri OX=623 GN=SAMEA3710404_03077 PE=4 SV=1	2.714	25	1	2	1	87	9	0
I6CFZ0	50S ribosomal protein L27 OS=Shigella flexneri K-315 OX=766150 GN=rpmA PE=3 SV=1	5.255	25	2	2	2	85	9.1	5.18
I6CAR9	Universal stress protein OS=Shigella flexneri K-315 OX=766150 GN=uspA PE=3 SV=1	3.474	22	2	2	2	144	16.1	0
A0A2Y5BIQ5	Lipoprotein NlpD OS=Shigella flexneri OX=623 GN=nlpD_1 PE=4 SV=1	3.228	21	2	2	2	112	12.1	0
I6C9X6	30S ribosomal protein S14 OS=Shigella flexneri K-315 OX=766150 GN=rpsN PE=3 SV=1	5.208	19	2	2	2	96	11	3.47
A0A1Q8N7Q2	50S ribosomal protein L11 OS=Shigella dysenteriae OX=622 GN=rplK PE=3 SV=1	7.311	17	2	2	2	142	14.9	6.42
D2A8T7	Cytochrome d ubiquinol oxidase subunit III OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yhcB PE=4 SV=1	5.905	16	2	2	2	134	15.2	6.06
F5P3D3	50S ribosomal protein L17 OS=Shigella flexneri K-227 OX=766147 GN=rplQ PE=3 SV=1	4.666	15	2	2	2	127	14.4	4.41
A0A2S4N174	50S ribosomal protein L22 OS=Shigella flexneri OX=623 GN=rplV PE=3 SV=1	3.951	13	1	2	1	110	12.2	2.08
A0A2Y4YUV3	Peptidyl-prolyl cis-trans isomerase OS=Shigella flexneri OX=623 GN=fklB PE=3 SV=1	7.366	13	2	2	2	206	22.2	5.43
A0A383JXW9	UPF0145 protein YbjQ OS=Shigella flexneri OX=623 GN=ybjQ PE=3 SV=1	4.296	11	1	2	1	107	11.4	3.27
I6C9X3	50S ribosomal protein L14 OS=Shigella flexneri K-315 OX=766150 GN=rplN PE=3 SV=1	2.081	11	2	2	2	117	12.8	1.98
F5NQR5	Tol-Pal system protein TolQ OS=Shigella flexneri K-227 OX=766147 GN=tolQ PE=3 SV=1	3.278	11	2	2	2	210	23.3	0
A0A2S8DFP5	Cell division protein DedD OS=Shigella dysenteriae OX=622 GN=dedD PE=3 SV=1	4.751	10	2	2	2	220	22.9	2.43
Q5IPA5	Aerobic respiratory control protein (Fragment) OS=Shigella flexneri OX=623 GN=arcA PE=4 SV=1	5.283	10	2	2	2	182	21	2.18
I6CI71	Sigma-E factor regulatory protein rseB OS=Shigella flexneri K-315 OX=766150 GN=rseB PE=3 SV=1	6.432	10	2	2	2	318	35.7	4.66
A0A6N3QNZ8	Inorganic pyrophosphatase OS=Shigella flexneri CDC 796-83 OX=945360 GN=ppa PE=3 SV=1	5.662	10	2	2	2	176	19.7	4.01
F5P2M7	Uncharacterized protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4769 PE=4 SV=1	8.148	9	2	2	2	236	25.6	5.77
A0A1Q8M6D1	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase OS=Shigella boydii OX=621 GN=gpmA PE=3 SV=1	6.013	9	2	2	2	250	28.5	3.98
A0A2S8DXT0	High frequency lysogenization protein HflD homolog OS=Shigella boydii OX=621 GN=hflD PE=3 SV=1	4.004	9	2	2	2	213	22.9	4.46
A0A658YZ45	Glutamine ABC transporter ATP-binding protein OS=Shigella flexneri OX=623 GN=glnQ PE=3 SV=1	7.387	9	2	2	2	240	26.6	5.32
F5NXQ5	NAD dependent epimerase/dehydratase family protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2932 PE=3 SV=1	3.231	8	2	2	2	297	32.7	1.67
A0A6N3RIF3	Enoyl-[acyl-carrier-protein] reductase [NADH] OS=Shigella flexneri 1485-80 OX=766155 GN=fabI PE=3 SV=1	7.803	8	2	2	2	262	27.8	5.94
A0A3Y2RTA3	Translation initiation factor IF-3 OS=Shigella flexneri OX=623 GN=infC PE=3 SV=1	3.116	8	1	2	1	180	20.6	4.69
I6CXD2	UTP--glucose-1-phosphate uridylyltransferase OS=Shigella flexneri K-315 OX=766150 GN=galU PE=3 SV=1	6.376	8	2	2	2	302	33	4.33
Q0T5C6	Putative protease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=sohB PE=3 SV=1	3.942	8	2	2	2	296	33.4	2.79
Q0SZ45	Thiol:disulfide interchange protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dsbA PE=3 SV=1	2.983	8	2	2	2	208	23.1	3.97
F5P2C5	L-threonine 3-dehydrogenase OS=Shigella flexneri K-227 OX=766147 GN=tdh PE=3 SV=1	6.363	8	2	2	2	341	37.3	4.6
I6CJU1	Acetate kinase OS=Shigella flexneri K-315 OX=766150 GN=ackA PE=3 SV=1	10.273	8	2	2	2	400	43.3	6.14
A0A3T3S7B2	ParB/RepB/Spo0J family partition protein OS=Shigella flexneri OX=623 GN=sopB_2 PE=3 SV=1	7.385	7	2	2	2	326	36.8	5.2
A0A3T2USI5	Glucokinase OS=Shigella flexneri OX=623 GN=glk PE=3 SV=1	3.477	7	2	2	2	321	34.7	1.91
Q0SZY4	50S ribosomal protein L4 OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=rplD PE=3 SV=1	2.365	6	2	2	2	201	22.1	3.44
A0A380B6A5	BAX inhibitor (BI)-1/YccA family protein OS=Shigella flexneri OX=623 GN=ybhL_2 PE=3 SV=1	3.356	6	2	2	2	234	25.9	2.39
A0A3T6J5Y5	Involved in lipopolysaccharide biosynthesis OS=Shigella flexneri OX=623 GN=htrL PE=4 SV=1	5.182	6	2	2	2	285	33.5	2.24
A0A6N3QM36	Protein QmcA OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_01963 PE=3 SV=1	10.802	6	2	2	2	305	33.7	6.8

P0A6L6	N-acetylneuraminase lyase OS=Shigella flexneri OX=623 GN=nanA PE=3 SV=2	3.418	6	2	2	2	297	32.6	2.22
A0A6N3RE59	S-adenosylmethionine synthase OS=Shigella flexneri 1485-80 OX=766155 GN=metK PE=3 SV=1	6.003	6	2	2	2	384	41.9	4.36
F5NTG0	2-dehydro-3-deoxyphosphooctonate aldolase OS=Shigella flexneri K-227 OX=766147 GN=kdsA PE=3 SV=1	2.834	6	2	2	2	284	30.8	1.91
A0A2Y4B916	Outer membrane-specific lipoprotein transporter subunit LoLe OS=Shigella flexneri OX=623 GN=loLe PE=3 SV=1	5.491	6	2	2	2	412	45.1	4.81
A0A4P7TP07	Tol-Pal system protein TolB OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=tolB PE=3 SV=1	6.97	6	2	2	2	430	45.9	4.74
A0A2S8D993	H(+)/Cl(-) exchange transporter ClcA OS=Shigella dysenteriae OX=622 GN=clcA PE=3 SV=1	3.952	5	2	2	2	473	50.4	1.71
D2ACQ6	Sulfatase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yijP PE=4 SV=1	6.983	5	2	2	2	577	66.5	5.9
A0A2S8DRX8	Sulfate ABC transporter substrate-binding protein OS=Shigella boydii OX=621 GN=cysP PE=3 SV=1	3.228	5	2	2	2	338	37.7	1.91
A0A6N3RHJ8	Aminotransferase OS=Shigella flexneri 1485-80 OX=766155 GN=aspC PE=3 SV=1	4.31	5	2	2	2	396	43.6	5.11
I6CD34	Undecaprenyl-phosphate alpha-N-acetylglucosaminyl 1-phosphate transferase OS=Shigella flexneri K-315 OX=766150 GN=wecA PE=3 SV=1	2.595	5	2	2	2	367	40.9	0
Q0T2K3	NADH-quinone oxidoreductase subunit F OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nuoF PE=3 SV=1	4.218	5	2	2	2	445	49.4	2.39
A0A383K210	Lipopolysaccharide heptosyltransferase RfaC OS=Shigella flexneri OX=623 GN=rfaC PE=4 SV=1	3.779	5	2	2	2	330	36.7	3.5
A0A6N3RFX8	N-acetylmuramoyl-L-alanine amidase OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_3202 PE=4 SV=1	2.977	5	2	2	2	417	45.6	4.74
A0A4P7TKN2	O13/O129/O135 family O-antigen flippase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=wzx PE=4 SV=1	4.44	5	2	2	2	418	46.3	3.76
D2A8B1	UDP-N-acetylglucosamine 1-carboxyvinyltransferase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=murA PE=3 SV=1	4.631	5	2	2	2	419	44.8	4.89
Q0T2J7	Putative aminotransferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_2357 PE=4 SV=1	2.838	5	2	2	2	405	45.5	1.65
A0A2S8DI03	Signal recognition particle-docking protein FtsY (Fragment) OS=Shigella boydii OX=621 GN=C5K23_31365 PE=3 SV=1	4.263	5	2	2	2	486	53.2	2.78
Q0T3A5	Histidine biosynthesis bifunctional protein HisB OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hisB PE=3 SV=1	2.075	5	2	2	2	355	40.1	1.93
A0A0H2V2Y1	Inositol-1-monophosphatase OS=Shigella flexneri OX=623 GN=suhB PE=3 SV=1	6.601	4	2	2	2	267	29.1	5.02
A0A4P7TR77	Maltoporin OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=lambB PE=2 SV=1	2.864	4	2	2	2	446	50	1.67
A0A658YX65	Phosphopentomutase OS=Shigella flexneri OX=623 GN=deoB PE=3 SV=1	3.901	4	2	2	2	407	44.4	2.21
Q0SZ40	Protoporphyrin oxidase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hemG PE=3 SV=1	2.338	4	1	2	1	181	21.2	3.91
A0A2Y4XRM3	CTP synthase OS=Shigella flexneri OX=623 GN=pyrG PE=3 SV=1	4.292	4	2	2	2	545	60.4	2.38
Q0T7Q8	Aminoacyl-histidine dipeptidase (Peptidase D) OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=pepD PE=4 SV=1	3.825	4	2	2	2	485	52.9	2
A0A658Y016	Glucan biosynthesis protein G OS=Shigella flexneri OX=623 GN=mdoG PE=3 SV=1	2.688	4	2	2	2	500	56.6	0
A0A2S8DWY7	Lysine--tRNA ligase OS=Shigella boydii OX=621 GN=lysS PE=3 SV=1	3.913	4	2	2	2	505	57.6	2.11
F5P3W0	UDP-N-acetylmuramate--L-alanyl-gamma-D-glutamyl-meso-2,6-diaminoheptandioate ligase OS=Shigella flexneri K-227 OX=766147 GN=mpl PE=3 SV=1	4.478	4	2	2	2	457	49.9	4.12
A0A2Y4X2P5	Proline/glycine betaine transporter OS=Shigella flexneri OX=623 GN=proP_1 PE=4 SV=1	3.781	4	2	2	2	366	40.2	4.33
F5NS05	2-octaprenyl-3-methyl-6-methoxy-1,4-benzoquinol hydroxylase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0893 PE=3 SV=1	3.142	4	2	2	2	391	43	0
A0A4P7TTF4	Methionine import ATP-binding protein MetN OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=metN PE=3 SV=1	3.044	4	2	2	2	343	37.8	0
I6C193	Serine hydroxymethyltransferase OS=Shigella flexneri K-315 OX=766150 GN=glyA PE=3 SV=1	2.21	4	2	2	2	417	45.3	0
F5NPR9	Proline--tRNA ligase OS=Shigella flexneri K-227 OX=766147 GN=proS PE=3 SV=1	5.542	4	2	2	2	572	63.6	4.37
A0A3T2UUK6	Energy-dependent translational throttle protein EttA OS=Shigella flexneri OX=623 GN=yijk PE=3 SV=1	4.674	4	2	2	2	555	62.4	2.67
F5P3E9	3'(2),5'-biphosphate nucleotidase CysQ OS=Shigella flexneri K-227 OX=766147 GN=cysQ PE=3 SV=1	1.743	4	1	2	1	230	25.4	1.92
Q0T2P1	sn-glycerol-3-phosphate permease OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=glpT PE=3 SV=1	4.32	4	2	2	2	452	50.4	3.61
A0A6N3QJK5	AsmA protein OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04099 PE=4 SV=1	4.692	4	2	2	2	617	68.9	2.27
Q0T3B2	Putative enzyme of sugar metabolism OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=SFV_2075 PE=4 SV=1	2.746	4	1	2	1	274	29.7	1.98
A0A3U1D8Q6	C4-dicarboxylic acid transporter DauA OS=Shigella flexneri OX=623 GN=dauA PE=3 SV=1	4.525	4	2	2	2	559	59.4	2.96
A0A6N3RBT1	GTP-binding protein TypA/BipA OS=Shigella flexneri 1485-80 OX=766155 GN=typA PE=4 SV=1	4.227	3	2	2	2	607	67.4	2.19
I6CVL0	Phenylalanine--tRNA ligase beta subunit OS=Shigella flexneri K-315 OX=766150 GN=pheT PE=3 SV=1	7.042	3	2	2	2	795	87.3	4.69
A0A2S8DD51	Cell division protein ZipA OS=Shigella dysenteriae OX=622 GN=zipA PE=3 SV=1	4.968	3	1	2	1	328	36.5	5.43
D2ADW1	Putative transport protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yegH PE=3 SV=1	2.127	3	2	2	2	549	62.1	0
A0A6N3QUN6	Zinc/cadmium/mercury/lead-transporting ATPase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00074 PE=3 SV=1	5.542	3	2	2	2	732	76.7	4.18
D2AF97	Carbon starvation family protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yjiY PE=3 SV=1	5.001	3	2	2	2	721	77.8	4.76
A0A6G5Z4D2	Ferric aerobactin receptor LutA OS=Shigella flexneri OX=623 GN=iutA PE=3 SV=1	2.189	3	2	2	2	731	80.9	0
I6CB04	Alpha-1,4 glucan phosphorylase OS=Shigella flexneri K-315 OX=766150 GN=malP PE=3 SV=1	3.646	3	2	2	2	797	90.5	2.17
F5NRH8	Efflux pump membrane transporter OS=Shigella flexneri K-227 OX=766147 GN=SFK227_0715 PE=3 SV=1	5.46	2	2	2	2	1049	113.6	1.77

Q83SN2	Protein translocase subunit SecA OS=Shigella flexneri OX=623 GN=secA PE=3 SV=1	5.683	2	2	2	2	901	102	2.3
Q0SYG1	3-deoxy-D-manno-octulosonic acid transferase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=kdtA PE=3 SV=1	2.996	2	2	2	2	425	47.2	0
F5P300	Glucose-1-phosphate adenyltransferase OS=Shigella flexneri K-227 OX=766147 GN=glgC PE=3 SV=1	2.698	2	1	2	1	431	48.7	3.91
A0A2S8DL49	NADH-quinone oxidoreductase subunit N OS=Shigella boydii OX=621 GN=nuoN PE=3 SV=1	3.558	2	1	2	1	485	52	4.09
F5NXM7	NADH-quinone oxidoreductase subunit L OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2904 PE=3 SV=1	3.933	2	1	2	1	611	66.1	5.45
A0A6N3RCV1	Alanine--tRNA ligase OS=Shigella flexneri 1485-80 OX=766155 GN=alaS PE=3 SV=1	4.929	2	2	2	2	876	96	4.11
A0A658YTU3	Response regulator of RpoS OS=Shigella flexneri OX=623 GN=hnr PE=4 SV=1	1.519	2	1	2	1	337	37.3	1.66
A0A6N3QPX8	Prophage P4 integrase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_03306 PE=4 SV=1	2.687	2	1	2	1	422	48.2	4.35
A0A658Y003	DNA-directed RNA polymerase subunit OS=Shigella flexneri OX=623 GN=rpoC PE=3 SV=1	2.789	1	2	2	2	1407	155.1	1.73
A0A6N3QTX3	Sensor histidine kinase RcsC OS=Shigella flexneri CDC 796-83 OX=945360 GN=rscC PE=3 SV=1	1.124	1	1	2	1	949	106.5	3.29
A0A1Q8M4D3	30S ribosomal protein S18 OS=Shigella boydii OX=621 GN=rpsR PE=3 SV=1	7.976	24	1	1	1	75	9	3.65
A0A2Y5Q6A1	YmiA family putative membrane protein OS=Shigella flexneri OX=623 GN=ymlA PE=4 SV=1	1.581	21	1	1	1	42	4.9	0
A0A2Y5BFL6	Uncharacterized protein OS=Shigella flexneri OX=623 GN=SAMEA3710404_02763 PE=4 SV=1	0.832	18	1	1	1	141	15.8	0
Q83R19	Cold shock-like protein CspC OS=Shigella flexneri OX=623 GN=cspC PE=3 SV=3	2.113	17	1	1	1	69	7.4	0
A0A2S8DPE3	Replication protein (Fragment) OS=Shigella boydii OX=621 GN=C5K23_18305 PE=3 SV=1	0.837	17	1	1	1	117	13.3	0
A0A3Y2RQZ3	TRAP transporter small permease OS=Shigella flexneri OX=623 GN=DTY77_11710 PE=4 SV=1	1.381	16	1	1	1	170	18.6	0
A0A1Q8M2L6	50S ribosomal protein L30 OS=Shigella boydii OX=621 GN=rpmD PE=3 SV=1	1.665	15	1	1	1	59	6.5	1.6
A0A6N3QU25	Thiol peroxidase OS=Shigella flexneri CDC 796-83 OX=945360 GN=tpx PE=3 SV=1	1.978	14	1	1	1	168	17.8	0
F5P1R3	Multidrug resistance protein D OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4445 PE=4 SV=1	1.864	12	1	1	1	378	40.3	0
A0A6N3RRG2	Outer membrane lipoprotein RcsF OS=Shigella flexneri 1485-80 OX=766155 GN=rscF PE=3 SV=1	3.724	12	1	1	1	117	12.4	2.47
A0A6D2X1F2	30S ribosomal protein S19 OS=Shigella flexneri OX=623 GN=rpsS PE=3 SV=1	2.832	12	1	1	1	92	10.4	2.09
F5P090	50S ribosomal protein L21 OS=Shigella flexneri K-227 OX=766147 GN=rplU PE=3 SV=1	3.143	12	1	1	1	103	11.5	1.87
A0A3T2UZW2	Gamma-glutamylcyclotransferase family protein OS=Shigella flexneri OX=623 GN=DK174_18910 PE=3 SV=1	3.154	12	1	1	1	113	12.9	1.68
Q0T0G7	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yqjD PE=3 SV=1	0.942	10	1	1	1	101	11.1	0
A0A2S8DEU7	UPF0325 protein YaeH OS=Shigella dysenteriae OX=622 GN=yaeH PE=3 SV=1	2.287	9	1	1	1	128	15.1	2.29
A0A3T2UV43	Fumarate reductase subunit D OS=Shigella flexneri OX=623 GN=frdD PE=3 SV=1	4.159	9	1	1	1	119	13.1	3.12
Q5IP18	Heat shock protein (Fragment) OS=Shigella flexneri OX=623 GN=grpE PE=3 SV=1	7.889	9	1	1	1	158	17.6	3.86
A0A2S8DHH6	30S ribosomal protein S15 OS=Shigella dysenteriae OX=622 GN=rpsO PE=3 SV=1	1.137	8	1	1	1	89	10.3	1.97
I6CGK0	Uncharacterized protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_3457 PE=4 SV=1	1.27	8	1	1	1	191	19.8	0
A0A6N3RC82	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SFI48580_3360 PE=4 SV=1	2.555	8	1	1	1	117	13.8	2.01
I6D700	UDP-3-O-(3-hydroxymyristoyl)glucosamine N-acyltransferase OS=Shigella flexneri K-315 OX=766150 GN=lpxD PE=3 SV=1	3.779	8	1	1	1	341	36	0
A0A6N3QMX0	Serine transporter OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_02214 PE=4 SV=1	2.555	7	1	1	1	134	14.6	2.25
A0A4P7TQF0	50S ribosomal protein L9 OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=rplI PE=3 SV=1	3.311	7	1	1	1	149	15.8	2.97
D2AD60	Protein translocase subunit SecE OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=secE PE=3 SV=1	2.625	7	1	1	1	127	13.6	2.6
A0A2S8D6V8	Transcription elongation factor GreA OS=Shigella dysenteriae OX=622 GN=greA PE=3 SV=1	3.167	7	1	1	1	158	17.7	0
A0A6N3QEL1	50S ribosomal protein L19 OS=Shigella flexneri CDC 796-83 OX=945360 GN=rplS PE=3 SV=1	1.774	7	1	1	1	115	13.1	0
A0A6N3RJ42	Glutaredoxin OS=Shigella flexneri 1485-80 OX=766155 GN=ydhD PE=3 SV=1	1.261	7	1	1	1	115	12.9	1.76
A0A2Y4XEM5	sn-glycerol-3-phosphate dehydrogenase subunit C OS=Shigella flexneri OX=623 GN=glpC PE=4 SV=1	2.081	7	1	1	1	470	51.8	0
I6C8L3	Fumarate reductase subunit C OS=Shigella flexneri K-315 OX=766150 GN=frdC PE=3 SV=1	1.872	7	1	1	1	120	13.6	1.65
I6CI69	RNA polymerase sigma factor OS=Shigella flexneri K-315 OX=766150 GN=rpoE PE=3 SV=1	3.731	7	1	1	1	151	17.1	2.93
I6CFV1	Putative stringent starvation protein A OS=Shigella flexneri K-315 OX=766150 GN=sspA PE=3 SV=1	5.489	7	1	1	1	212	24.3	3.32
A0A2Y4XVQ2	Outer membrane protein assembly complex subunit YfgL OS=Shigella flexneri OX=623 GN=yfgL PE=4 SV=1	3.693	7	1	1	1	184	20.1	3.24
I6CCX4	Sec-independent protein translocase protein TatB OS=Shigella flexneri K-315 OX=766150 GN=tatB PE=3 SV=1	2.564	6	1	1	1	171	18.5	2.75
A0A2S8DK85	Single-stranded DNA-binding protein (Fragment) OS=Shigella boydii OX=621 GN=C5K23_27980 PE=3 SV=1	1.272	6	1	1	1	156	16.5	1.82
Q0SY57	ATP-dependent protease subunit HsIV OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=hsIV PE=3 SV=1	2.796	6	1	1	1	176	19.1	2.48
A0A2S8E8N3	Superoxide dismutase OS=Shigella dysenteriae OX=622 GN=sodB PE=3 SV=1	2.067	6	1	1	1	193	21.3	1.92
A0A6N3QJ9	UPF0301 protein YqgE OS=Shigella flexneri CDC 796-83 OX=945360 GN=yqgE PE=3 SV=1	4.345	6	1	1	1	187	20.7	2.92
Q0SY18	Transcription termination/antitermination protein NusG OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=nusG PE=3 SV=1	2.037	6	1	1	1	181	20.5	0
I6CP87	UPF0265 protein YeeX OS=Shigella flexneri K-315 OX=766150 GN=yeeX PE=3 SV=1	1.092	6	1	1	1	109	12.8	0
P0A7Z3	Ribose-5-phosphate isomerase A OS=Shigella flexneri OX=623 GN=rpiA PE=3 SV=1	2.225	5	1	1	1	219	22.8	2.16

A0A4P7TS42	Elongation factor P OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=efp PE=3 SV=1	1.653	5	1	1	1	188	20.6	0
A0A6N3RN87	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ OS=Shigella flexneri 1485-80 OX=766155 GN=fabZ PE=3 SV=1	2.029	5	1	1	1	151	17	1.81
A0A1Q8NKE7	Phosphoheptose isomerase OS=Shigella dysenteriae OX=622 GN=gmhA PE=3 SV=1	3.627	5	1	1	1	192	20.8	2.63
I6CGW6	Inner membrane protein ygaP OS=Shigella flexneri K-315 OX=766150 GN=ygaP PE=4 SV=1	1.117	5	1	1	1	174	18.7	0
A0A2S8DMQ5	MobA/MobL family protein (Fragment) OS=Shigella boydii OX=621 GN=C5K23_21360 PE=4 SV=1	1.027	5	1	1	1	117	13.9	0
F5NZU7	2-oxoglutarate reductase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3700 PE=3 SV=1	2.09	5	1	1	1	410	44.2	0
A0A4V1CTV8	Disulfide bond formation protein B OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=dsbB PE=3 SV=1	2.725	5	1	1	1	176	20.1	2.18
A0A2S8D7U4	Phosphoribosylaminoimidazole-succinocarboxamide synthase OS=Shigella dysenteriae OX=622 GN=purC PE=3 SV=1	2.653	5	1	1	1	237	27	2.69
Q2EUS5	Ribonuclease 3 (Fragment) OS=Shigella flexneri OX=623 GN=rnc PE=3 SV=1	1.793	5	1	1	1	178	19.9	0
A0A1Q8NZW8	DNA-binding transcriptional regulator PhoP OS=Shigella dysenteriae OX=622 GN=phoP PE=4 SV=1	1.691	5	1	1	1	223	25.5	1.72
Q0T811	Putative transport system permease protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yaeE PE=3 SV=1	1.482	5	1	1	1	212	22.6	0
Q0SZA9	Triosephosphate isomerase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=tpiA PE=3 SV=1	2.868	5	1	1	1	255	27	3.16
Q0T0F5	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yhaR PE=3 SV=1	1.044	5	1	1	1	150	16.4	0
A0A3T2UX13	Probable transcriptional regulatory protein YeeN OS=Shigella flexneri OX=623 GN=yeeN PE=3 SV=1	1.362	5	1	1	1	238	25.8	1.93
A0A6N3QV54	YciO family OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_00295 PE=4 SV=1	1.465	5	1	1	1	218	24.5	0
Q0SZX1	Uncharacterized protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yheO PE=4 SV=1	4.793	5	1	1	1	240	26.8	2.41
I6D2B3	Amino ABC transporter, permease, 3-TM region, His/Glu/Gln/Arg/opine family domain protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_0742 PE=3 SV=1	2.294	5	1	1	1	219	24.3	2.37
A0A6N3RJ20	Pyridoxal kinase PdxY OS=Shigella flexneri 1485-80 OX=766155 GN=pxdY PE=3 SV=1	3.736	5	1	1	1	286	31.2	2.2
P0A8A1	Probable transcriptional regulatory protein YebC OS=Shigella flexneri OX=623 GN=yebC PE=3 SV=1	3.738	4	1	1	1	246	26.4	2.64
A0A2X2HWV5	Phospholipid ABC transporter ATP-binding protein MlaF OS=Shigella boydii OX=621 GN=mlaF PE=4 SV=1	2.194	4	1	1	1	269	29.1	0
A0A6N3RMT3	ABC transporter family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_2023 PE=4 SV=1	1.947	4	1	1	1	202	22.7	0
A0A6N3RFE9	Adenosylcobinamide-GDP ribazoletransferase OS=Shigella flexneri 1485-80 OX=766155 GN=cobS PE=3 SV=1	1.701	4	1	1	1	247	26.4	1.66
A0A2S8DBY7	Iron ABC transporter ATP-binding protein FetA OS=Shigella dysenteriae OX=622 GN=C5K18_13250 PE=4 SV=1	1.965	4	1	1	1	225	25.4	1.71
I6CY67	UPF0227 protein YcFP OS=Shigella flexneri K-315 OX=766150 GN=ycFP PE=3 SV=1	1.964	4	1	1	1	180	21.2	1.65
A0A383JXR7	Lactoylglutathione lyase OS=Shigella flexneri OX=623 GN=gloA PE=3 SV=1	0.894	4	1	1	1	135	14.9	0
I6CBX6	Lipopolysaccharide heptosyltransferase III, putative OS=Shigella flexneri K-315 OX=766150 GN=SFK315_4420 PE=4 SV=1	4.077	4	1	1	1	254	28	3.1
A0A6N3QRI2	ATP synthase epsilon chain OS=Shigella flexneri CDC 796-83 OX=945360 GN=atpC PE=3 SV=1	1.122	4	1	1	1	139	15	1.62
D2AEJ0	RNA chaperone ProQ OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=proQ PE=3 SV=1	1.985	4	1	1	1	232	25.8	0
A0A6N3QIM1	Polysaccharide deacetylase OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04901 PE=4 SV=1	2.776	4	1	1	1	280	32.9	1.7
A0A6N3RR48	Uncharacterized protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0546 PE=3 SV=1	2.649	4	1	1	1	259	28.2	2.39
A0A088CC14	Uncharacterized protein OS=Shigella phage POCJ13 OX=1498227 PE=4 SV=1	1.07	4	1	1	1	237	27.3	0
I6CFX7	Lipopolysaccharide export system protein LptC OS=Shigella flexneri K-315 OX=766150 GN=lptC PE=3 SV=1	2.687	4	1	1	1	191	21.7	2.38
I6CB18	Transcriptional regulatory protein ompR OS=Shigella flexneri K-315 OX=766150 GN=ompR PE=4 SV=1	3.284	4	1	1	1	239	27.4	1.9
A0A2S8DUK8	Methionine aminopeptidase OS=Shigella boydii OX=621 GN=map PE=3 SV=1	2.491	4	1	1	1	264	29.3	2.04
A0A6N3RLN2	PotC OS=Shigella flexneri 1485-80 OX=766155 GN=potC PE=3 SV=1	2.963	4	1	1	1	264	29.1	2.19
A0A3Y2RTQ4	Hydroxymethylbilane synthase OS=Shigella flexneri OX=623 GN=DTY77_17110 PE=3 SV=1	0.99	4	1	1	1	313	33.9	0
A0A383JXU2	Alkaline phosphatase isozyme conversion aminopeptidase OS=Shigella flexneri OX=623 GN=iap PE=4 SV=1	7.87	4	1	1	1	345	37.9	3.41
A0A3T3S0R7	Glutamine ABC transporter periplasmic protein OS=Shigella flexneri OX=623 GN=glnH PE=3 SV=1	1.654	4	1	1	1	248	27.2	1.64
Q0T3Y0	Fumarate and nitrate reduction Regulatory protein OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=fnr PE=4 SV=1	2.347	4	1	1	1	250	27.9	0
Q2EW05	Galactokinase OS=Shigella flexneri OX=623 GN=galK PE=3 SV=1	2.726	4	1	1	1	382	41.4	0
A0A6L6UVT0	Lipid A biosynthesis lauroyltransferase OS=Shigella flexneri OX=623 GN=lpXL PE=3 SV=1	5.23	4	1	1	1	306	35.4	3.89
I6CGG2	Biopolymer transport protein ExbB OS=Shigella flexneri K-315 OX=766150 GN=exbB PE=3 SV=1	1.861	4	1	1	1	230	24.8	1.73
A0A380B899	Replication protein P OS=Shigella flexneri OX=623 GN=DTY77_04360 PE=4 SV=1	1.018	4	1	1	1	233	26.5	0
Q0SY71	Glutamate decarboxylase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=gadA PE=3 SV=1	4.794	4	1	1	1	466	52.7	3.08
I6C9W3	50S ribosomal protein L3 OS=Shigella flexneri K-315 OX=766150 GN=rplC PE=3 SV=1	1.181	4	1	1	1	209	22.2	0
A0A1W2MD82	Protease HtpX OS=Shigella flexneri OX=623 GN=htpX PE=3 SV=1	4.001	4	1	1	1	293	31.9	2.26
A0A237F1C5	Arginine ABC transporter ATP-binding protein ArtP OS=Shigella boydii OX=621 GN=artP PE=3 SV=1	2.595	4	1	1	1	242	27	2.69
Q0SZ34	NAD(P)H-flavin reductase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=ubiB PE=4 SV=1	1.467	4	1	1	1	244	27.6	0
A0A2S8DH97	Pyridoxine/pyridoxamine 5'-phosphate oxidase OS=Shigella dysenteriae OX=622 GN=pxdH PE=3 SV=1	1.558	4	1	1	1	218	25.6	2
A0A0H2V3T2	L-lactate permease OS=Shigella flexneri OX=623 GN=SF4369 PE=3 SV=1	0.952	4	1	1	1	300	32.5	0

A0A380B7X8	Carbonic anhydrase OS=Shigella flexneri OX=623 GN=can PE=3 SV=1	2.574	4	1	1	1	220	25.1	0
A0A6L6UWY8	Flavodoxin family protein OS=Shigella flexneri OX=623 GN=mdaB PE=4 SV=1	1.021	4	1	1	1	193	22	0
I6D6P9	Ribosomal RNA small subunit methyltransferase H OS=Shigella flexneri K-315 OX=766150 GN=mraW PE=3 SV=1	1.363	4	1	1	1	313	34.9	0
A0A2Y5A0I3	Repressor protein for FtsI OS=Shigella flexneri OX=623 GN=sufI PE=4 SV=1	3.477	4	1	1	1	370	40.8	2.41
A0A2Y4ELK4	Phosphate import ATP-binding protein PstB OS=Shigella flexneri OX=623 GN=pstB PE=3 SV=1	2.523	4	1	1	1	257	29	2.23
A0A379ZT68	Putative ATP-binding protein yghT OS=Shigella flexneri OX=623 GN=yghT PE=4 SV=1	0.961	3	1	1	1	230	25.4	0
F5P2B3	Lipopolysaccharide core biosynthesis protein rfaG OS=Shigella flexneri K-227 OX=766147 GN=SFK227_4654 PE=4 SV=1	3.796	3	1	1	1	374	42.5	2.18
A0A2S8DKM1	Putrescine-binding periplasmic protein OS=Shigella boydii OX=621 GN=potD PE=3 SV=1	2.565	3	1	1	1	348	38.9	2.74
P69985	Yop proteins translocation protein T OS=Yersinia pseudotuberculosis serotype I (strain IP32953) OX=273123 GN=yscT PE=3 SV=1	0.93	3	1	1	1	261	28.4	0
F5NWP2	Glucose-1-phosphate thymidyltransferase OS=Shigella flexneri K-227 OX=766147 GN=SFK227_2560 PE=3 SV=1	2.487	3	1	1	1	292	32.5	2.13
I6CG52	tRNA-modifying protein YgfZ OS=Shigella flexneri K-315 OX=766150 GN=ygfZ PE=3 SV=1	1.361	3	1	1	1	326	36.1	0
A0A6N3RH36	Zinc transport protein ZntB OS=Shigella flexneri 1485-80 OX=766155 GN=zntB PE=3 SV=1	2.889	3	1	1	1	327	36.5	2.28
F5NVI8	Lipid A biosynthesis myristoyltransferase OS=Shigella flexneri K-227 OX=766147 GN=lpzM PE=3 SV=1	2.297	3	1	1	1	331	38.3	1.86
I6CGI6	1-acyl-sn-glycerol-3-phosphate acyltransferase OS=Shigella flexneri K-315 OX=766150 GN=plsC PE=3 SV=1	1.767	3	1	1	1	211	23.4	1.65
A0A0F3PAC1	Shigella flexneri OspC family protein OS=Orientia tsutsugamushi str. TA716 OX=1359175 GN=OTSTA716_0900 PE=4 SV=1	1.679	3	1	1	1	214	23.9	0
A0A2S8DU80	Agmatinase OS=Shigella boydii OX=621 GN=speB PE=3 SV=1	2.975	3	1	1	1	306	33.5	2.37
D2ADS0	Orotidine 5'-phosphate decarboxylase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=pyrF PE=3 SV=1	0.85	3	1	1	1	245	26.3	1.85
D2A6V5	Chain length determinant protein OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=SFxv_2324 PE=4 SV=1	2.398	3	1	1	1	337	37.9	1.62
D2A6N5	Chaperone protein hchA OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=hchA PE=4 SV=1	2.39	3	1	1	1	283	31.2	1.99
A0A6N3QLJ7	Magnesium transport protein CorA OS=Shigella flexneri CDC 796-83 OX=945360 GN=corA PE=3 SV=1	3.319	3	1	1	1	316	36.6	0
A0A658YYG3	Nitrite extrusion protein 2 OS=Shigella flexneri OX=623 GN=narU_1 PE=4 SV=1	2.037	3	1	1	1	412	43.8	0
A0A2S8D3M5	tRNA pseudouridine synthase D OS=Shigella dysenteriae OX=622 GN=truD PE=3 SV=1	2.12	3	1	1	1	349	39	2.05
A0A658Y2W3	Mannose-6-phosphate isomerase OS=Shigella flexneri OX=623 GN=manA PE=3 SV=1	2.629	3	1	1	1	391	42.9	1.87
A0A6N3QWE0	33 kDa chaperonin OS=Shigella flexneri CDC 796-83 OX=945360 GN=hsI0 PE=3 SV=1	1.495	3	1	1	1	294	32.7	0
A0A2S8DMY7	1-deoxy-D-xylulose-5-phosphate synthase (Fragment) OS=Shigella boydii OX=621 GN=dxs PE=3 SV=1	1.417	3	1	1	1	295	32.1	1.92
A0A2S8DDB6	Peptide ABC transporter ATP-binding protein SapD OS=Shigella dysenteriae OX=622 GN=sapD PE=4 SV=1	0.847	3	1	1	1	330	37.6	0
I6D6M8	L-ribulose-5-phosphate 4-epimerase OS=Shigella flexneri K-315 OX=766150 GN=araD PE=3 SV=1	1.46	3	1	1	1	231	25.5	0
I6CVA3	L-serine dehydratase OS=Shigella flexneri K-315 OX=766150 GN=SFK315_1603 PE=3 SV=1	1.473	3	1	1	1	432	46.6	0
A0A6G5YYG3	Lipoprotein-releasing system ATP-binding protein LoLD OS=Shigella flexneri OX=623 GN=loLD PE=3 SV=1	1.527	3	1	1	1	233	25.5	0
D2AJ35	Delta-aminolevulinic acid dehydratase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=hemb PE=3 SV=1	2.14	3	1	1	1	335	36.9	2.6
QOT0X8	Putative actin OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=yggE PE=4 SV=1	1.537	3	1	1	1	235	25.4	2.46
A0A384KK93	Putative UDP-glucose:(Galactosyl) LPS alpha1,2-glucosyltransferase OS=Shigella flexneri OX=623 GN=waaJ PE=4 SV=1	0.93	3	1	1	1	337	40	0
F5NZS7	Thiol:disulfide interchange protein OS=Shigella flexneri K-227 OX=766147 GN=SFK227_3680 PE=3 SV=1	0.879	3	1	1	1	236	25.6	0
A0A6N3R946	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin) OS=Shigella flexneri 1485-80 OX=766155 GN=ispG PE=3 SV=1	1.528	3	1	1	1	372	40.7	1.64
A0A6N3QLB6	Peptidase T OS=Shigella flexneri CDC 796-83 OX=945360 GN=pepT PE=3 SV=1	2.617	3	1	1	1	408	44.8	1.99
A0A3Y2RM52	Phosphoglucosamine mutase OS=Shigella flexneri OX=623 GN=glmM PE=3 SV=1	4.87	3	1	1	1	445	47.5	2.59
A0A6N3RMN1	Aldose 1-epimerase OS=Shigella flexneri 1485-80 OX=766155 GN=galM PE=3 SV=1	1.24	3	1	1	1	346	38.2	0
I6CG61	Aminomethyltransferase OS=Shigella flexneri K-315 OX=766150 GN=gcvT PE=3 SV=1	2.609	3	1	1	1	347	38.2	2.7
I6D5T1	Gamma-glutamyl phosphate reductase OS=Shigella flexneri K-315 OX=766150 GN=proA PE=3 SV=1	2.996	3	1	1	1	417	44.5	1.79
A0A2S8D6C3	Proline-specific permease ProY OS=Shigella dysenteriae OX=622 GN=C5K18_23740 PE=4 SV=1	3.058	3	1	1	1	457	50.1	0
A0A658YXY5	Aminopeptidase B OS=Shigella flexneri OX=623 GN=pepB PE=3 SV=1	1.52	3	1	1	1	427	46.2	1.7
A0A658Y1R7	Putative dimethyl sulfoxide reductase chain ynfF OS=Shigella flexneri OX=623 GN=ynfF PE=4 SV=1	1.164	3	1	1	1	605	66.9	0
D2A949	Bifunctional protein FoID OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=foID PE=3 SV=1	1.039	3	1	1	1	288	30.9	1.75
A0A379ZZF8	23S rRNA (uracil(1939)-C(5))-methyltransferase RlmD OS=Shigella flexneri OX=623 GN=rmaA PE=3 SV=1	1.232	3	1	1	1	433	48	1.81
A0A2Y4Y231	Lipid A-core, surface polymer ligase OS=Shigella flexneri OX=623 GN=DTY77_19500 PE=4 SV=1	2.342	3	1	1	1	402	46.1	2.06
A0A6N3RFW5	Penicillin-binding protein 7 OS=Shigella flexneri 1485-80 OX=766155 GN=pbpG PE=3 SV=1	1.84	3	1	1	1	293	32.5	0
A0A2S8DPX0	Flagellar regulator flk OS=Shigella boydii OX=621 GN=flk PE=3 SV=1	2.443	3	1	1	1	331	36.7	2.16
F5P0Q9	Maltose/maltodextrin import ATP-binding protein MalK OS=Shigella flexneri K-227 OX=766147 GN=malK PE=3 SV=1	1.477	3	1	1	1	371	41	0
A0A6N3QJ05	Macrolide-specific efflux protein MacA OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04622 PE=3 SV=1	1.829	3	1	1	1	371	40.6	0
D2AIK6	Putative electron transfer flavoprotein subunit ygcQ OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=ygcQ PE=4 SV=1	1.15	3	1	1	1	297	31.6	0

A0A6N3RRT0	Probable peptidoglycan glycosyltransferase FtsW OS=Shigella flexneri 1485-80 OX=766155 GN=ftsW PE=3 SV=1	3.063	3	1	1	1	372	41.2	2.01
A0A3T2UU59	Metalloprotease PmbA OS=Shigella flexneri OX=623 GN=DK174_07880 PE=3 SV=1	2.477	3	1	1	1	450	48.4	2.05
A0A658Y5Z7	L-1,2-propanediol oxidoreductase OS=Shigella flexneri OX=623 GN=fucO PE=4 SV=1	1.936	3	1	1	1	382	40.4	0
I6CI34	Histidine--tRNA ligase OS=Shigella flexneri K-315 OX=766150 GN=hisS PE=3 SV=1	1.553	3	1	1	1	424	47.1	2.07
A0A1Q8M994	ADP-L-glycero-D-manno-heptose-6-epimerase OS=Shigella boydii OX=621 GN=rfad PE=3 SV=1	1.269	3	1	1	1	310	34.9	0
D2AAC5	Putative lipase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=yhjY PE=4 SV=1	0.885	3	1	1	1	234	26.2	0
F5NQ21	Cell division protein FtsQ OS=Shigella flexneri K-227 OX=766147 GN=ftsQ PE=3 SV=1	1.366	3	1	1	1	276	31.4	1.84
A0A2Y4XSE7	Uncharacterized protein OS=Shigella flexneri OX=623 GN=SAMEA3710404_02312 PE=4 SV=1	0.85	3	1	1	1	316	36.5	0
A0A3T3S2E7	Pyruvate kinase OS=Shigella flexneri OX=623 GN=pyk PE=3 SV=1	1.297	3	1	1	1	480	51.3	0
Q83RC9	Dihydroneopterin reductase OS=Shigella flexneri OX=623 GN=folM PE=3 SV=1	0.854	3	1	1	1	240	26.3	0
A0A6N3REG1	NADH-quinone oxidoreductase subunit H OS=Shigella flexneri 1485-80 OX=766155 GN=nuoH PE=3 SV=1	1.502	2	1	1	1	325	36.2	0
A0A2S8DHD8	AI-2E family transporter OS=Shigella dysenteriae OX=622 GN=C5K18_00760 PE=3 SV=1	1.246	2	1	1	1	370	39.9	1.6
I6CAX8	HTH-type transcriptional regulator gntR OS=Shigella flexneri K-315 OX=766150 GN=gntR PE=4 SV=1	1.297	2	1	1	1	331	36.3	2.05
F5NZS4	Peptide chain release factor 2 OS=Shigella flexneri K-227 OX=766147 GN=prfB PE=3 SV=1	1.626	2	1	1	1	293	32.8	0
D2ADZ6	Glucose-6-phosphate isomerase OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=pgi PE=3 SV=1	3.463	2	1	1	1	549	61.5	2.83
Q9AJW3	ORF94 OS=Shigella flexneri OX=623 PE=4 SV=1	0.875	2	1	1	1	258	28.5	0
Q7UBI8	Bifunctional protein HldE OS=Shigella flexneri OX=623 GN=hldE PE=3 SV=2	2.042	2	1	1	1	477	51.1	0
A0A0H2VZX0	Thiol:disulfide interchange protein DsbD OS=Shigella flexneri OX=623 GN=dsbD PE=3 SV=1	5.026	2	1	1	1	565	61.8	2.57
F5NRC2	Curved DNA-binding protein OS=Shigella flexneri K-227 OX=766147 GN=cbpA PE=3 SV=1	0.84	2	1	1	1	306	34.4	0
A0A6G5Z373	Sensor protein QseC OS=Shigella flexneri OX=623 GN=qseC PE=4 SV=1	1.655	2	1	1	1	449	50.3	0
A0A2S8D9I9	D-alanine--D-alanine ligase OS=Shigella dysenteriae OX=622 GN=ddl_2 PE=3 SV=1	2.84	2	1	1	1	364	39.3	2.55
A0A0H2V3I9	Putative amino acid/amine transport protein OS=Shigella flexneri OX=623 GN=yifK PE=4 SV=1	1.782	2	1	1	1	417	45.3	1.8
A0A6N3RFD0	Dihydrofolate synthase/folypolyglutamase synthase OS=Shigella flexneri 1485-80 OX=766155 GN=folC PE=3 SV=1	1.532	2	1	1	1	422	45.3	0
A0A6N3QN40	Glutamate--tRNA ligase OS=Shigella flexneri CDC 796-83 OX=945360 GN=gltx PE=3 SV=1	3.145	2	1	1	1	471	53.8	2.58
A0A6G5YUI2	Pantothenate synthetase OS=Shigella flexneri OX=623 GN=panC PE=3 SV=1	1.201	2	1	1	1	283	31.5	0
A0A380AZ64	Phosphoenolpyruvate--glycerone phosphotransferase OS=Shigella flexneri OX=623 GN=yecC PE=3 SV=1	2.525	2	1	1	1	472	51.4	2.32
A0A6N3RLZ1	Cytochrome d ubiquinol oxidase, subunit II OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_0759 PE=3 SV=1	2.47	2	1	1	1	379	42.4	1.81
A0A379ZAE3	Dihydroxy-acid dehydratase OS=Shigella flexneri OX=623 GN=ilvD PE=3 SV=1	2.198	2	1	1	1	616	65.5	2.51
A0A6G5Z350	Serine-type D-Ala-D-Ala carboxypeptidase OS=Shigella flexneri OX=623 GN=dacB PE=3 SV=1	2.646	2	1	1	1	477	51.8	2.39
A0A6N3RBC1	EnvC murein hydrolase OS=Shigella flexneri 1485-80 OX=766155 GN=yibP PE=4 SV=1	1.285	2	1	1	1	393	43.7	0
A0A2Y4Y1C7	Fumarate hydratase class I OS=Shigella flexneri OX=623 GN=fumA PE=3 SV=1	1.711	2	1	1	1	548	60.3	0
A0A6G5Z3B5	Biotin carboxylase OS=Shigella flexneri OX=623 GN=accC PE=4 SV=1	3.046	2	1	1	1	449	49.3	2.13
F6GSD9	Cytochrome c-type biogenesis protein OS=Shigella flexneri OX=623 GN=ccmH PE=3 SV=1	1.014	2	1	1	1	350	39.1	0
A0A2S8D9Z2	Major capsid protein OS=Shigella dysenteriae OX=622 GN=C5K18_16185 PE=4 SV=1	0.924	2	1	1	1	352	39.2	0
D2ACS1	Vitamin B12 transporter BtuB OS=Shigella flexneri serotype X (strain 2002017) OX=591020 GN=btuB PE=3 SV=1	1.172	2	1	1	1	629	70.1	0
A0A658Z2S5	GMP synthase OS=Shigella flexneri OX=623 GN=guaA PE=4 SV=1	1.104	2	1	1	1	525	58.7	0
I6CB41	Tryptophan--tRNA ligase OS=Shigella flexneri K-315 OX=766150 GN=trpS PE=3 SV=1	1.058	2	1	1	1	319	35.9	0
I6CMG4	Inner membrane protein YejM OS=Shigella flexneri K-315 OX=766150 GN=yejM PE=4 SV=1	1.891	2	1	1	1	586	67.2	0
A0A3T2V110	Aspartate--tRNA ligase OS=Shigella flexneri OX=623 GN=aspS PE=3 SV=1	2.64	2	1	1	1	590	65.8	0
A0A6N3QF78	Metal-dependent hydrolase involved in phosphonate metabolism OS=Shigella flexneri CDC 796-83 OX=945360 GN=SGF_04684 PE=4 SV=1	0.979	2	1	1	1	378	42	0
P0A6A2	Probable protein kinase UbiB OS=Shigella flexneri OX=623 GN=ubiB PE=3 SV=1	1.11	2	1	1	1	546	63.2	0
A0A6N3RA69	Proton/glutamate-aspartate symporter OS=Shigella flexneri 1485-80 OX=766155 GN=glrT PE=3 SV=1	1.191	2	1	1	1	437	47.1	1.71
I6CE82	Fumarate hydratase class I OS=Shigella flexneri K-315 OX=766150 GN=fumB PE=3 SV=1	2.428	2	1	1	1	548	60.1	2.5
A0A3T2UTG1	UvrABC system protein C OS=Shigella flexneri OX=623 GN=uvrC PE=3 SV=1	1.299	2	1	1	1	610	68.2	1.74
A0A3T2UQ06	Penicillin-binding protein activator LpoA OS=Shigella flexneri OX=623 GN=lpoA PE=3 SV=1	2.3	2	1	1	1	675	72.6	0
A0A658Y4L9	Exopolyphosphatase OS=Shigella flexneri OX=623 GN=ppx PE=3 SV=1	2.128	2	1	1	1	513	58.1	0
A0A2S8DX13	Aromatic amino acid transporter AroP OS=Shigella boydii OX=621 GN=C5K23_02025 PE=4 SV=1	1.878	2	1	1	1	457	49.7	1.76
A0A4V1CTY5	ATP-dependent Clp protease ATP-binding subunit ClpA OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=clpA PE=3 SV=1	1.225	2	1	1	1	758	84.2	0
A0A658YRM6	Peptidase, M48 family OS=Shigella flexneri OX=623 GN=SAMEA3710514_00010 PE=4 SV=1	2.959	2	1	1	1	487	53.9	2.19



A0A2S8DTV4	Phosphoenolpyruvate synthase OS=Shigella boydii OX=621 GN=ppsA PE=3 SV=1	1.241	2	1	1	1	792	87.4	0
A0A2S8D986	SelB translation factor OS=Shigella dysenteriae OX=622 GN=C5K18_18765 PE=4 SV=1	1.383	2	1	1	1	614	68.8	0
A0A4P7TS84	Probable cytosol aminopeptidase OS=Shigella flexneri serotype 5a (strain M90T) OX=1086030 GN=pepA PE=3 SV=1	1.909	2	1	1	1	503	54.8	1.62
F5NPQ4	Lipid-A-disaccharide synthase OS=Shigella flexneri K-227 OX=766147 GN=lpxB PE=3 SV=1	0.914	2	1	1	1	382	42.4	0
F5NVB6	Glutamate/gamma-aminobutyrate antiporter OS=Shigella flexneri K-227 OX=766147 GN=gadC PE=3 SV=1	1.401	2	1	1	1	511	55.1	2.23
Q83PW3	Fe(2+) transporter FeoB OS=Shigella flexneri OX=623 GN=feoB PE=3 SV=4	1.258	2	1	1	1	773	84.4	1.73
Q0T0J7	DNA primase OS=Shigella flexneri serotype 5b (strain 8401) OX=373384 GN=dnaG PE=3 SV=1	3.312	2	1	1	1	581	65.6	1.99
A0A2S8DBJ8	Diguanylate cyclase OS=Shigella dysenteriae OX=622 GN=C5K18_12670 PE=4 SV=1	1.789	2	1	1	1	646	73.3	1.98
A0A3T2UZX6	Aldehyde dehydrogenase EutE OS=Shigella flexneri OX=623 GN=DK174_19145 PE=4 SV=1	1.153	1	1	1	1	467	49	1.86
A0A658YSH2	Dehydrogenase OS=Shigella flexneri OX=623 GN=yrfF PE=3 SV=1	0.964	1	1	1	1	711	79.5	0
A0A2S8D9Y1	AAA domain-containing protein OS=Shigella dysenteriae OX=622 GN=C5K18_16210 PE=4 SV=1	0.844	1	1	1	1	507	57.7	0
I6CBQ2	DNA gyrase subunit B OS=Shigella flexneri K-315 OX=766150 GN=gyrB PE=3 SV=1	2.188	1	1	1	1	804	89.9	0
I6CBM2	Glutamine-fructose-6-phosphate aminotransferase [isomerizing] OS=Shigella flexneri K-315 OX=766150 GN=glmS PE=3 SV=1	1.57	1	1	1	1	609	66.9	2.01
I6CCW2	Trk system potassium uptake protein OS=Shigella flexneri K-315 OX=766150 GN=SFK315_4180 PE=3 SV=1	1.405	1	1	1	1	463	50.7	0
Q83Q93	Biosynthetic arginine decarboxylase OS=Shigella flexneri OX=623 GN=speA PE=3 SV=2	2.022	1	1	1	1	662	74.3	2.25
A0A2S8DE29	Low affinity potassium transport system protein kup OS=Shigella dysenteriae OX=622 GN=trkD PE=3 SV=1	1.347	1	1	1	1	587	65.6	0
A0A2Y5I422	Diguanylate cyclase OS=Shigella flexneri OX=623 GN=yfgF_2 PE=4 SV=1	1.443	1	1	1	1	505	57.7	0
I6CI73	Elongation factor 4 OS=Shigella flexneri K-315 OX=766150 GN=lepA PE=3 SV=1	1.668	1	1	1	1	599	66.6	1.65
A0A3T2UXW2	Efflux pump membrane transporter OS=Shigella flexneri OX=623 GN=DK174_15180 PE=3 SV=1	0.834	1	1	1	1	1037	111.4	0
A0A658XY46	Glucose dehydrogenase OS=Shigella flexneri OX=623 GN=gcd PE=3 SV=1	1.03	1	1	1	1	796	86.7	0
A0A6N3RGX9	Reverse transcriptase family protein OS=Shigella flexneri 1485-80 OX=766155 GN=SF148580_5106 PE=4 SV=1	0.889	1	1	1	1	550	64	0
A0A2S8D9R8	Penicillin-binding protein 1B OS=Shigella dysenteriae OX=622 GN=mrCB PE=3 SV=1	2.291	1	1	1	1	840	93.8	1.95
A0A3T2V040	Formate dehydrogenase-N subunit alpha OS=Shigella flexneri OX=623 GN=fdnG PE=3 SV=1	0.951	1	1	1	1	1016	112.5	0
A0A380AKI1	Phage tail protein OS=Shigella flexneri OX=623 GN=DTY77_05925 PE=4 SV=1	0.923	1	1	1	1	693	73.7	1.63
A0A3T2UZZ4	Glucans biosynthesis glucosyltransferase H OS=Shigella flexneri OX=623 GN=mdoH PE=3 SV=1	1.799	1	1	1	1	847	96.9	1.82
A0A2Y3Y011	ATPase OS=Shigella flexneri OX=623 GN=tiba_1 PE=4 SV=1	0.986	1	1	1	1	863	91.1	0

## Appendix E – Whole genome sequencing full data set

### Appx. E.1: All mutations found in *AsdhC::kan<sup>R</sup>* Keio mutant

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
3538370	3538372	3	GCC -> CAA	Substitution	97.1% -> 97.2%	A -> Q	gntX CDS	130	GCC -> CAA	Substitution
3538415	3538417	3	CCG -> TAT	Substitution	96.2% -> 96.6%	P -> Y	gntX CDS	175	CCG -> TAT	Substitution
3538382	3538384	3	CAT -> TGA	Substitution	94.6% -> 94.7%		gntX CDS	142		Truncation
3538451	3538452	2	GC -> TG	Substitution	94.6% -> 94.7%	A -> W	gntX CDS	211	GCG -> TGG	Substitution
3538424	3538429	5	CAAAGA -> TGGTGC	Substitution	94.4% -> 94.8%	QR -> WC	gntX CDS	184	CAA,AGA -> TGG,TGC	Substitution
3538437	3538440	4	CGGT -> TCTC	Substitution	94.3% -> 94.6%	TV -> IS	gntX CDS	197	ACG,GTT -> ATC,TCT	Substitution
3538410	3538413	4	AACC -> GTGG	Substitution	93.4% -> 93.8%	KP -> SG	gntX CDS	170	AAA,CCG -> AGT,GGG	Substitution
3538359	3538368	10	GTGGATTACC -> CACATGGGTA	Substitution	92.1% -> 92.6%	CGLP -> SHGY	gntX CDS	119	TGT,GGA,TTA,CCC -> TCA,CAT,GGG,TAC	Substitution
3538431	3538435	5	TGGTC -> GCAAT	Substitution	91.9% -> 92.7%	LV -> RN	gntX CDS	191	CTG,GTC -> CGC,AAT	Substitution
3538443	3538446	4	CCGA -> TTCC	Substitution	91.8% -> 93.5%	AD -> VP	gntX CDS	203	GCC,GAC -> GTT,CCC	Substitution
2310527	2310532	6	GACCCT -> TGTAGG	Substitution	89.1% -> 89.6%	RV -> PT	rcsC CDS	2824	AGG,GTC -> CCT,ACA	Substitution
2310873	2310878	6	AGAATC -> CCCGGA	Substitution	88.1% -> 89.3%	MIL -> IPG	rcsC CDS	2478	ATG,ATT,CTG -> ATT,CCG,GGG	Substitution
3539751	3539752	2	GT -> CC	Substitution	88.0% -> 88.5%					
2310860	2310863	4	ATCA -> CAGG	Substitution	87.0% -> 87.9%	DD -> DL	rcsC CDS	2493	GAT,GAT -> GAC,CTG	Substitution
3538454	3538459	6	CCGCCG -> GATAAT	Substitution	85.4% -> 88.9%	PP -> DN	gntX CDS	214	CCG,CCG -> GAT,AAT	Substitution

3538389	3538408	20	CCTGCGGTCGCTGCCTGCAA -> TGACTACAATAATTAGTTTT	Substitution	85.0% -> 86.6%	PCGRCLQ -> LTTHSF	gntX CDS	149	CCC,TGC,GGT,CGC,TGC,CTG,CAA -> CTG,ACT,ACA,ATA,ATT,AGT,TTT	Substitution
3538461	3538462	2	TA -> AG	Substitution	85.0% -> 86.4%		gntX CDS	221		Truncation
2310561	2310565	4	ATCAC -> TCTAG	Substitution	82.5% -> 84.7%	VI -> LE	rcsC CDS	2791	GTG,ATA -> CTA,GAA	Substitution
2310548	2310550	3	CAG -> AGT	Substitution	80.4% -> 80.7%	L -> T	rcsC CDS	2806	CTG -> ACT	Substitution
2310551	2310558	8	CGTCTGTT -> TCCTATAC	Substitution	78.7% -> 80.0%	KQT -> SIG	rcsC CDS	2798	AAA,CAG,ACG -> AGT,ATA,GGA	Substitution
2310849	2310852	4	TTAA -> AACT	Substitution	75.5% -> 80.0%	IN -> KF	rcsC CDS	2504	ATT,AAC -> AAG,TTC	Substitution
3539736	3539739	4	CGCC -> GATA	Substitution	68.8% -> 86.0%					
2310842	2310846	5	ACGCC -> GAATA	Substitution	68.0% -> 71.1%	RR -> LF	rcsC CDS	2510	CGG,CGT -> CTA,TTC	Substitution
2310870	2310871	1	AC -> GAT	Insertion	67.8% -> 76.6%		rcsC CDS	2485		Frame Shift
3539728	3539729	2	TT -> CC	Substitution	67.7% -> 71.4%					
3538466	3538467	2	CC -> TT	Substitution	66.7% -> 72.7%	P -> L	gntX CDS	226	CCG -> TTG	Substitution
2310537	2310544	7	GCATATAA -> AGCTGCTT	Substitution	62.7% -> 72.0%	LYA -> KQL	rcsC CDS	2812	TTA,TAT,GCC -> AAG,CAG,CTC	Substitution
3539725	3539726	2	TG -> CC	Substitution	60.0% -> 68.0%					
2310837	2310840	4	AGCA -> CTAG	Substitution	53.8% -> 63.2%	LL -> SR	rcsC CDS	2516	TTG,CTG -> TCT,AGG	Substitution
2310567	2310579	12	TCCAGCGTTACCG -> GAATAGGAACTTC	Substitution	47.8% -> 67.2%	PVTLD -> RSSYS	rcsC CDS	2777	CCG,GTA,ACG,CTG,GAT -> CGA,AGT,TCC,TAT,TCT	Substitution
2310581	2310595	14	CTTCGACAGGCAGCT -> GAATAGGAACTTCAA	Substitution	36.4% -> 60.7%	SCLSK -> LKFLF	rcsC CDS	2761	AGC,TGC,CTG,TCG,AAG -> TTG,AAG,TTC,CTA,TTC	Substitution
3797101	3797104	3	CGGC -> GCGA	Substitution	33.4% -> 34.2%	AD -> SH	waaB CDS	394	GCC,GAC -> TCG,CAC	Substitution
3797097	3797098	2	TA -> AG	Substitution	33.2% -> 33.4%	Y -> L	waaB CDS	400	TAT -> CTT	Substitution

3797093	3797094	2	AT -> G	Deletion	33.1% -> 33.3%		waaB CDS	404		Frame Shift
3797087	3797088	2	TG -> CC	Substitution	32.6% -> 33.3%	A -> G	waaB CDS	410	GCA -> GGG	Substitution
3797111	3797115	5	GATAT -> CCCTG	Substitution	27.4% -> 32.1%	YI -> SG	waaB CDS	383	TAT,ATC -> TCA,GGG	Substitution
3538469	3538470	2	CT -> TC	Substitution	25.0% -> 40.0%	L -> S	gntX CDS	229	CTT -> TCT	Substitution
3797118	3797120	3	TCA -> ATG	Substitution	24.7% -> 26.0%	AE -> AI	waaB CDS	378	GCT,GAA -> GCC,ATA	Substitution
3797080	3797085	6	AGCTGA -> GCACCT	Substitution	24.5% -> 29.5%	ISS -> KVP	waaB CDS	413	ATC,AGC,TCT -> AAG,GTG,CCT	Substitution
2310567	2310572	6	TCCAGC -> GAATAG	Substitution	13.0% -> 33.3%	TLD -> TYS	rcsC CDS	2784	ACG,CTG,GAT -> ACC,TAT,TCT	Substitution
2310581	2310587	7	CTTCGAC -> GAATAGG	Substitution	12.9% -> 40.0%	LSK -> LLF	rcsC CDS	2769	CTG,TCG,AAG -> CTC,CTA,TTC	Substitution
3538422	3538422	1	G -> C	SNP (transversion)	98.70%	W -> S	gntX CDS	182	TGG -> TCG	Substitution
3538386	3538386	1	T -> A	SNP (transversion)	98.40%	L -> H	gntX CDS	146	CTT -> CAT	Substitution
2719426	2719426	1	T -> C	SNP (transition)	98.20%					
3538376	3538376	1	C -> T	SNP (transition)	98.20%	H -> Y	gntX CDS	136	CAC -> TAC	Substitution
3538419	3538420	2	CC -> AG	Substitution	98.20%	P -> Q	gntX CDS	179	CCC -> CAG	Substitution
3538357	3538357	1	A -> T	SNP (transversion)	97.50%	Q -> H	gntX CDS	117	CAA -> CAT	Substitution
2310535	2310535	1	C -> G	SNP (transversion)	96.20%	E -> Q	rcsC CDS	2821	GAG -> CAG	Substitution
3538448	3538448	1	T -> G	SNP (transversion)	91.60%	Y -> D	gntX CDS	208	TAT -> GAT	Substitution
2310856	2310856	1	G -> A	SNP (transition)	88.40%	P -> S	rcsC CDS	2500	CCG -> TCG	Substitution
3539734	3539734	1	A -> C	SNP (transversion)	84.90%					
2310866	2310866	1	C -> G	SNP (transversion)	84.50%		rcsC CDS	2490	GTG -> GTC	None

3539731	3539731	1	T -> G	SNP (transversion)	82.10%					
2310547	2310547	1	T -> A	SNP (transversion)	81.30%	T -> S	rcsC CDS	2809	ACG -> TCG	Substitution
2310858	2310857	0	+C	Insertion	67.60%		rcsC CDS	2499		Frame Shift
3539031	3539031	1	G -> C	SNP (transversion)	66.70%	A -> P	nfuA CDS	49	GCA -> CCA	Substitution
3538490	3538490	1	T -> C	SNP (transition)	42.90%	S -> P	gntX CDS	250	TCC -> CCC	Substitution
4076057	4076057	1	A -> G	SNP (transition)	42.70%		fdoH CDS	63	CCT -> CCC	None
3538658	3538658	1	C -> A	SNP (transversion)	40.00%	P -> T	gntX CDS	418	CCG -> ACG	Substitution
3797108	3797108	1	G -> A	SNP (transition)	34.80%		waaB CDS	390	ACC -> ACT	None
4076055	4076055	1	T -> G	SNP (transversion)	33.60%	Q -> P	fdoH CDS	65	CAG -> CCG	Substitution
2310835	2310835	1	C -> T	SNP (transition)	33.30%	A -> T	rcsC CDS	2521	GCA -> ACA	Substitution
3538645	3538645	1	T -> G	SNP (transversion)	33.30%	D -> E	gntX CDS	405	GAT -> GAG	Substitution
3539487	3539487	1	A -> G	SNP (transition)	30.00%	N -> D	nfuA CDS	505	AAC -> GAC	Substitution
3991413	3991413	1	A -> C	SNP (transversion)	28.90%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
3538642	3538642	1	C -> G	SNP (transversion)	28.60%	S -> R	gntX CDS	402	AGC -> AGG	Substitution
3991428	3991428	1	C -> G	SNP (transversion)	25.30%	A -> G	uvrD CDS	86	GCG -> GGG	Substitution
4076055	4076055	1	T -> C	SNP (transition)	25.00%	Q -> R	fdoH CDS	65	CAG -> CGG	Substitution

**Appx E.2: All mutations found in *ΔargP::kan<sup>R</sup>* Keio mutant**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
3053964	3053965	2	GC -> TT	Substitution	98.8% -> 98.9%	A -> L	argP CDS	853	GCG -> TTG	Substitution
3053982	3053984	3	CAC -> ACA	Substitution	98.3% -> 98.4%	H -> T	argP CDS	871	CAC -> ACA	Substitution
3053220	3053221	2	CA -> GG	Substitution	98.3% -> 98.4%	Q -> G	argP CDS	109	CAG -> GGG	Substitution
3053235	3053236	2	CT -> GC	Substitution	98.0% -> 98.1%	L -> A	argP CDS	124	CTG -> GCG	Substitution
3053224	3053228	4	GCATT -> TGCTA	Substitution	97.2% -> 97.8%	RI -> LL	argP CDS	113	CGC,ATT -> CTG,CTA	Substitution
3053212	3053215	4	CCGT -> GGCG	Substitution	97.1% -> 97.2%	AV -> GR	argP CDS	101	GCC,GTC -> GGG,CGC	Substitution
3053116	3053117	2	AA -> TT	Substitution	96.2% -> 96.3%	K -> I	argP CDS	5	AAA -> ATT	Substitution
3053199	3053201	3	ATT -> CGC	Substitution	95.8% -> 95.9%	I -> R	argP CDS	88	ATT -> CGC	Substitution
3053119	3053122	4	GCCC -> CGGG	Substitution	95.6% -> 95.8%	RP -> PG	argP CDS	8	CGC,CCG -> CCG,GGG	Substitution
3053262	3053266	5	TTGGT -> AAACG	Substitution	95.5% -> 96.0%	LV -> KR	argP CDS	151	TTG,GTG -> AAA,CGG	Substitution
3053910	3053914	5	CTCTA -> GGGAT	Substitution	95.4% -> 95.6%	LY -> GI	argP CDS	799	CTC,TAC -> GGG,ATC	Substitution
3053133	3053137	4	ACATT -> CCTGC	Substitution	94.6% -> 95.5%	TL -> PA	argP CDS	22	ACA,TTA -> CCT,GCA	Substitution
3053932	3053938	7	CTGAAAG -> GAAGTTC	Substitution	94.4% -> 94.7%	PES -> RSS	argP CDS	821	CCT,GAA,AGC -> CGA,AGT,TCC	Substitution
3053169	3053171	3	GGA -> ACT	Substitution	93.5% -> 93.6%	G -> T	argP CDS	58	GGA -> ACT	Substitution
3053869	3053870	2	AA -> CG	Substitution	92.2% -> 95.0%	E -> A	argP CDS	758	GAA -> GCG	Substitution
3053127	3053130	4	TACA -> CGTC	Substitution	91.8% -> 92.0%	YR -> RR	argP CDS	16	TAC,AGA -> CGT,CGA	Substitution
3053283	3053288	6	CGCCCG -> AATGTC	Substitution	91.7% -> 93.1%	RP -> NV	argP CDS	172	CGC,CCG -> AAT,GTC	Substitution
3053241	3053252	9	AATATGTTCCGGG -> CACGTAGAAAGC	Substitution	91.6% -> 92.9%	NMFG -> HVES	argP CDS	130	AAT,ATG,TTC,GGG -> CAC,GTA,GAA,AGC	Substitution
3053925	3053930	5	TTTGCT -> CTATTC	Substitution	90.7% -> 95.1%	FA -> LF	argP CDS	814	TTT,GCT -> CTA,TTC	Substitution
3053940	3053951	11	CGCATGATGCGT -> TATTCTCTAGAA	Substitution	90.6% -> 92.9%	RMMR -> YSLE	argP CDS	829	CGC,ATG,ATG,CGT -> TAT,TCT,CTA,GAA	Substitution
3053919	3053923	4	CACCG -> AAGTT	Substitution	90.0% -> 94.2%	HR -> KF	argP CDS	808	CAC,CGC -> AAG,TTC	Substitution
3053971	3053980	9	TCGATTATGG -> AGCTCCAGCC	Substitution	89.9% -> 91.3%	LDYG -> QLQP	argP CDS	860	CTC,GAT,TAT,GGT -> CAG,CTC,CAG,CCT	Substitution
3053290	3053297	7	CGGAACAA -> GCTACTGG	Substitution	89.4% -> 92.7%	TEQ -> SYW	argP CDS	179	ACG,GAA,CAA -> AGC,TAC,TGG	Substitution

3053181	3053185	5	GCGGC -> TTTTG	Substitution	89.2% -> 89.5%		argP CDS	70		Truncation
3053145	3053148	3	CTGG -> GTTCCT	Insertion	88.7% -> 92.1%		argP CDS	34		Frame Shift
3053898	3053906	9	CAACGACGG -> TCTTCTAAT	Substitution	88.6% -> 89.8%	QRR -> SSN	argP CDS	787	CAA,CGA,CGG -> TCT,TCT,AAT	Substitution
3053187	3053192	6	CAAAAG -> AGCTCA	Substitution	88.0% -> 88.4%	QK -> SS	argP CDS	76	CAA,AAG -> AGC,TCA	Substitution
3053139	3053143	4	CAGGC -> GTTCGA	Insertion	87.9% -> 88.3%		argP CDS	28		Frame Shift
3053151	3053158	6	GCGGTGAT -> TCTCTAGA	Substitution	87.8% -> 90.2%	AVI -> SLE	argP CDS	40	GCG,GTG,ATA -> TCT,CTA,GAA	Substitution
3053866	3053867	2	GT -> CA	Substitution	87.3% -> 87.5%	G -> A	argP CDS	755	GGT -> GCA	Substitution
3053953	3053962	9	AAGTCACTGA -> GTATAGGAAC	Substitution	86.1% -> 90.1%	KVTD -> SIGT	argP CDS	842	AAA,GTC,ACT,GAT -> AGT,ATA,GGA,ACT	Substitution
3053268	3053276	8	CGTACCGTA -> TGCTGACCC	Substitution	86.1% -> 87.5%		argP CDS	157		Truncation
3053872	3053879	8	TGATTGAC -> ATCGCCTT	Substitution	84.0% -> 85.6%	LID -> HRL	argP CDS	761	CTG,ATT,GAC -> CAT,CGC,CTT	Substitution
3053299	3053311	13	GGCAAAAAGTCT -> CTATCTGGACAAG	Substitution	76.7% -> 83.6%	GQKLL -> AIWTR	argP CDS	188	GGG,CAA,AAA,CTG,CTG -> GCT,ATC,TGG,ACA,AGG	Substitution
3053889	3053893	5	GGGCT -> TCTTG	Substitution	75.6% -> 82.5%		argP CDS	778		Truncation
3053895	3053896	2	TT -> CGAG	Insertion	70.3% -> 76.1%		argP CDS	784		Frame Shift
3053846	3053847	2	CG -> GT	Substitution	22.2% -> 40.0%		argP CDS	735		Truncation
3053846	3053863	17	CGAGAAAGAGCTGGCCAG -> GTATCGCCGCTCCCGATT	Substitution	20.0% -> 71.4%	IEKELAS -> MYRRSRF	argP CDS	735	ATC,GAG,AAA,GAG,CTG,GCC,AGC -> ATG,TAT,CGC,CGC,TCC,CGA,TTC	Substitution
3053316	3053322	7	CTGCTGC -> AACGCAA	Substitution	100.00%	LLR -> NAS	argP CDS	205	CTG,CTG,CGC -> AAC,GCA,AGC	Substitution
3053260	3053260	1	T -> A	SNP (transversion)	100.00%	L -> Q	argP CDS	149	CTG -> CAG	Substitution
3053217	3053218	2	TC -> A	Deletion	99.70%		argP CDS	106		Frame Shift
2400028	2400036	9	=-TGCGGCAGC	Deletion	99.60%	AAA ->	lrhA CDS	85	GCT,GCC,GCA ->	Deletion
2098363	2098363	1	(A)9 -> (A)8	Deletion (tandem repeat)	99.60%		wbbJ CDS	203		Frame Shift
3053256	3053255	0	=+T	Insertion	99.50%		argP CDS	145		Frame Shift

3053917	3053917	1	G -> T	SNP (transversion)	99.20%	W -> L	argP CDS	806	TGG -> TTG	Substitution
3053280	3053281	2	CC -> AT	Substitution	99.20%	P -> M	argP CDS	169	CCG -> ATG	Substitution
3053207	3053207	1	A -> C	SNP (transversion)	99.10%	Q -> H	argP CDS	96	CAA -> CAC	Substitution
3053232	3053232	1	C -> G	SNP (transversion)	98.90%	Q -> E	argP CDS	121	CAA -> GAA	Substitution
3053173	3053174	2	TT -> CA	Substitution	98.90%	F -> S	argP CDS	62	TTT -> TCA	Substitution
3053166	3053166	1	C -> G	SNP (transversion)	98.70%	R -> G	argP CDS	55	CGA -> GGA	Substitution
3053160	3053160	1	C -> A	SNP (transversion)	98.70%	R -> S	argP CDS	49	CGT -> AGT	Substitution
3053203	3053204	2	CA -> AG	Substitution	98.60%	T -> K	argP CDS	92	ACA -> AAG	Substitution
3053278	3053278	1	C -> G	SNP (transversion)	98.50%	P -> R	argP CDS	167	CCG -> CGG	Substitution
3053163	3053164	2	GA -> AT	Substitution	98.50%	E -> I	argP CDS	52	GAA -> ATA	Substitution
3053908	3053908	1	T -> A	SNP (transversion)	98.10%	M -> K	argP CDS	797	ATG -> AAG	Substitution
2719426	2719426	1	T -> C	SNP (transition)	98.10%					
3053880	3053880	1	T -> C	SNP (transition)	96.30%		argP CDS	769	TTA -> CTA	None
3053883	3053883	1	A -> T	SNP (transversion)	95.70%	T -> S	argP CDS	772	ACG -> TCG	Substitution
3053314	3053314	1	C -> A	SNP (transversion)	95.20%	A -> E	argP CDS	203	GCA -> GAA	Substitution
3053194	3053195	2	TG -> GC	Substitution	95.00%	L -> R	argP CDS	83	CTG -> CGC	Substitution
3053124	3053125	2	GA -> AT	Substitution	94.70%	D -> I	argP CDS	13	GAC -> ATC	Substitution
3053967	3053969	3	TTA -> AAG	Substitution	94.60%	L -> K	argP CDS	856	TTA -> AAG	Substitution
118490	118490	1	C -> T	SNP (transition)	72.80%					
3053509	3053509	1	A -> G	SNP (transition)	50.00%	E -> G	argP CDS	398	GAA -> GGA	Substitution
4076057	4076057	1	A -> G	SNP (transition)	45.60%		fdoH CDS	63	CCT -> CCC	None



1203245	1203245	1	C -> G	SNP (transversion)	25.90%	L -> V	stfP CDS	289	CTT -> GTT	Substitution
---------	---------	---	--------	-----------------------	--------	--------	----------	-----	------------	--------------

### Appx. E.3: All mutations found in *AyedV::kan<sup>R</sup>* Keio mutant

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
2031550	2031551	2	AG -> GC	Substitution	98.6% -> 99.0%	T -> S	yedV CDS	83	ACT -> AGC	Substitution
2031574	2031576	2	AAC -> TAT	Substitution	97.3% -> 97.4%	V -> I	yedV CDS	58	GTT -> ATA	Substitution
2031578	2031582	5	GACAG -> CTTC	Substitution	95.9% -> 96.0%	LS -> ES	yedV CDS	52	CTG,TCT -> GAA,AGT	Substitution
2031478	2031480	3	GAT -> CTG	Substitution	95.7% -> 96.4%	I -> Q	yedV CDS	154	ATC -> CAG	Substitution
2031508	2031513	5	ATCATC -> CCTTTA	Substitution	95.6% -> 95.9%		yedV CDS	121		Truncation
2031529	2031534	6	ACTTGC -> TGAGTG	Substitution	95.0% -> 95.4%	AS -> HS	yedV CDS	100	GCA,AGT -> CAC,TCA	Substitution
2030315	2030316	2	TT -> GA	Substitution	94.2% -> 94.3%	K -> S	yedV CDS	1318	AAG -> TCG	Substitution
2031625	2031629	5	TCTTT -> GAA	Deletion	93.7% -> 93.8%		yedV CDS	5		Frame Shift
2031500	2031506	7	AGTGTTG -> TTCCGCT	Substitution	93.1% -> 93.6%	TTL -> KRN	yedV CDS	128	ACA,ACA,CTC -> AAG,CGG,AAC	Substitution
2031597	2031600	4	GCAA -> CTTC	Substitution	93.1% -> 93.3%	LL -> EV	yedV CDS	34	TTG,CTT -> GAA,GTT	Substitution
2030329	2030334	5	ATACGT -> TTCTAG	Substitution	92.4% -> 92.6%	TY -> LE	yedV CDS	1300	ACG,TAT -> CTA,GAA	Substitution
2031524	2031525	2	AA -> G	Deletion	91.8% -> 92.0%		yedV CDS	109		Frame Shift
2031439	2031442	4	TAAC -> CCCA	Substitution	90.9% -> 92.9%	TL -> TG	yedV CDS	192	ACG,TTA -> ACT,GGG	Substitution
2030357	2030360	4	GCAA -> AACT	Substitution	90.8% -> 91.2%	IA -> KF	yedV CDS	1274	ATT,GCC -> AAG,TTC	Substitution
2030302	2030313	9	GAACACATTATG -> CTGGAGCTGCTT	Substitution	90.4% -> 90.7%	HNVF -> KQLQ	yedV CDS	1321	CAT,AAT,GTG,TTC -> AAG,CAG,CTC,CAG	Substitution
2030363	2030369	6	GCTTTGA -> AAGATCCC	Insertion	90.1% -> 92.5%		yedV CDS	1265		Frame Shift
2031602	2031608	7	GTAAAC -> AACTGCA	Substitution	89.2% -> 89.6%	RLT -> LQF	yedV CDS	26	CGT,TTA,ACC -> CTG,CAG,TTC	Substitution
2030339	2030348	9	CTTCCCCCAT -> TAGGAACTTC	Substitution	88.1% -> 89.9%	HGGS -> RSSY	yedV CDS	1286	CAT,GGG,GGA,AGT -> CGA,AGT,TCC,TAT	Substitution
2030320	2030328	9	GAGATAGTG -> TCCTATACT	Substitution	87.9% -> 88.9%	HYL -> SIG	yedV CDS	1306	CAC,TAT,CTC -> AGT,ATA,GGA	Substitution

2031540	2031549	10	CATTATAGAG -> GGCAGCGTGA	Substitution	86.2% -> 86.9%	LYNG -> SRCR	yedV CDS	85	CTC,TAT,AAT,GGC -> TCA,CGC,TGC,CGC	Substitution
2031614	2031623	10	GTTATAGATA -> ACGGATCCCC	Substitution	86.0% -> 87.2%	LSIT -> RGSV	yedV CDS	11	CTA,TCT,ATA,ACC -> CGG,GGA,TCC,GTC	Substitution
2031565	2031572	7	GGCGCCAG -> TGAAGTTC	Substitution	85.1% -> 87.3%	AGA -> GTS	yedV CDS	62	GCT,GGC,GCC -> GGA,ACT,TCA	Substitution
2031556	2031563	7	GACAATTC -> AAAGCGCT	Substitution	82.9% -> 87.9%	GIV -> ERF	yedV CDS	71	GGA,ATT,GTC -> GAG,CGC,TTT	Substitution
2031484	2031497	12	CGCTGTCCGGTTAA - > ACTGGCTTTCTACG	Substitution	80.4% -> 90.6%		yedV CDS	137		Truncation
2030393	2030394	2	CC -> GG	Substitution	76.0% -> 79.2%	G -> P	yedV CDS	1240	GGT -> CCT	Substitution
2031458	2031459	2	CC -> AT	Substitution	67.3% -> 68.4%	G -> M	yedV CDS	175	GGG -> ATG	Substitution
2031435	2031437	3	CAG -> ATA	Substitution	66.7% -> 83.3%	PV -> LL	yedV CDS	197	CCT,GTG -> CTA,TTG	Substitution
2030395	2030400	6	TACGGA -> CGATAG	Substitution	58.3% -> 70.0%	SV -> LS	yedV CDS	1234	TCC,GTA -> CTA,TCG	Substitution
2031460	2031465	6	ATCAAT -> CCGGGG	Substitution	56.3% -> 67.8%	ID -> PR	yedV CDS	169	ATT,GAT -> CCC,CGG	Substitution
2030374	2030376	2	AGA -> TTAG	Insertion	53.8% -> 61.3%		yedV CDS	1258		Frame Shift
2031451	2031456	6	ATTTAC -> GACATT	Substitution	48.5% -> 62.2%	VN -> NV	yedV CDS	178	GTA,AAT -> AAT,GTC	Substitution
1972971	1972972	2	TT -> GG	Substitution	47.4% -> 47.8%					
2031455	2031456	2	AC -> CA	Substitution	35.6% -> 37.2%		yedV CDS	178		Truncation
2030374	2030375	2	AG -> TT	Substitution	33.7% -> 35.2%		yedV CDS	1259		Truncation
2030407	2030414	8	CGAATTAT -> ATGCGCTG	Substitution	33.3% -> 75.0%	DNS -> AAH	yedV CDS	1220	GAT,AAT,TCG -> GCA,GCG,CAT	Substitution
2030377	2030379	2	AAG -> GAA	Substitution	27.3% -> 30.0%	L -> F	yedV CDS	1255	CTT -> TTC	Substitution
1972943	1972948	6	AACAGA -> TTTCCC	Substitution	25.2% -> 28.6%					
2031447	2031450	4	CTGG -> AGCT	Substitution	25.0% -> 40.0%	PD -> SY	yedV CDS	184	CCA,GAT -> AGC,TAT	Substitution
2031460	2031463	4	ATCA -> GGGG	Substitution	25.0% -> 27.6%	ID -> IP	yedV CDS	171	ATT,GAT -> ATC,CCC	Substitution
1972976	1972980	5	TACAA -> GTGCG	Substitution	24.8% -> 30.2%					
360287	360292	6	GGGAAT -> AATTCC	Substitution	23.5% -> 25.6%					
2031475	2031475	1	C -> T	SNP (transition)	100.00%		yedV CDS	159	AAG -> AAA	None
2031434	2031434	1	A -> C	SNP (transversion)	100.00%	V -> G	yedV CDS	200	GTG -> GGG	Substitution

2031432	2031432	1	A -> G	SNP (transition)	100.00%	Y -> H	yedV CDS	202	TAC -> CAC	Substitution
2031527	2031527	1	T -> C	SNP (transition)	99.60%	E -> G	yedV CDS	107	GAG -> GGG	Substitution
2031519	2031519	1	A -> C	SNP (transversion)	99.50%	W -> G	yedV CDS	115	TGG -> GGG	Substitution
2031516	2031515	0	=+A	Insertion	99.50%		yedV CDS	119		Frame Shift
2031482	2031482	1	T -> G	SNP (transversion)	99.20%	Q -> P	yedV CDS	152	CAG -> CCG	Substitution
2030296	2030297	2	AA -> TG	Substitution	99.00%	I -> T	yedV CDS	1337	ATT -> ACA	Substitution
2719426	2719426	1	T -> C	SNP (transition)	98.90%					
2030372	2030372	1	A -> T	SNP (transversion)	98.90%		yedV CDS	1262		Truncation
2031536	2031537	2	AG -> TT	Substitution	98.80%	L -> K	yedV CDS	97	CTG -> AAG	Substitution
2030318	2030318	1	T -> G	SNP (transversion)	98.70%	N -> T	yedV CDS	1316	AAT -> ACT	Substitution
2031467	2031467	1	A -> C	SNP (transversion)	98.60%		yedV CDS	167		Truncation
2030299	2030300	2	CC -> AG	Substitution	98.60%	R -> P	yedV CDS	1334	CGG -> CCT	Substitution
2031473	2031473	1	T -> C	SNP (transition)	97.90%	Q -> R	yedV CDS	161	CAG -> CGG	Substitution
2030337	2030337	1	C -> A	SNP (transversion)	97.90%	A -> S	yedV CDS	1297	GCT -> TCT	Substitution
2031469	2031470	2	CA -> GC	Substitution	97.50%	L -> C	yedV CDS	164	TTG -> TGC	Substitution
2030350	2030350	1	C -> G	SNP (transversion)	97.40%	L -> F	yedV CDS	1284	TTG -> TTC	Substitution
2031586	2031589	3	CAAT -> AGA	Deletion	97.30%		yedV CDS	45		Frame Shift
2031522	2031522	1	T -> G	SNP (transversion)	97.20%	K -> Q	yedV CDS	112	AAA -> CAA	Substitution
2031593	2031594	2	AA -> GG	Substitution	96.90%	F -> P	yedV CDS	40	TTT -> CCT	Substitution
2030354	2030355	2	TC -> AG	Substitution	96.60%	E -> L	yedV CDS	1279	GAA -> CTA	Substitution
2031611	2031611	1	A -> T	SNP (transversion)	94.30%	V -> D	yedV CDS	23	GTC -> GAC	Substitution

4075572	4075572	1	G -> T	SNP (transversion)	93.80%	A -> E	fdoH CDS	548	GCG -> GAG	Substitution
2031553	2031553	1	C -> T	SNP (transition)	92.90%		yedV CDS	81		Truncation
2030402	2030402	1	T -> A	SNP (transversion)	87.50%	H -> L	yedV CDS	1232	CAT -> CTT	Substitution
2030388	2030388	1	C -> A	SNP (transversion)	80.60%		yedV CDS	1246		Truncation
2030404	2030404	1	A -> G	SNP (transition)	80.00%		yedV CDS	1230	CGT -> CGC	None
2030389	2030391	3	CTG -> AGA	Substitution	78.90%	Q -> S	yedV CDS	1243	CAG -> TCT	Substitution
2030384	2030384	1	A -> C	SNP (transversion)	76.40%	L -> R	yedV CDS	1250	CTA -> CGA	Substitution
2031446	2031446	1	T -> G	SNP (transversion)	72.70%	D -> A	yedV CDS	188	GAT -> GCT	Substitution
2030380	2030381	2	GC -> AA	Substitution	68.00%	G -> V	yedV CDS	1253	GGC -> GTT	Substitution
2031450	2031450	1	G -> A	SNP (transition)	50.00%	P -> S	yedV CDS	184	CCA -> TCA	Substitution
2030416	2030416	1	T -> G	SNP (transversion)	50.00%		yedV CDS	1218	GGA -> GGC	None
2031451	2031452	2	AT -> CA	Substitution	48.50%	N -> M	yedV CDS	182	AAT -> ATG	Substitution
4177534	4177534	1	T -> C	SNP (transition)	46.20%	S -> P	rpoC CDS	2257	TCC -> CCC	Substitution
2031457	2031457	1	C -> T	SNP (transition)	32.70%		yedV CDS	177	GGG -> GGA	None
2634568	2634568	1	A -> T	SNP (transversion)	31.90%	I -> N	ispG CDS	596	ATC -> AAC	Substitution
677146	677146	1	A -> G	SNP (transition)	31.60%					
1714635	1714635	1	G -> C	SNP (transversion)	31.50%					
161088	161088	1	T -> C	SNP (transition)	30.90%					
4076057	4076057	1	A -> G	SNP (transition)	30.80%		fdoH CDS	63	CCT -> CCC	None

1972989	1972989	1	A -> C	SNP (transversion)	30.80%					
161082	161082	1	T -> C	SNP (transition)	30.80%					
3991413	3991413	1	A -> C	SNP (transversion)	30.40%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
4076055	4076055	1	T -> C	SNP (transition)	28.10%	Q -> R	fdoH CDS	65	CAG -> CGG	Substitution
4076055	4076055	1	T -> G	SNP (transversion)	27.20%	Q -> P	fdoH CDS	65	CAG -> CCG	Substitution
1881082	1881082	1	A -> G	SNP (transition)	27.10%					
284974	284974	1	A -> G	SNP (transition)	27.00%					
2030381	2030381	1	C -> A	SNP (transversion)	26.70%	G -> V	yedV CDS	1253	GGC -> GTC	Substitution
1688395	1688395	1	A -> C	SNP (transversion)	25.80%		uidB CDS	126	GGT -> GGG	None
2077674	2077674	1	T -> C	SNP (transition)	25.70%					
910664	910664	1	C -> G	SNP (transversion)	25.60%					
4076049	4076049	1	C -> G	SNP (transversion)	25.50%	R -> P	fdoH CDS	71	CGG -> CCG	Substitution
3991428	3991428	1	C -> G	SNP (transversion)	25.10%	A -> G	uvrD CDS	86	GCG -> GGG	Substitution

#### Appx E.4: All mutations found in *AldhA::kan<sup>R</sup>* Keio mutant

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
1436972	1436973	2	AT -> GC	Substitution	97.7% -> 97.8%	N -> S	ldhA CDS	128	AAT -> AGC	Substitution
1436991	1436994	4	TTTT -> GCGC	Substitution	97.5% -> 97.6%	EK -> GA	ldhA CDS	107	GAA,AAA -> GGC,GCA	Substitution
1436211	1436215	5	GCCTG -> TAGAA	Substitution	97.2% -> 97.5%		ldhA CDS	886		Truncation
1436294	1436295	2	TT -> AG	Substitution	96.9% -> 97.0%	E -> A	ldhA CDS	806	GAA -> GCT	Substitution

1436315	1436319	4	GTTCT -> TTCAG	Substitution	96.6% -> 98.1%	EN -> AE	ldhA CDS	782	GAG,AAC -> GCT,GAA	Substitution
1437027	1437028	2	AA -> GC	Substitution	96.5% -> 96.7%	F -> A	ldhA CDS	73	TTT -> GCT	Substitution
1436931	1436932	2	CT -> GG	Substitution	96.5% -> 96.6%	S -> P	ldhA CDS	169	AGC -> CCC	Substitution
1436187	1436191	3	GTCAG -> AATAGG	Insertion	96.1% -> 96.8%		ldhA CDS	910		Frame Shift
1436967	1436969	3	CAG -> TGTT	Insertion	95.7% -> 96.1%		ldhA CDS	132		Frame Shift
1436273	1436277	4	GATCA -> TAAAG	Substitution	95.6% -> 95.8%	VI -> AL	ldhA CDS	824	GTG,ATC -> GCT,TTA	Substitution
1436975	1436976	2	GG -> TC	Substitution	95.2% -> 95.3%	A -> G	ldhA CDS	125	GCC -> GGA	Substitution
1436223	1436224	2	GT -> TC	Substitution	95.1% -> 95.4%	T -> D	ldhA CDS	877	ACC -> GAC	Substitution
1436261	1436263	3	TAC -> GCG	Substitution	94.9% -> 95.1%	V -> R	ldhA CDS	838	GTA -> CGC	Substitution
1437047	1437053	5	CTGTTGC -> TTCCTAG	Substitution	94.8% -> 95.2%		ldhA CDS	48		Truncation
1436936	1436937	2	GT -> AG	Substitution	94.6% -> 94.7%	D -> A	ldhA CDS	164	GAC -> GCT	Substitution
1436198	1436203	6	TGCTGT -> AAGATC	Substitution	94.4% -> 94.8%	TA -> DL	ldhA CDS	898	ACA,GCA -> GAT,CTT	Substitution
1436252	1436254	3	GCG -> CGA	Substitution	93.9% -> 94.2%	R -> S	ldhA CDS	847	CGC -> TCG	Substitution
1436231	1436236	5	CACGTT -> GCGATA	Substitution	93.8% -> 94.4%	NV -> YR	ldhA CDS	865	AAC,GTG -> TAT,CGC	Substitution
1436265	1436266	2	TC -> CG	Substitution	93.7% -> 93.8%	D -> R	ldhA CDS	835	GAC -> CGC	Substitution
1436918	1436920	3	CAG -> AGA	Substitution	92.7% -> 93.3%	L -> S	ldhA CDS	181	CTG -> TCT	Substitution
1436322	1436325	4	TACA -> ATAT	Substitution	92.5% -> 93.0%	VY -> DI	ldhA CDS	776	GTG,TAT -> GAT,ATT	Substitution
1436173	1436177	4	TCTGA -> AATAG	Substitution	92.3% -> 93.0%	SQT -> SYS	ldhA CDS	924	TCT,CAG,ACT -> TCC,TAT,TCT	Substitution
1436923	1436929	6	CCGGGCG -> TTCATCC	Substitution	92.2% -> 93.4%		ldhA CDS	172		Truncation
1437084	1437090	6	TAAACGG -> CGGATCC	Substitution	91.4% -> 92.7%	AVY -> GIR	ldhA CDS	11	GCC,GTT,TAT -> GGG,ATC,CGT	Substitution
1436897	1436905	9	GCCGTGCTT -> TTGTCCAGA	Substitution	91.3% -> 95.8%	KHG -> SGQ	ldhA CDS	196	AAG,CAC,GGC -> TCT,GGA,CAA	Substitution
1436910	1436916	7	AGCTCTT -> CCAGTAG	Substitution	91.2% -> 93.0%	EEL -> ATG	ldhA CDS	185	GAA,GAG,CTG -> GCT,ACT,GGG	Substitution
1436244	1436248	5	GCAGA -> ATGCG	Substitution	91.1% -> 91.5%	SA -> RI	ldhA CDS	853	TCT,GCC -> CGC,ATC	Substitution
1436997	1437007	11	GTCAGCAGAAA -> TGAGTGCTTGC	Substitution	90.6% -> 90.9%	FLLT -> ASTQ	ldhA CDS	94	TTT,CTG,CTG,ACG -> GCA,AGC,ACT,CAG	Substitution
1437074	1437082	7	TTTTGTGCT -> CAGGTCCG	Deletion	89.3% -> 90.3%		ldhA CDS	19		Frame Shift

1436300	1436307	8	GAATAGAT -> TTCGCCGC	Substitution	88.9% -> 90.6%	DLF -> GGE	ldhA CDS	794	GAT,CTA,TTC -> GGC,GGC,GAA	Substitution
1436309	1436310	2	GC -> AA	Substitution	88.6% -> 88.9%	R -> L	ldhA CDS	791	CGC -> CTT	Substitution
1436157	1436160	4	AAGT -> CCTA	Substitution	88.5% -> 88.9%	NL -> IG	ldhA CDS	941	AAC,TTA -> ATA,GGA	Substitution
1437020	1437025	6	CAGCTC -> TTCAAA	Substitution	88.3% -> 88.7%	EL -> FE	ldhA CDS	76	GAG,CTG -> TTT,GAA	Substitution
1436162	1436170	9	TTGCAGCGT -> ACTTTCTAG	Substitution	87.8% -> 88.8%	TLQ -> LES	ldhA CDS	931	ACG,CTG,CAA -> CTA,GAA,AGT	Substitution
1437012	1437018	7	AAAAATT -> GCGTGAG	Substitution	86.7% -> 87.5%	EFF -> AHA	ldhA CDS	83	GAA,TTT,TTT -> GCT,CAC,GCT	Substitution
1437056	1437065	10	GTA CTTCTTG -> AATAGGAACT	Substitution	86.5% -> 87.8%	DKKY -> EVPI	ldhA CDS	36	GAC,AAG,AAG,TAC -> GAA,GTT,CCT,ATT	Substitution
1436949	1436961	12	AAAATACATACCG -> CGGACTGGCTTT	Deletion	84.1% -> 89.9%		ldhA CDS	140		Frame Shift
1436135	1436146	11	TTCGCCTTTTTC -> AGGCTGGAGCTG	Substitution	81.7% -> 82.8%	EKGE -> QLQP	ldhA CDS	955	GAA,AAA,GGC,GAA -> CAG,CTC,CAG,CCT	Substitution
1436279	1436291	13	GTCGTTGGATTTA -> ACGAGGAAGCGG	Deletion	81.5% -> 86.9%		ldhA CDS	810		Frame Shift
1437031	1437038	8	CAAAGGAC -> TCTGAAGT	Substitution	81.1% -> 82.1%	ESFG -> ELQS	ldhA CDS	63	GAG,TCC,TTT,GGC -> GAA,CTT,CAG,AGC	Substitution
1436148	1436154	7	AGATTGC -> TTCGAAG	Substitution	75.1% -> 78.4%	SNL -> TSK	ldhA CDS	947	AGC,AAT,CTG -> ACT,TCG,AAG	Substitution
4581508	4581509	2	=-TG	Deletion	42.4% -> 42.8%		tsr CDS	35		Frame Shift
4581497	4581499	3	GAC -> TCG	Substitution	40.3% -> 41.0%	VT -> VR	tsr CDS	24	GTG,ACC -> GTT,CGC	Substitution
4581480	4581481	2	AA -> TC	Substitution	30.0% -> 30.7%	K -> S	tsr CDS	7	AAA -> TCA	Substitution
4581490	4581490	1	A -> GGG	Insertion	29.8% -> 30.1%		tsr CDS	17		Frame Shift
1436894	1436895	2	AA -> CC	Substitution	100.00%	V -> G	ldhA CDS	206	GTT -> GGG	Substitution
1436889	1436890	2	TA -> GT	Substitution	100.00%	Y -> T	ldhA CDS	211	TAT -> ACT	Substitution
1436341	1436342	2	AA -> CT	Substitution	100.00%	GS -> GA	ldhA CDS	759	GGT,TCG -> GGA,GCG	Substitution
1436334	1436334	1	C -> G	SNP (transversion)	100.00%	G -> A	ldhA CDS	767	GGT -> GCT	Substitution
1436327	1436331	5	GTCCA -> ACGGG	Substitution	100.00%	MD -> TR	ldhA CDS	770	ATG,GAC -> ACC,CGT	Substitution
1436269	1436269	1	C -> A	SNP (transversion)	100.00%	D -> Y	ldhA CDS	832	GAT -> TAT	Substitution

1436259	1436259	1	A -> G	SNP (transition)	100.00%	F -> S	ldhA CDS	842	TTC -> TCC	Substitution
1436271	1436271	1	T -> C	SNP (transition)	99.50%	Q -> R	ldhA CDS	830	CAG -> CGG	Substitution
2719426	2719426	1	T -> C	SNP (transition)	99.40%					
1436940	1436940	1	T -> C	SNP (transition)	99.30%	D -> G	ldhA CDS	161	GAT -> GGT	Substitution
1436297	1436298	2	AA -> CC	Substitution	99.20%	F -> W	ldhA CDS	803	TTT -> TGG	Substitution
1436987	1436988	2	GG -> CC	Substitution	98.90%	T -> R	ldhA CDS	113	ACC -> AGG	Substitution
1436946	1436947	2	AC -> CT	Substitution	98.80%	V -> R	ldhA CDS	154	GTA -> AGA	Substitution
1436250	1436250	1	A -> T	SNP (transversion)	98.80%	L -> Q	ldhA CDS	851	CTG -> CAG	Substitution
1436978	1436979	2	AG -> TT	Substitution	98.70%	T -> K	ldhA CDS	122	ACT -> AAA	Substitution
1436256	1436255	0	=+T	Insertion	98.30%		ldhA CDS	846		Frame Shift
1436227	1436228	2	AC -> GA	Substitution	98.10%	LF -> LL	ldhA CDS	873	CTG,TTT -> CTT,CTT	Substitution
1436193	1436193	1	G -> A	SNP (transition)	98.10%	A -> V	ldhA CDS	908	GCT -> GTT	Substitution
1436181	1436181	1	A -> C	SNP (transversion)	98.10%	I -> S	ldhA CDS	920	ATT -> AGT	Substitution
1436964	1436964	1	T -> A	SNP (transversion)	98.00%	E -> V	ldhA CDS	137	GAA -> GTA	Substitution
1436238	1436239	2	TG -> AA	Substitution	97.90%	H -> F	ldhA CDS	862	CAC -> TTC	Substitution
1436220	1436220	1	C -> T	SNP (transition)	97.90%	G -> E	ldhA CDS	881	GGG -> GAG	Substitution
1436205	1436208	4	AGGA -> CCTT	Substitution	97.90%		ldhA CDS	893		Truncation
1436217	1436218	2	TG -> AA	Substitution	97.70%	H -> F	ldhA CDS	883	CAC -> TTC	Substitution
1436132	1436132	1	G -> T	SNP (transversion)	97.70%		ldhA CDS	969	ACC -> ACA	None
1437009	1437009	1	T -> G	SNP (transversion)	97.60%	D -> A	ldhA CDS	92	GAC -> GCC	Substitution
1437092	1437093	2	GA -> CG	Substitution	97.50%	L -> P	ldhA CDS	8	CTC -> CCG	Substitution
1437041	1437043	2	GTT -> CTA	Substitution	97.20%		ldhA CDS	58		Truncation
1436981	1436983	3	TTT -> AGC	Substitution	97.20%	K -> A	ldhA CDS	118	AAA -> GCT	Substitution



1436312	1436312	1	T -> C	SNP (transition)	97.00%		ldhA CDS	789	GAA -> GAG	None
1437095	1437096	2	TT -> AA	Substitution	96.60%	K -> I	ldhA CDS	5	AAA -> ATT	Substitution
1436128	1436128	1	G -> A	SNP (transition)	96.20%	P -> S	ldhA CDS	973	CCG -> TCG	Substitution
1437069	1437069	1	T -> A	SNP (transversion)	95.20%	Y -> F	ldhA CDS	32	TAC -> TTC	Substitution
1436241	1436242	2	CA -> GC	Substitution	94.90%	C -> A	ldhA CDS	859	TGC -> GCC	Substitution
1436934	1436934	1	C -> T	SNP (transition)	94.40%	G -> D	ldhA CDS	167	GGC -> GAC	Substitution
1436907	1436908	2	TT -> AG	Substitution	94.40%	K -> L	ldhA CDS	193	AAA -> CTA	Substitution
1436183	1436185	2	ACT -> TCG	Substitution	94.10%	S -> R	ldhA CDS	916	AGT -> CGA	Substitution
3074216	3074216	1	G -> A	SNP (transition)	70.00%	S -> F	tktA CDS	779	TCC -> TTC	Substitution
4581502	4581502	1	G -> C	SNP (transversion)	46.30%	S -> T	tsr CDS	29	AGC -> ACC	Substitution
4581506	4581506	1	A -> C	SNP (transversion)	43.40%	L -> F	tsr CDS	33	TTA -> TTC	Substitution
4581492	4581492	1	A -> C	SNP (transversion)	41.40%	I -> L	tsr CDS	19	ATT -> CTT	Substitution
4076057	4076057	1	A -> G	SNP (transition)	39.30%		fdoH CDS	63	CCT -> CCC	None
4076055	4076055	1	T -> G	SNP (transversion)	33.60%	Q -> P	fdoH CDS	65	CAG -> CCG	Substitution
3991413	3991413	1	A -> C	SNP (transversion)	28.20%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
1701277	1701277	1	G -> C	SNP (transversion)	26.80%		rsxC CDS	102	CTG -> CTC	None
4581477	4581477	1	T -> A	SNP (transversion)	26.20%	L -> I	tsr CDS	4	TTA -> ATA	Substitution
446820	446820	1	A -> G	SNP (transition)	25.50%	S -> P	cyoA CDS	247	TCA -> CCA	Substitution

**Appx. E.5: All mutations found in *Alhr::kan<sup>R</sup>* Keio mutant**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
1727820	1727822	3	ACT -> CGC	Substitution	96.7% -> 96.8%	T -> R	lhr CDS	4477	ACT -> CGC	Substitution
1727828	1727833	5	ACTGCG -> CTCTA	Substitution	96.4% -> 96.7%	ALR -> AFY	lhr CDS	4485	GCA,CTG,CGT -> GCC,TTC,TAT	Substitution
1727881	1727883	3	CGG -> TAT	Substitution	95.9% -> 96.0%	PV -> LF	lhr CDS	4538	CCG,GTC -> CTA,TTC	Substitution
1723495	1723496	2	CA -> TC	Substitution	95.9% -> 96.0%	A -> V	lhr CDS	152	GCA -> GTC	Substitution
1723455	1723460	6	TGGCAT -> GCAAGG	Substitution	95.8% -> 96.0%	WH -> AR	lhr CDS	112	TGG,CAT -> GCA,AGG	Substitution
1727856	1727859	4	ACGC -> CTAA	Substitution	95.5% -> 95.8%	TL -> LI	lhr CDS	4513	ACG,CTA -> CTA,ATA	Substitution
1723466	1723470	5	GGCGC -> TAAAG	Substitution	95.5% -> 95.8%	AAR -> AKG	lhr CDS	123	GCG,GCG,CGA -> GCT,AAA,GGA	Substitution
1723432	1723436	4	CGACC -> ACGCT	Substitution	95.1% -> 95.2%	PT -> HA	lhr CDS	89	CCG,ACC -> CAC,GCT	Substitution
1723498	1723499	2	CG -> GC	Substitution	94.9% -> 95.1%	P -> R	lhr CDS	155	CCG -> CGC	Substitution
1723393	1723396	4	CGGC -> TCTA	Substitution	94.4% -> 94.7%		lhr CDS	50		Truncation
1723414	1723416	3	TTC -> AGA	Substitution	94.3% -> 94.8%	LR -> QS	lhr CDS	71	CTT,CGC -> CAG,AGC	Substitution
1723347	1723355	9	GCAGATAAT -> ATTC	Deletion	93.9% -> 94.1%		lhr CDS	4		Frame Shift
1723491	1723493	3	ATT -> CCA	Substitution	93.9% -> 94.0%	I -> P	lhr CDS	148	ATT -> CCA	Substitution
1727848	1727853	6	TGCGCT -> GAGTTC	Substitution	93.0% -> 94.0%	LRF -> RVL	lhr CDS	4505	CTG,CGC,TTT -> CGA,GTT,CTT	Substitution
1723377	1723379	3	CCG -> TTC	Substitution	92.6% -> 92.9%	P -> F	lhr CDS	34	CCG -> TTC	Substitution
1727868	1727879	9	GTGAATGATCTA -> TCTTGAAGTTC	Deletion	92.3% -> 93.4%		lhr CDS	4525		Truncation
1727838	1727846	7	GAACCACGG -> CTTCTTGA	Deletion	91.9% -> 93.5%		lhr CDS	4495		Frame Shift
1727811	1727814	3	CACG -> GATT	Substitution	91.4% -> 91.9%	HA -> DS	lhr CDS	4468	CAC,GCG -> GAT,TCG	Substitution
1727788	1727794	6	AATTA CT -> TGCTTTA	Substitution	90.5% -> 92.7%	ELL -> VLY	lhr CDS	4445	GAA,TTA,CTC -> GTG,CTT,TAC	Substitution
1723428	1723430	3	CAG -> GCT	Substitution	90.5% -> 90.8%	Q -> A	lhr CDS	85	CAG -> GCT	Substitution
1723506	1723508	3	TCC -> GGT	Substitution	89.4% -> 91.1%	S -> G	lhr CDS	163	TCC -> GGT	Substitution
1723479	1723487	9	CATGCGCTG -> ACACGTAGA	Substitution	88.5% -> 89.0%	HAL -> TRR	lhr CDS	136	CAT,GCG,CTG -> ACA,CGT,AGA	Substitution

1723501	1723504	4	CCGG -> GAAA	Substitution	88.4% -> 88.7%	TG -> RN	lhr CDS	158	ACC,GGC -> AGA,AAC	Substitution
1727888	1727891	4	GCAA -> AAGT	Substitution	87.6% -> 87.9%	RQ -> RS	lhr CDS	4545	CGG,CAA -> CGA,AGT	Substitution
1723399	1723410	11	CCCGCGACTGGT -> AAGTATAGGAAC	Substitution	87.3% -> 87.8%		lhr CDS	56		Truncation
1723532	1723535	4	CTAC -> ACTG	Substitution	87.2% -> 88.2%	LY -> LL	lhr CDS	189	CTC,TAC -> CTA,CTG	Substitution
1727797	1727801	5	CCCCA -> GTATC	Substitution	87.1% -> 87.9%	AP -> GI	lhr CDS	4454	GCC,CCA -> GGT,ATC	Substitution
1723510	1723520	9	GGAAAACGCTG -> CTGACCCCGGAT	Insertion	86.7% -> 89.2%		lhr CDS	167		Frame Shift
1723548	1723550	3	CTC -> AAG	Substitution	85.7% -> 90.0%	L -> K	lhr CDS	205	CTC -> AAG	Substitution
1723524	1723529	6	GCA TTT -> TGTCAG	Substitution	85.7% -> 87.1%	AF -> CQ	lhr CDS	181	GCA,TTT -> TGT,CAG	Substitution
1727755	1727766	12	GCAAAAAAATGC -> CTTGGCGGCGAA	Substitution	82.1% -> 88.3%	GKKML -> AWRRM	lhr CDS	4412	GGC,AAA,AAA,ATG,CTG -> GCT,TGG,CGG,CGA,ATG	Substitution
1723360	1723370	10	ACCCTTCATCG -> GATCCGTCGAC	Substitution	81.6% -> 85.4%	DPSS -> GSVD	lhr CDS	17	GAC,CCT,TCA,TCG -> GGA,TCC,UTC,GAC	Substitution
1727741	1727749	9	TCTGGCGCA -> ATATTGCTG	Substitution	80.0% -> 100.0%		lhr CDS	4398		Truncation
1727922	1727937	15	GGATTTTCAAGTTTCGC -> AAGCAGCTCCAGCCTA	Substitution	78.6% -> 81.3%	GFSSSP -> KQLQPT	lhr CDS	4579	GGA,TTT,TCA,AGT,TCG,CCA -> AAG,CAG,CTC,CAG,CCT,ACA	Substitution
1727778	1727780	3	GAA -> CT	Deletion	68.0% -> 73.1%		lhr CDS	4435		Frame Shift
1727892	1727919	24	ACGCCGATGTTTAC GCTGCTGCGTGAGG -> TCCTATTCTCTAGA AAGTATAGGAACTT	Substitution	66.5% -> 76.9%	TPMFTLLREA -> SYSLESIGTS	lhr CDS	4549	ACG,CCG,ATG,TTT,ACG,CTG,CTG,CGT,GAG,GCG -> TCC,TAT,TCT,CTA,GAA,AGT,ATA,GGA,ACT,TCG	Substitution
1727781	1727786	6	AAAGAG -> TCCTC	Deletion	66.0% -> 69.2%		lhr CDS	4438		Frame Shift
1723539	1723547	9	CTCGATCGG -> TATCTGGAC	Substitution	60.0% -> 76.0%	LDR -> YLD	lhr CDS	196	CTC,GAT,CGG -> TAT,CTG,GAC	Substitution
1727818	1727818	1	T -> A	SNP (transversion)	100.00%	L -> Q	lhr CDS	4475	CTG -> CAG	Substitution
1727808	1727809	2	TT -> CC	Substitution	100.00%	F -> P	lhr CDS	4465	TTC -> CCC	Substitution
1727803	1727804	2	AG -> CC	Substitution	100.00%	E -> A	lhr CDS	4460	GAG -> GCC	Substitution
1727751	1727753	2	GGT -> AGA	Substitution	100.00%	G -> R	lhr CDS	4408	GGT -> AGA	Substitution

1726787	1726787	1	A -> G	SNP (transition)	100.00%		lhr CDS	3444	GGA -> GGG	None
1724785	1724785	1	A -> G	SNP (transition)	100.00%	E -> G	lhr CDS	1442	GAA -> GGA	Substitution
1723552	1723553	2	TC -> AA	Substitution	100.00%		lhr CDS	209		Truncation
1723488	1723489	2	GT -> AA	Substitution	100.00%	V -> K	lhr CDS	145	GTG -> AAG	Substitution
1723441	1723441	1	=-T	Deletion	100.00%		lhr CDS	98		Frame Shift
1723375	1723375	1	T -> A	SNP (transversion)	99.60%	L -> Q	lhr CDS	32	CTG -> CAG	Substitution
1727806	1727806	1	T -> C	SNP (transition)	99.40%	V -> A	lhr CDS	4463	GTT -> GCT	Substitution
1723425	1723425	1	A -> G	SNP (transition)	99.40%	K -> E	lhr CDS	82	AAA -> GAA	Substitution
1723443	1723443	1	C -> A	SNP (transversion)	99.30%	Q -> K	lhr CDS	100	CAG -> AAG	Substitution
1727866	1727866	1	A -> G	SNP (transition)	99.10%	E -> G	lhr CDS	4523	GAA -> GGA	Substitution
1723477	1723477	1	A -> G	SNP (transition)	99.10%	E -> G	lhr CDS	134	GAA -> GGA	Substitution
1723373	1723373	1	C -> G	SNP (transversion)	99.10%		lhr CDS	30	CTC -> CTG	None
3048197	3048197	1	C -> A	SNP (transversion)	99.00%		pepP CDS	3	ATG -> ATT	None
1723447	1723448	1	CG -> ACT	Insertion	99.00%		lhr CDS	104		Frame Shift
1723439	1723439	1	T -> C	SNP (transition)	98.70%		lhr CDS	96	GCT -> GCC	None
1723451	1723453	3	AAC -> GGG	Substitution	98.60%	QT -> QG	lhr CDS	108	CAA,ACC -> CAG,GGC	Substitution
1723382	1723382	1	C -> A	SNP (transversion)	98.20%	D -> E	lhr CDS	39	GAC -> GAA	Substitution
1723357	1723358	2	CA -> GG	Substitution	98.00%	P -> R	lhr CDS	14	CCA -> CGG	Substitution
1727863	1727864	2	CA -> GG	Substitution	97.40%	T -> R	lhr CDS	4520	ACA -> AGG	Substitution
1727770	1727770	1	T -> G	SNP (transversion)	97.40%	V -> G	lhr CDS	4427	GTC -> GGC	Substitution
1723462	1723463	2	TG -> CT	Substitution	96.90%	V -> A	lhr CDS	119	GTG -> GCT	Substitution

1723421	1723421	1	C -> T	SNP (transition)	96.70%		lhr CDS	78	GCC -> GCT	None
1723385	1723387	3	GTT -> TCC	Substitution	96.60%	VF -> VP	lhr CDS	42	GTG,TTT -> GTT,CCT	Substitution
1723522	1723522	1	C -> A	SNP (transversion)	96.30%	A -> E	lhr CDS	179	GCA -> GAA	Substitution
1723389	1723391	3	TCA -> ATT	Substitution	95.90%	S -> I	lhr CDS	46	TCA -> ATT	Substitution
1727824	1727824	1	C -> T	SNP (transition)	91.80%	T -> I	lhr CDS	4481	ACC -> ATC	Substitution
1723537	1723537	1	C -> G	SNP (transversion)	91.70%	A -> G	lhr CDS	194	GCC -> GGC	Substitution
1727774	1727774	1	G -> A	SNP (transition)	88.20%		lhr CDS	4431		Truncation
1727776	1727776	1	A -> C	SNP (transversion)	84.30%	Q -> P	lhr CDS	4433	CAG -> CCG	Substitution
4076057	4076057	1	A -> G	SNP (transition)	40.50%		fdoH CDS	63	CCT -> CCC	None
1726937	1726937	1	A -> C	SNP (transversion)	33.30%		lhr CDS	3594	GCA -> GCC	None
4076055	4076055	1	T -> G	SNP (transversion)	29.50%	Q -> P	fdoH CDS	65	CAG -> CCG	Substitution
1727780	1727780	1	A -> C	SNP (transversion)	29.00%	E -> D	lhr CDS	4437	GAA -> GAC	Substitution
4076055	4076055	1	T -> C	SNP (transition)	27.90%	Q -> R	fdoH CDS	65	CAG -> CGG	Substitution
1688398	1688398	1	G -> C	SNP (transversion)	25.80%		uidB CDS	123	GTC -> GTG	None
3991428	3991428	1	C -> G	SNP (transversion)	25.70%	A -> G	uvrD CDS	86	GCG -> GGG	Substitution
3123500	3123500	1	A -> G	SNP (transition)	25.10%		yghO CDS	3	AAT -> AAC	None
3991413	3991413	1	A -> C	SNP (transversion)	25.00%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution

**Appx. E.6: All mutations found in *AyegQ::kan<sup>R</sup>* Keio mutant**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
2160398	2160399	2	CA -> TT	Substitution	98.6% -> 98.8%	P -> L	yegQ CDS	1250	CCA -> CTT	Substitution
2159309	2159309	1	A -> CTG	Insertion	97.9% -> 98.0%		yegQ CDS	161		Frame Shift
2159291	2159295	5	TTCAG -> CAGTC	Substitution	97.8% -> 98.0%	LQ -> PV	yegQ CDS	143	CTT,CAG -> CCA,GTC	Substitution
2159353	2159354	2	GC -> AA	Substitution	97.5% -> 97.6%	A -> K	yegQ CDS	205	GCA -> AAA	Substitution
2159254	2159259	5	CTGCGT -> GCAAGG	Substitution	97.3% -> 97.4%	LR -> AR	yegQ CDS	106	CTG,CGT -> GCA,AGG	Substitution
2159324	2159325	2	GG -> TC	Substitution	97.1% -> 97.2%	G -> V	yegQ CDS	176	GGG -> GTC	Substitution
2159305	2159306	2	AA -> GG	Substitution	97.1% -> 97.2%	N -> G	yegQ CDS	157	AAT -> GGT	Substitution
2159248	2159252	4	TATTC -> CAGGG	Substitution	96.9% -> 97.0%	YS -> QG	yegQ CDS	100	TAT,TCC -> CAG,GGC	Substitution
2160405	2160408	4	TGGT -> GTTC	Substitution	96.3% -> 96.5%	DG -> EF	yegQ CDS	1257	GAT,GGT -> GAG,TTC	Substitution
2160434	2160437	4	AGGA -> CTAT	Substitution	95.8% -> 96.0%	QD -> PI	yegQ CDS	1286	CAG,GAT -> CCT,ATT	Substitution
2159220	2159222	3	AGA -> TTT	Substitution	95.4% -> 95.5%	AD -> AF	yegQ CDS	72	GCA,GAT -> GCT,TTT	Substitution
2159239	2159246	8	CAGCCGCG -> GCAAGCAC	Substitution	94.7% -> 94.9%	QPR -> AST	yegQ CDS	91	CAG,CCG,CGT -> GCA,AGC,ACT	Substitution
2160379	2160381	3	GAA -> ATC	Substitution	94.6% -> 94.9%	E -> I	yegQ CDS	1231	GAA -> ATC	Substitution
2159261	2159265	5	TGCGC -> CTGCT	Substitution	94.3% -> 94.5%	VR -> AA	yegQ CDS	113	GTG,CGC -> GCT,GCT	Substitution
2160479	2160482	4	GGGA -> CTCC	Substitution	94.2% -> 94.7%	GE -> AP	yegQ CDS	1331	GGG,GAA -> GCT,CCA	Substitution
2159315	2159318	4	ACGC -> GGAT	Substitution	93.6% -> 94.0%	HA -> RM	yegQ CDS	167	CAC,GCG -> CGG,ATG	Substitution
2159282	2159285	4	ACGA -> GTAG	Substitution	93.6% -> 93.8%	HE -> RR	yegQ CDS	134	CAC,GAA -> CGT,AGA	Substitution
2159320	2159322	3	CTG -> AAT	Substitution	93.6% -> 93.7%	L -> N	yegQ CDS	172	CTG -> AAT	Substitution
2159155	2159159	5	AAACC -> CCGGG	Substitution	93.5% -> 93.7%	KP -> PG	yegQ CDS	7	AAA,CCG -> CCG,GGG	Substitution
2160293	2160294	2	AA -> CC	Substitution	93.3% -> 95.0%	K -> T	yegQ CDS	1145	AAA -> ACC	Substitution
2159327	2159331	4	AAAAG -> GCTAC	Substitution	93.0% -> 93.5%	KK -> SY	yegQ CDS	179	AAA,AAG -> AGC,TAC	Substitution
2159161	2159163	3	GAA -> ATC	Substitution	92.8% -> 92.9%	E -> I	yegQ CDS	13	GAA -> ATC	Substitution
2159165	2159166	2	TC -> GT	Substitution	92.1% -> 92.3%	L -> R	yegQ CDS	17	CTC -> CGT	Substitution
2159177	2159179	3	CGG -> TTC	Substitution	91.7% -> 92.1%	AG -> VR	yegQ CDS	29	GCG,GGA -> GTT,CGA	Substitution
2160428	2160432	5	TCCCG -> AAGTT	Substitution	91.6% -> 91.9%	VP -> EV	yegQ CDS	1280	GTC,CCG -> GAA,GTT	Substitution

2160367	2160376	7	AACGCCAAAG -> GATTCGCAGC	Substitution	91.4% -> 94.6%	NAKG -> DSQR	yegQ CDS	1219	AAC,GCC,AAA,GGC -> GAT,TCG,CAG,CGC	Substitution
2159206	2159209	4	TTCG -> GGAA	Substitution	91.4% -> 91.7%	FA -> GT	yegQ CDS	58	TTC,GCT -> GGA,ACT	Substitution
2160355	2160359	5	GAGCA -> ATCGC	Substitution	91.0% -> 91.4%	EH -> IA	yegQ CDS	1207	GAG,CAC -> ATC,GCC	Substitution
2159168	2159175	6	TTTCCCG -> GACCTGCA	Substitution	90.6% -> 91.1%	LSP -> RPA	yegQ CDS	20	CTT,TCC,CCG -> CGA,CCT,GCA	Substitution
2160410	2160418	9	ATACTGTGT -> TCTAATAAG	Substitution	90.5% -> 91.4%		yegQ CDS	1262		Truncation
2160463	2160468	5	ATGCGT -> TATAGG	Substitution	90.5% -> 90.6%	MR -> YR	yegQ CDS	1315	ATG,CGT -> TAT,AGG	Substitution
2159268	2159277	10	CAACGAATTC -> AGGAAGCGGA	Substitution	90.4% -> 91.4%	NNEF -> KGSG	yegQ CDS	120	AAC,AAC,GAA,TTC -> AAA,GGA,AGC,GGA	Substitution
2160454	2160461	7	GCGCTGCT -> TCTAGAAA	Substitution	90.0% -> 92.7%	ALL -> SRK	yegQ CDS	1306	GCG,CTG,CTG -> TCT,AGA,AAG	Substitution
2159183	2159192	9	CGCTGAAAAA -> GTTCCTATTC	Substitution	89.3% -> 91.3%	TLKN -> SSYS	yegQ CDS	35	ACG,CTG,AAA,AAT -> AGT,TCC,TAT,TCT	Substitution
2159333	2159336	4	TTTA -> GGGC	Substitution	89.2% -> 90.3%	FY -> WA	yegQ CDS	185	TTT,TAT -> TGG,GCT	Substitution
2160361	2160366	6	ATGGAA -> GCTCCC	Substitution	89.2% -> 90.0%	ME -> AP	yegQ CDS	1213	ATG,GAA -> GCT,CCC	Substitution
2160328	2160334	5	CCGCAAG -> GCTGACC	Substitution	87.4% -> 94.8%	PQG -> ADR	yegQ CDS	1180	CCG,CAA,GGC -> GCT,GAC,CGC	Substitution
2160340	2160346	6	ATTAATT -> CTCGTGC	Substitution	86.2% -> 97.4%	INF -> LVL	yegQ CDS	1192	ATT,AAT,TTT -> CTC,GTG,CTT	Substitution
2159349	2159352	4	CATT -> GGGA	Substitution	85.4% -> 87.9%	NI -> KG	yegQ CDS	201	AAC,ATT -> AAG,GGA	Substitution
2160475	2160477	2	TCC -> GAAGCA	Insertion	85.2% -> 85.8%	S -> EA	yegQ CDS	1327	TCC -> GAA,GCA	Insertion
2160384	2160396	12	TATGCCGATAGCA -> CTTCTATCGCCTT	Substitution	83.9% -> 92.2%	AMPIA -> AFYRL	yegQ CDS	1236	GCT,ATG,CCG,ATA,GCA -> GCC,TTC,TAT,CGC,CTT	Substitution
2159194	2159205	11	ATGCGTTACGCT -> CTAGAAAGTATA	Substitution	81.1% -> 88.1%	MRYA -> LESI	yegQ CDS	46	ATG,CGT,TAC,GCT -> CTA,GAA,AGT,ATA	Substitution
2160317	2160318	2	AG -> GC	Substitution	75.3% -> 76.7%	E -> G	yegQ CDS	1169	GAG -> GGC	Substitution
2160319	2160326	8	CTGATGAC -> GGCGAATG	Substitution	67.6% -> 75.0%	LMT -> GEW	yegQ CDS	1171	CTG,ATG,ACG -> GGC,GAA,TGG	Substitution
2160310	2160312	3	AGC -> GAG	Substitution	65.6% -> 68.2%	S -> E	yegQ CDS	1162	AGC -> GAG	Substitution
2160305	2160306	2	GC -> CT	Substitution	61.5% -> 62.7%	G -> A	yegQ CDS	1157	GGC -> GCT	Substitution
2160295	2160297	2	TTC -> GTG	Substitution	50.0% -> 52.0%	F -> V	yegQ CDS	1147	TTC -> GTG	Substitution
2160298	2160300	3	TCC -> GAT	Substitution	46.4% -> 55.6%	S -> D	yegQ CDS	1150	TCC -> GAT	Substitution

2160298	2160300	3	TCC -> ATA	Substitution	38.9% -> 50.0%	S -> I	yegQ CDS	1150	TCC -> ATA	Substitution
121658	121659	2	AC -> CT	Substitution	38.8% -> 39.7%	T -> L	aceE CDS	2155	ACT -> CTT	Substitution
121643	121645	3	ATC -> GG	Deletion	38.3% -> 38.7%		aceE CDS	2140		Frame Shift
2101605	2101606	1	AC -> CCC	Insertion	38.3% -> 38.6%		glf CDS	205		Frame Shift
121648	121650	3	CAA -> ATG	Substitution	38.1% -> 38.7%		aceE CDS	2145		Truncation
2160295	2160297	3	TTC -> CGT	Substitution	37.5% -> 40.9%	F -> R	yegQ CDS	1147	TTC -> CGT	Substitution
2101615	2101618	4	ATAT -> GGTG	Substitution	37.0% -> 41.0%	IW -> HR	glf CDS	193	ATA,TGG -> CAC,CGG	Substitution
2159357	2159359	3	CGC -> GCA	Substitution	35.0% -> 44.0%	PH -> RN	yegQ CDS	209	CCG,CAC -> CGC,AAC	Substitution
121666	121667	2	AG -> TA	Substitution	31.4% -> 32.1%	EG -> DS	aceE CDS	2163	GAA,GGT -> GAT,AGT	Substitution
2160301	2160306	5	GTTGGC -> TTGCTG	Substitution	27.9% -> 30.8%	VG -> LL	yegQ CDS	1153	GTT,GGC -> TTG,CTG	Substitution
360287	360292	6	GGGAAT -> AATCC	Substitution	26.2% -> 28.3%					
121670	121674	5	AGCAA -> GTTTT	Substitution	25.9% -> 28.3%	SK -> VL	aceE CDS	2167	AGC,AAA -> GTT,TTA	Substitution
2101621	2101627	6	ATTTATC -> GCTGCCA	Substitution	25.6% -> 28.8%	DKY -> WQH	glf CDS	184	GAT,AAA,TAT -> TGG,CAG,CAT	Substitution
2159357	2159363	6	CGCACAA -> GCAAGCG	Substitution	25.0% -> 76.9%	PHN -> RKR	yegQ CDS	209	CCG,CAC,AAC -> CGC,AAG,CGC	Substitution
2160307	2160309	2	GAC -> AAG	Substitution	24.6% -> 26.3%	D -> K	yegQ CDS	1159	GAC -> AAG	Substitution
2160313	2160315	2	CTT -> TTG	Substitution	23.7% -> 26.1%		yegQ CDS	1165	CTT -> TTG	None
121623	121630	8	AGGAAGGT -> GCAGCATC	Substitution	22.6% -> 25.6%	EEG -> GSI	aceE CDS	2120	GAG,GAA,GGT -> GGC,AGC,ATC	Substitution
2160289	2160290	2	AA -> GC	Substitution	20.0% -> 33.3%	N -> A	yegQ CDS	1141	AAT -> GCT	Substitution
2160506	2160510	5	--AGTGA	Deletion	100.00%		yegQ CDS	1358		Extension
2159371	2159372	2	CT -> AG	Substitution	100.00%	L -> R	yegQ CDS	223	CTG -> AGG	Substitution
2159367	2159367	1	C -> A	SNP (transversion)	100.00%		yegQ CDS	219	GCC -> GCA	None
2159311	2159311	1	G -> C	SNP (transversion)	100.00%	A -> P	yegQ CDS	163	GCC -> CCC	Substitution
2159303	2159303	1	T -> A	SNP (transversion)	100.00%	I -> N	yegQ CDS	155	ATC -> AAC	Substitution
2159289	2159289	1	T -> G	SNP (transversion)	100.00%	N -> K	yegQ CDS	141	AAT -> AAG	Substitution
2159235	2159235	1	G -> T	SNP (transversion)	100.00%		yegQ CDS	87	GCG -> GCT	None
2160489	2160489	1	G -> A	SNP (transition)	99.80%		yegQ CDS	1341	ACG -> ACA	None
2160352	2160353	2	CT -> GG	Substitution	99.50%	L -> G	yegQ CDS	1204	CTT -> GGT	Substitution
2159230	2159232	2	TAT -> CAC	Substitution	99.30%	Y -> H	yegQ CDS	82	TAT -> CAC	Substitution



2159228	2159228	1	T -> C	SNP (transition)	99.30%	V -> A	yegQ CDS	80	GTT -> GCT	Substitution
2160471	2160471	1	T -> C	SNP (transition)	98.90%		yegQ CDS	1323	AAT -> AAC	None
2160452	2160452	1	A -> T	SNP (transversion)	98.90%	Y -> F	yegQ CDS	1304	TAC -> TTC	Substitution
2159338	2159338	1	G -> A	SNP (transition)	98.90%	V -> I	yegQ CDS	190	GTC -> ATC	Substitution
2159279	2159280	2	AC -> CA	Substitution	98.90%	N -> T	yegQ CDS	131	AAC -> ACA	Substitution
2159213	2159214	2	AT -> CA	Substitution	98.70%	Y -> S	yegQ CDS	65	TAT -> TCA	Substitution
2160484	2160484	1	A -> G	SNP (transition)	98.50%	T -> A	yegQ CDS	1336	ACC -> GCC	Substitution
2159237	2159237	1	G -> C	SNP (transversion)	98.50%	G -> A	yegQ CDS	89	GGC -> GCC	Substitution
2160349	2160350	2	AC -> TA	Substitution	98.40%	T -> Y	yegQ CDS	1201	ACC -> TAC	Substitution
2160425	2160426	2	CG -> TT	Substitution	97.80%	P -> L	yegQ CDS	1277	CCG -> CTT	Substitution
2159297	2159297	1	T -> G	SNP (transversion)	97.80%	L -> R	yegQ CDS	149	CTC -> CGC	Substitution
2159152	2159152	1	T -> A	SNP (transversion)	97.50%	F -> I	yegQ CDS	4	TTT -> ATT	Substitution
2160445	2160445	1	C -> T	SNP (transition)	97.40%	L -> F	yegQ CDS	1297	CTC -> TTC	Substitution
2160337	2160338	2	AA -> TT	Substitution	96.60%	N -> F	yegQ CDS	1189	AAC -> TTC	Substitution
2159299	2159301	2	GGC -> AGA	Substitution	95.90%	G -> R	yegQ CDS	151	GGC -> AGA	Substitution
2160401	2160401	1	G -> A	SNP (transition)	95.40%	G -> D	yegQ CDS	1253	GGC -> GAC	Substitution
2159216	2159215	0	=+A	Insertion	95.20%		yegQ CDS	68		Frame Shift
2160448	2160450	3	AAT -> CTA	Substitution	95.10%	N -> L	yegQ CDS	1300	AAT -> CTA	Substitution
2160440	2160442	3	TTG -> CGA	Substitution	94.40%	LE -> PK	yegQ CDS	1292	CTT,GAG -> CCG,AAG	Substitution
2159225	2159226	2	CT -> AA	Substitution	94.20%	A -> E	yegQ CDS	77	GCT -> GAA	Substitution
2160421	2160423	3	CTC -> GAT	Substitution	94.10%	L -> D	yegQ CDS	1273	CTC -> GAT	Substitution
2159345	2159345	1	T -> A	SNP (transversion)	84.10%	V -> D	yegQ CDS	197	GTC -> GAC	Substitution
2159341	2159341	1	=-G	Deletion	74.70%		yegQ CDS	193		Frame Shift
2160309	2160309	1	C -> A	SNP (transversion)	65.60%	D -> E	yegQ CDS	1161	GAC -> GAA	Substitution
2160290	2160290	1	A -> C	SNP (transversion)	60.00%	N -> T	yegQ CDS	1142	AAT -> ACT	Substitution
2160301	2160301	1	G -> A	SNP (transition)	59.00%	V -> I	yegQ CDS	1153	GTT -> ATT	Substitution
2160286	2160288	3	AAA -> GCT	Substitution	50.00%	K -> A	yegQ CDS	1138	AAA -> GCT	Substitution
2160283	2160283	1	G -> C	SNP (transversion)	50.00%	V -> L	yegQ CDS	1135	GTG -> CTG	Substitution
2160283	2160283	1	G -> T	SNP (transversion)	50.00%	V -> L	yegQ CDS	1135	GTG -> TTG	Substitution
2159363	2159363	1	A -> G	SNP (transition)	50.00%	N -> S	yegQ CDS	215	AAC -> AGC	Substitution

121655	121655	1	G -> C	SNP (transversion)	41.20%	E -> Q	aceE CDS	2152	GAA -> CAA	Substitution
4076057	4076057	1	A -> G	SNP (transition)	38.30%		fdoH CDS	63	CCT -> CCC	None
4076055	4076055	1	T -> G	SNP (transversion)	35.20%	Q -> P	fdoH CDS	65	CAG -> CCG	Substitution
2101595	2101595	1	T -> G	SNP (transversion)	34.60%	L -> F	glf CDS	216	TTA -> TTC	Substitution
2160289	2160289	1	A -> C	SNP (transversion)	33.30%	N -> H	yegQ CDS	1141	AAT -> CAT	Substitution
2101593	2101593	1	A -> T	SNP (transversion)	32.10%	V -> E	glf CDS	218	GTA -> GAA	Substitution
2101590	2101590	1	T -> A	SNP (transversion)	29.70%	E -> V	glf CDS	221	GAA -> GTA	Substitution
121632	121632	1	T -> C	SNP (transition)	29.70%	I -> T	aceE CDS	2129	ATC -> ACC	Substitution
4076052	4076052	1	G -> C	SNP (transversion)	29.60%	A -> G	fdoH CDS	68	GCG -> GGG	Substitution
4076068	4076068	1	T -> G	SNP (transversion)	26.30%	T -> P	fdoH CDS	52	ACC -> CCC	Substitution
2101629	2101629	1	T -> C	SNP (transition)	25.80%	N -> S	glf CDS	182	AAT -> AGT	Substitution
121676	121676	1	G -> T	SNP (transversion)	25.50%	G -> C	aceE CDS	2173	GGT -> TGT	Substitution
2159341	2159341	1	G -> T	SNP (transversion)	25.30%	V -> L	yegQ CDS	193	GTG -> TTG	Substitution
2159363	2159363	1	=-A	Deletion	25.00%		yegQ CDS	215		Frame Shift

**Appx. E.7: All mutations found in *AycbU::kan<sup>R</sup>* Keio mutant**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
998390	998391	2	AT -> TC	Substitution	97.6% -> 97.7%	I -> S	ycbU CDS	46	ATT -> TCT	Substitution
998791	998795	5	CACAC -> GGGGA	Substitution	96.9% -> 97.0%	NTL -> KGI	ycbU CDS	447	AAC,ACA,CTC -> AAG,GGG,ATC	Substitution
998745	998748	4	CGCT -> GCAG	Substitution	96.4% -> 96.7%	PL -> RS	ycbU CDS	401	CCG,CTC -> CGC,AGC	Substitution
998723	998723	1	G -> TA	Insertion	95.5% -> 95.7%		ycbU CDS	379		Frame Shift
998447	998448	2	GG -> CA	Substitution	95.4% -> 95.5%	G -> Q	ycbU CDS	103	GGG -> CAG	Substitution
998838	998839	2	CG -> TA	Substitution	95.0% -> 95.3%	T -> I	ycbU CDS	494	ACG -> ATA	Substitution
998349	998356	8	AGAAAAAA -> TTCCGGGG	Substitution	94.8% -> 95.1%	KKK -> IPG	ycbU CDS	5	AAG,AAA,AAA -> ATT,CCG,GGG	Substitution
998442	998445	4	TTTA -> GCAC	Substitution	94.6% -> 94.9%	IY -> ST	ycbU CDS	98	ATT,TAT -> AGC,ACT	Substitution
998408	998410	3	GGG -> TTC	Substitution	94.3% -> 94.5%	G -> F	ycbU CDS	64	GGG -> TTC	Substitution
998750	998754	5	AATCA -> GCATC	Substitution	93.5% -> 93.8%	NQ -> AS	ycbU CDS	406	AAT,CAG -> GCA,TCG	Substitution
998412	998413	2	TG -> GA	Substitution	93.5% -> 93.6%	M -> R	ycbU CDS	68	ATG -> AGA	Substitution
998702	998703	2	AT -> GC	Substitution	92.7% -> 92.9%	I -> A	ycbU CDS	358	ATT -> GCT	Substitution
998450	998452	3	AAT -> GGC	Substitution	92.6% -> 92.8%	N -> G	ycbU CDS	106	AAT -> GGC	Substitution
998435	998440	6	ATGCAT -> GCCGCG	Substitution	92.5% -> 93.2%	MH -> AA	ycbU CDS	91	ATG,CAT -> GCC,GCG	Substitution
998454	998458	5	TGGTC -> CAAGG	Substitution	92.3% -> 93.5%	VV -> AR	ycbU CDS	110	GTG,GTC -> GCA,AGG	Substitution
998855	998860	6	GCGGTT -> CTCCAG	Substitution	91.8% -> 92.4%	AV -> LQ	ycbU CDS	511	GCG,GTT -> CTC,CAG	Substitution
998375	998379	5	ATCTT -> TCGAA	Substitution	91.1% -> 91.6%	IL -> SK	ycbU CDS	31	ATC,TTG -> TCG,AAG	Substitution
998778	998786	8	AAGCCGGGG -> GTTCTTCTA	Substitution	91.0% -> 93.5%	KAGD -> SSSN	ycbU CDS	434	AAA,GCC,GGG,GAT -> AGT,TCT,TCT,AAT	Substitution
998426	998433	8	CAAGTTAG -> GCTCACGC	Substitution	91.0% -> 92.7%	QVS -> AHA	ycbU CDS	82	CAA,GTT,AGT -> GCT,CAC,GCT	Substitution
998822	998830	9	ACAAAGGCG -> TATTCTCTA	Substitution	90.6% -> 90.8%	TKA -> YSL	ycbU CDS	478	ACA,AAG,GCG -> TAT,TCT,CTA	Substitution
998809	998815	7	TCGTTAT -> ATTCCGA	Substitution	90.3% -> 90.8%	LRY -> LFR	ycbU CDS	465	CTT,CGT,TAT -> CTA,TTC,CGA	Substitution
998492	998494	2	TTA -> GTC	Substitution	90.0% -> 92.9%	L -> V	ycbU CDS	148	TTA -> GTC	Substitution
998798	998806	8	AAATATCAA -> TTGAAGTTC	Substitution	89.9% -> 93.1%	KYQ -> LKF	ycbU CDS	454	AAA,TAT,CAA -> TTG,AAG, TTC	Substitution

998398	998406	9	CATCCATGT -> GTATAGGAA	Substitution	89.6% -> 90.9%	NIHV -> KYRN	ycbU CDS	54	AAC,ATC,CAT,GTC -> AAG,TAT,AGG,AAC	Substitution
998729	998731	3	AGT -> TCG	Substitution	88.4% -> 88.6%		ycbU CDS	385	AGT -> TCG	None
998736	998740	5	AAGAA -> TCCCG	Substitution	88.3% -> 89.0%	QE -> LP	ycbU CDS	392	CAA,GAA -> CTC,CCG	Substitution
998358	998365	8	CGATATTT -> TCCGTCGA	Substitution	88.0% -> 88.5%	TIF -> IRR	ycbU CDS	14	ACG,ATA,TTT -> ATC,CGT,CGA	Substitution
998462	998464	3	CAG -> GCT	Substitution	86.4% -> 87.0%	Q -> A	ycbU CDS	118	CAG -> GCT	Substitution
998846	998850	5	AATGC -> TCGAA	Substitution	86.2% -> 86.9%	NA -> SK	ycbU CDS	502	AAT,GCG -> TCG,AAG	Substitution
998418	998421	4	GGCC -> CTTT	Substitution	85.7% -> 86.4%	GP -> AF	ycbU CDS	74	GGG,CCT -> GCT,TTT	Substitution
998487	998489	3	GTG -> AGC	Substitution	81.0% -> 83.3%	SA -> KP	ycbU CDS	143	AGT,GCA -> AAG,CCA	Substitution
998694	998699	6	CAACGG -> GCGAAT	Substitution	80.9% -> 84.6%	ATG -> GEW	ycbU CDS	350	GCA,ACG,GGG -> GGC,GAA,TGG	Substitution
998708	998721	12	GTGCAAATCTTGA -> CGCTTCTCTCGTGCT	Substitution	78.9% -> 80.8%	VQILD -> RFLVL	ycbU CDS	364	GTG,CAA,ATT,CTT,GAT -> CGC,TTC,CTC,GTG,CTT	Substitution
998756	998776	21	GTCCAGCCTC TTACGCCCTTA -> CCTTCTATCGC CTTCTTGACG	Substitution	77.7% -> 82.5%	VQPLTPL - > PSIAFLT	ycbU CDS	412	GTC,CAG,CCT,CTT, ACG,CCC,TTA -> CCT,TCT,ATC,GCC, TTC,TTG,ACG	Substitution
998685	998688	4	GTGG -> AGCT	Substitution	76.2% -> 80.6%	GG -> EL	ycbU CDS	341	GGT,GGT -> GAG,CTT	Substitution
998465	998473	8	GGCTGTGAT -> AAAGGAAGC	Substitution	76.1% -> 82.8%	GCD -> KGS	ycbU CDS	121	GGC,TGT,GAT -> AAA,GGA,AGC	Substitution
998690	998692	3	ATG -> GGC	Substitution	74.4% -> 80.6%	M -> G	ycbU CDS	346	ATG -> GGC	Substitution
998679	998683	5	GAAGC -> CTGAA	Substitution	72.7% -> 82.4%	GS -> AE	ycbU CDS	335	GGA,AGC -> GCT,GAA	Substitution
998475	998482	7	TCGCCACC -> GAACACGT	Substitution	71.4% -> 77.8%	VAT -> GTR	ycbU CDS	131	GTC,GCC,ACC -> GGA,ACA,CGT	Substitution
998496	998497	2	AA -> GC	Substitution	66.7% -> 83.3%	Q -> R	ycbU CDS	152	CAA -> CGC	Substitution
998672	998674	3	ACA -> TAT	Substitution	100.00%	T -> Y	ycbU CDS	328	ACA -> TAT	Substitution
998574	998574	1	T -> C	SNP (transition)	100.00%	I -> T	ycbU CDS	230	ATC -> ACC	Substitution
998367	998368	2	AG -> C	Deletion	100.00%		ycbU CDS	23		Frame Shift
998416	998416	1	=-T	Deletion	99.40%		ycbU CDS	72		Frame Shift
998733	998733	1	A -> C	SNP (transversion)	99.30%	Q -> P	ycbU CDS	389	CAG -> CCG	Substitution
998726	998727	2	CA -> GT	Substitution	99.20%	Q -> V	ycbU CDS	382	CAA -> GTA	Substitution
2719426	2719426	1	T -> C	SNP (transition)	99.10%					
998852	998853	2	AC -> CA	Substitution	98.50%	T -> Q	ycbU CDS	508	ACG -> CAG	Substitution

998372	998373	2	GT -> AG	Substitution	98.40%	V -> S	ycbU CDS	28	GTT -> AGT	Substitution
998862	998866	5	TCTAT -> CTACA	Substitution	97.80%	LY -> PT	ycbU CDS	518	CTC,TAT -> CCT,ACA	Substitution
998817	998818	2	AG -> GT	Substitution	97.60%	K -> S	ycbU CDS	473	AAG -> AGT	Substitution
998393	998395	3	CTT -> AGA	Substitution	97.10%	L -> R	ycbU CDS	49	CTT -> AGA	Substitution
998384	998388	4	TTTAG -> CTATT	Substitution	96.80%	FS -> LF	ycbU CDS	40	TTT,AGC -> CTA,TTC	Substitution
998834	998836	3	GCA -> AGT	Substitution	96.50%	A -> S	ycbU CDS	490	GCA -> AGT	Substitution
998743	998743	1	C -> T	SNP (transition)	96.30%		ycbU CDS	399	ATC -> ATT	None
3752513	3752513	1	T -> G	SNP (transversion)	95.90%	I -> L	selB CDS	709	ATC -> CTC	Substitution
998460	998460	1	A -> C	SNP (transversion)	95.70%	D -> A	ycbU CDS	116	GAT -> GCT	Substitution
998832	998832	1	G -> A	SNP (transition)	94.70%	G -> E	ycbU CDS	488	GGA -> GAA	Substitution
998842	998844	3	CGG -> AAC	Substitution	94.60%	GG -> GT	ycbU CDS	498	GGC,GGT -> GGA,ACT	Substitution
998484	998484	1	A -> G	SNP (transition)	91.70%	K -> R	ycbU CDS	140	AAA -> AGA	Substitution
998706	998706	1	C -> A	SNP (transversion)	88.90%	A -> D	ycbU CDS	362	GCC -> GAC	Substitution
121404	121404	1	T -> A	SNP (transversion)	87.80%	L -> Q	aceE CDS	1901	CTG -> CAG	Substitution
998675	998675	1	G -> T	SNP (transversion)	50.00%	G -> C	ycbU CDS	331	GGC -> TGC	Substitution
998519	998519	1	A -> C	SNP (transversion)	50.00%	N -> H	ycbU CDS	175	AAT -> CAT	Substitution
998499	998499	1	A -> G	SNP (transition)	50.00%	N -> S	ycbU CDS	155	AAT -> AGT	Substitution
4076057	4076057	1	A -> G	SNP (transition)	33.30%		fdoH CDS	63	CCT -> CCC	None
3991413	3991413	1	A -> C	SNP (transversion)	29.90%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
4076062	4076062	1	C -> G	SNP (transversion)	28.30%	A -> P	fdoH CDS	58	GCG -> CCG	Substitution
998683	998683	1	C -> A	SNP (transversion)	26.30%	S -> R	ycbU CDS	339	AGC -> AGA	Substitution

**Appx. E.8: All mutations found in *ybjJ::kan<sup>R</sup>* Keio mutant**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
881753	881756	4	TAAC -> AGCA	Substitution	98.1% -> 98.2%	GY -> VL	ybjJ CDS	1040	GGT,TAT -> GTG,CTT	Substitution
882715	882717	2	TAA -> GAG	Substitution	98.0% -> 98.2%		ybjJ CDS	79	TTA -> CTC	None
881690	881691	2	CT -> AG	Substitution	98.0% -> 98.1%	S -> L	ybjJ CDS	1105	AGT -> CTT	Substitution
882622	882623	2	GA -> AT	Substitution	97.9% -> 98.0%	I -> N	ybjJ CDS	173	ATC -> AAT	Substitution
881687	881688	2	GC -> AG	Substitution	97.5% -> 97.6%	A -> L	ybjJ CDS	1108	GCA -> CTA	Substitution
882786	882788	3	TTA -> CCC	Substitution	97.2% -> 97.4%	VN -> GD	ybjJ CDS	8	GTA,AAT -> GGG,GAT	Substitution
881696	881697	2	AA -> CG	Substitution	97.0% -> 97.1%	L -> R	ybjJ CDS	1099	TTA -> CGA	Substitution
882682	882686	4	GATAG -> CAGCC	Substitution	96.7% -> 97.3%	AI -> GL	ybjJ CDS	110	GCT,ATC -> GGG,CTG	Substitution
881749	881751	3	CAG -> GTA	Substitution	96.5% -> 96.6%	L -> Y	ybjJ CDS	1045	CTG -> TAC	Substitution
881783	881786	4	GTTG -> CCAA	Substitution	96.0% -> 96.3%	PT -> LG	ybjJ CDS	1010	CCA,ACC -> CTT,GGC	Substitution
881770	881771	2	CA -> AG	Substitution	95.9% -> 96.0%	V -> A	ybjJ CDS	1025	GTG -> GCT	Substitution
882650	882655	6	ATTCA -> GGA CTG	Substitution	95.5% -> 96.0%	AEM -> ASP	ybjJ CDS	141	GCT,GAA,ATG -> GCC,AGT,CCG	Substitution
882601	882604	4	GAGA -> CCAG	Substitution	95.0% -> 96.1%	IL -> IW	ybjJ CDS	192	ATT,CTC -> ATC,TGG	Substitution
882693	882696	3	TACG -> CCCT	Substitution	94.9% -> 95.2%	RT -> RA	ybjJ CDS	100	CGT,ACG -> AGG,GCG	Substitution
882792	882791	0	=+AA	Insertion	94.8% -> 95.1%		ybjJ CDS	5		Frame Shift
881773	881777	4	ACTGA -> CCATT	Substitution	94.2% -> 94.7%	VS -> EW	ybjJ CDS	1019	GTC,AGT -> GAA,TGG	Substitution
882673	882680	6	AATATCGC -> CTTCCTTTA	Insertion	94.1% -> 96.2%		ybjJ CDS	116		Frame Shift
882703	882708	5	CCAGGA -> TTGCGG	Substitution	94.0% -> 94.3%	SW -> PQ	ybjJ CDS	88	TCC,TGG -> CCG,CAA	Substitution
881741	881742	2	AG -> GC	Substitution	93.9% -> 94.2%	L -> A	ybjJ CDS	1054	CTC -> GCC	Substitution
882761	882763	3	CGT -> AAC	Substitution	93.6% -> 93.8%	KR -> KF	ybjJ CDS	33	AAA,CGC -> AAG,TTC	Substitution
881608	881616	7	CTGCGTTTT -> TGTAGGCTG	Substitution	93.5% -> 94.4%	KTQ -> QPT	ybjJ CDS	1180	AAA,ACG,CAG -> CAG,CCT,ACA	Substitution
881652	881654	3	CCG -> GAA	Substitution	93.5% -> 93.7%	AA -> VP	ybjJ CDS	1142	GCG,GCT -> GTT,CCT	Substitution
882664	882671	8	GACAGAGA -> CGTGTCC	Substitution	93.1% -> 93.7%	LSV -> RNT	ybjJ CDS	125	CTC,TCT,GTC -> CCG,AAC,ACG	Substitution
882631	882635	5	ACCAA -> GGGTC	Substitution	92.8% -> 93.2%		ybjJ CDS	161		Truncation

882737	882738	2	AA -> TT	Substitution	92.6% -> 92.9%	F -> N	ybjJ CDS	58	TTC -> AAC	Substitution
881716	881721	6	ATAGCC -> GCGATG	Substitution	91.4% -> 91.8%	GY -> HR	ybjJ CDS	1075	GGC,TAT -> CAT,CGC	Substitution
882746	882752	7	AGCGCCC -> TTTCTAG	Substitution	91.1% -> 91.6%	WAL -> SRK	ybjJ CDS	44	TGG,GCG,CTG -> TCT,AGA,AAG	Substitution
881794	881802	8	GGGGCCGGT -> AGCAATATC	Substitution	90.9% -> 92.6%	TGP -> DIA	ybjJ CDS	994	ACC,GGC,CCC -> GAT,ATT,GCT	Substitution
881635	881636	2	GG -> TATA	Insertion	90.5% -> 90.8%		ybjJ CDS	1160		Truncation
881759	881768	10	GTCGTTGCTA -> AGGAAGCGGT	Substitution	89.0% -> 90.3%	VATT -> DRFL	ybjJ CDS	1028	GTA,GCA,ACG,ACC -> GAC,CGC,TTC,CTC	Substitution
881707	881713	7	TTCGCCG -> CGATAGA	Substitution	89.0% -> 89.8%	LGE -> LLS	ybjJ CDS	1083	CTC,GGC,GAA -> CTT,CTA,TCG	Substitution
882638	882648	11	AGAACACCGCC -> CACCGTTTCTG	Substitution	88.8% -> 89.6%	GGVL -> QKRC	ybjJ CDS	148	GGC,GGT,GTT,CTC -> CAG,AAA,CGG,TGC	Substitution
882719	882725	7	AGGCCTG -> TTCAAAA	Substitution	87.7% -> 88.0%	PGL -> LLK	ybjJ CDS	71	CCA,GGC,CTG -> CTT,TTG,AAG	Substitution
881656	881661	6	GAGAAT -> TTCGGA	Substitution	87.7% -> 88.0%	IL -> SE	ybjJ CDS	1135	ATT,CTC -> TCC,GAA	Substitution
882728	882732	5	AAAAA -> GCTCT	Substitution	87.4% -> 88.1%	FL -> RA	ybjJ CDS	64	TTT,TTG -> AGA,GCG	Substitution
881663	881678	16	ACCAGCGC CAGTACAA -> TAGGAACTT CAAGATC	Substitution	85.8% -> 87.1%		ybjJ CDS	1118		Truncation
881730	881738	9	GCGGCCCGA -> AATCGGGAG	Substitution	85.8% -> 86.8%	VGPP -> APDS	ybjJ CDS	1058	GTC,GGG,CCG,CCG -> GCT,CCC,GAT,TCG	Substitution
882596	882600	5	GAGCA -> CTTGT	Substitution	83.8% -> 87.1%	CS -> TR	ybjJ CDS	196	TGC,TCG -> ACA,AGG	Substitution
882769	882777	9	TGCATTACG -> ACTGCAGGT	Substitution	83.8% -> 84.5%	RNA -> TCS	ybjJ CDS	19	CGT,AAT,GCA -> ACC,TGC,AGT	Substitution
881804	881805	2	TC -> CG	Substitution	83.3% -> 86.7%	D -> R	ybjJ CDS	991	GAT -> CGT	Substitution
882587	882593	7	AACCACG -> GCGTTTT	Substitution	80.0% -> 88.2%	AWL -> ENA	ybjJ CDS	203	GCG,TGG,TTA -> GAA,AAC,GCA	Substitution
881618	881633	14	GTATCGGG TTTGGCGA -> AGCTGCTTC GAAGTTC	Substitution	69.1% -> 73.1%	VAKPDT - > GTSKQL	ybjJ CDS	1163	GTC,GCC,AAA,CCC,GAT,ACC > GGA,ACT,TCG,AAG,CAG,CTC	Substitution
882610	882620	10	GCTCATCGAAC -> CCAGTAGCTGA	Substitution	63.7% -> 82.8%	GSMS -> VSYW	ybjJ CDS	176	GGT,TCG,ATG,AGC -> GTC,AGC,TAC,TGG	Substitution

2595130	2595139	10	GCCAATGGCG -> TAGCGCTTAT	Substitution	37.2% -> 40.3%		hyfA CDS	571		Truncation
2595125	2595128	4	GGTT -> TAAG	Substitution	36.1% -> 39.0%	WF -> LS	hyfA CDS	566	TGG,TTT -> TTA,AGT	Substitution
1203239	1203240	2	GA -> AT	Substitution	35.2% -> 35.8%	E -> I	stfP CDS	283	GAA -> ATA	Substitution
2595154	2595156	3	TCC -> AAG	Substitution	31.5% -> 32.5%	S -> K	hyfA CDS	595	TCC -> AAG	Substitution
2595161	2595162	2	CT -> AA	Substitution	25.7% -> 26.3%	T -> K	hyfA CDS	602	ACT -> AAA	Substitution
1203262	1203266	4	AGCAA -> CTCTG	Substitution	25.6% -> 31.2%	GAK -> GSE	stfP CDS	306	GGA,GCA,AAA -> GGC,TCT,GAA	Substitution
1205055	1205058	3	AGAG -> TGCT	Substitution	24.4% -> 25.7%	GS -> GA	stfE CDS	18	GGC,TCT -> GGA,GCA	Substitution
1203228	1203234	7	AAACGGC -> TCTTCAT	Substitution	23.9% -> 29.4%	QTA -> LFI	stfP CDS	272	CAA,ACG,GCT -> CTC,TTC,ATT	Substitution
190011	190013	3	AAG -> GCC	Substitution	23.4% -> 25.3%	K -> A	dxr CDS	4	AAG -> GCC	Substitution
882662	882662	1	G -> T	SNP (transversion)	100.00%		ybjJ CDS	134		Truncation
882584	882585	2	AC -> CT	Substitution	100.00%	V -> R	ybjJ CDS	211	GTG -> AGG	Substitution
881809	881809	1	G -> A	SNP (transition)	100.00%		ybjJ CDS	987	GCC -> GCT	None
881807	881807	1	C -> G	SNP (transversion)	100.00%	S -> T	ybjJ CDS	989	AGC -> ACC	Substitution
881781	881781	1	G -> C	SNP (transversion)	100.00%	R -> G	ybjJ CDS	1015	CGC -> GGC	Substitution
881723	881723	1	=-A	Deletion	100.00%		ybjJ CDS	1073		Frame Shift
882710	882711	2	GC -> AG	Substitution	99.70%	A -> L	ybjJ CDS	85	GCG -> CTG	Substitution
881700	881700	1	C -> A	SNP (transversion)	99.70%		ybjJ CDS	1096		Truncation
271022	271022	1	G -> A	SNP (transition)	99.60%					
882713	882713	1	A -> G	SNP (transition)	99.40%	M -> T	ybjJ CDS	83	ATG -> ACG	Substitution
2719426	2719426	1	T -> C	SNP (transition)	99.30%					
882778	882778	1	T -> C	SNP (transition)	99.30%		ybjJ CDS	18	TCA -> TCG	None
882629	882629	1	A -> C	SNP (transversion)	99.10%	L -> R	ybjJ CDS	167	CTG -> CGG	Substitution



882743	882743	1	A -> T	SNP (transversion)	99.00%	F -> Y	ybjJ CDS	53	TTT -> TAT	Substitution
882740	882740	1	A -> C	SNP (transversion)	99.00%	M -> R	ybjJ CDS	56	ATG -> AGG	Substitution
882658	882659	2	GA -> TT	Substitution	99.00%	I -> K	ybjJ CDS	137	ATC -> AAA	Substitution
881747	881747	1	G -> C	SNP (transversion)	99.00%	A -> G	ybjJ CDS	1049	GCT -> GGT	Substitution
881726	881726	1	A -> T	SNP (transversion)	98.80%	L -> Q	ybjJ CDS	1070	CTG -> CAG	Substitution
881788	881789	2	TG -> CT	Substitution	98.40%	A -> E	ybjJ CDS	1007	GCA -> GAG	Substitution
881705	881705	1	T -> G	SNP (transversion)	98.40%	H -> P	ybjJ CDS	1091	CAT -> CCT	Substitution
882626	882627	2	GA -> AT	Substitution	98.10%	S -> M	ybjJ CDS	169	TCG -> ATG	Substitution
882698	882699	2	GT -> AG	Substitution	98.00%	T -> L	ybjJ CDS	97	ACC -> CTC	Substitution
882688	882689	2	AG -> TT	Substitution	97.90%	P -> Q	ybjJ CDS	107	CCT -> CAA	Substitution
881646	881646	1	C -> G	SNP (transversion)	97.80%	V -> L	ybjJ CDS	1150	GTC -> CTC	Substitution
121919	121919	1	A -> G	SNP (transition)	97.80%	K -> E	aceE CDS	2416	AAA -> GAA	Substitution
881693	881694	2	CG -> AC	Substitution	97.70%	R -> V	ybjJ CDS	1102	CGT -> GTT	Substitution
881681	881683	3	AGC -> CTT	Substitution	97.70%	ML -> IR	ybjJ CDS	1113	ATG,CTG -> ATA,AGG	Substitution
882781	882783	2	AGA -> CGG	Substitution	97.40%	S -> P	ybjJ CDS	13	TCT -> CCG	Substitution
882767	882767	1	A -> G	SNP (transition)	97.10%	L -> S	ybjJ CDS	29	TTG -> TCG	Substitution
882758	882758	1	C -> A	SNP (transversion)	97.10%	R -> L	ybjJ CDS	38	CGA -> CTA	Substitution
881745	881745	1	A -> T	SNP (transversion)	97.00%	F -> I	ybjJ CDS	1051	TTC -> ATC	Substitution
881702	881702	1	T -> G	SNP (transversion)	96.90%	Y -> S	ybjJ CDS	1094	TAT -> TCT	Substitution
881642	881643	2	GC -> TA	Substitution	95.80%		ybjJ CDS	1153		Truncation
882755	882756	2	GT -> AA	Substitution	94.90%	T -> F	ybjJ CDS	40	ACC -> TTC	Substitution
881791	881791	1	A -> T	SNP (transversion)	94.70%	D -> E	ybjJ CDS	1005	GAT -> GAA	Substitution

882608	882608	1	C -> G	SNP (transversion)	91.40%	G -> A	ybjJ CDS	188	GGT -> GCT	Substitution
1203245	1203245	1	C -> G	SNP (transversion)	40.10%	L -> V	stfP CDS	289	CTT -> GTT	Substitution
4076057	4076057	1	A -> G	SNP (transition)	38.40%		fdoH CDS	63	CCT -> CCC	None
1205075	1205075	1	C -> G	SNP (transversion)	38.00%	V -> L	stfE CDS	1	GTT -> CTT	Substitution
2595152	2595152	1	T -> A	SNP (transversion)	35.60%	L -> H	hyfA CDS	593	CTT -> CAT	Substitution
4076055	4076055	1	T -> G	SNP (transversion)	35.10%	Q -> P	fdoH CDS	65	CAG -> CCG	Substitution
2595122	2595122	1	G -> C	SNP (transversion)	33.20%	S -> T	hyfA CDS	563	AGC -> ACC	Substitution
1203236	1203236	1	C -> T	SNP (transition)	32.40%	L -> F	stfP CDS	280	CTC -> TTC	Substitution
2595158	2595158	1	T -> G	SNP (transversion)	30.40%	L -> R	hyfA CDS	599	CTC -> CGC	Substitution
3991413	3991413	1	A -> C	SNP (transversion)	29.20%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
190008	190008	1	A -> C	SNP (transversion)	29.20%		dxr CDS	1	ATG -> CTG	None
3991425	3991425	1	T -> G	SNP (transversion)	28.10%	L -> R	uvrD CDS	83	CTG -> CGG	Substitution
3991428	3991428	1	C -> G	SNP (transversion)	26.20%	A -> G	uvrD CDS	86	GCG -> GGG	Substitution
1688395	1688395	1	A -> C	SNP (transversion)	25.90%		uidB CDS	126	GGT -> GGG	None
4076055	4076055	1	T -> C	SNP (transition)	25.60%	Q -> R	fdoH CDS	65	CAG -> CGG	Substitution

**Appx. E.9: All mutations found in *AmprA::kan<sup>R</sup>* Keio mutant**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
2804565	2804566	2	CA -> G	Deletion	99.4% -> 99.5%		mprA CDS	437		Frame Shift
2804268	2804272	5	TGCTG -> AAGCC	Substitution	99.3% -> 99.4%	ML -> KA	mprA CDS	140	ATG,CTG -> AAA,GCC	Substitution
2804568	2804569	2	AA -> GG	Substitution	99.2% -> 99.5%	E -> G	mprA CDS	440	GAA -> GGG	Substitution
2804624	2804626	2	GAA -> CAG	Substitution	98.1% -> 98.3%	E -> Q	mprA CDS	496	GAA -> CAG	Substitution
2804544	2804545	2	TC -> TTCT	Insertion	96.2% -> 96.5%		mprA CDS	416		Frame Shift
2804605	2804609	5	CTCCC -> AGAAA	Substitution	96.0% -> 96.5%	LSR -> LES	mprA CDS	477	CTC,TCC,CGT -> CTA,GAA,AGT	Substitution
2804300	2804302	2	TTT -> ATG	Substitution	96.0% -> 96.4%	F -> M	mprA CDS	172	TTT -> ATG	Substitution
2804221	2804226	6	TCTTTG -> CAAGCA	Substitution	95.7% -> 96.0%	RLC -> RKH	mprA CDS	93	CGT,CTT,TGC -> CGC,AAG,CAC	Substitution
2804612	2804614	2	CTC -> ATA	Substitution	95.7% -> 95.9%	L -> I	mprA CDS	484	CTC -> ATA	Substitution
2804571	2804576	6	AAGATC -> TCTTGA	Substitution	95.1% -> 95.2%	KDQ -> ILK	mprA CDS	443	AAA,GAT,CAG -> ATC,TTG,AAG	Substitution
2804274	2804278	4	AGGCT -> GTCCG	Substitution	94.6% -> 95.1%	KA -> SP	mprA CDS	146	AAG,GCT -> AGT,CCG	Substitution
2804262	2804266	4	ATAAA -> CGTAG	Substitution	94.5% -> 95.0%		mprA CDS	134		Truncation
2804541	2804542	2	AA -> GC	Substitution	94.5% -> 94.6%	Q -> R	mprA CDS	413	CAA -> CGC	Substitution
2804214	2804218	5	TGACT -> GCTGC	Substitution	94.3% -> 94.9%	LT -> RC	mprA CDS	86	CTG,ACT -> CGC,TGC	Substitution
2804548	2804551	4	GTCC -> ACGA	Substitution	94.0% -> 94.3%		mprA CDS	420		Truncation
2804243	2804250	8	AAGCTGCT -> TGCTAAAG	Substitution	92.8% -> 93.3%		mprA CDS	115		Truncation
2804592	2804595	4	CCCG -> GTTC	Substitution	92.8% -> 92.9%	TR -> SS	mprA CDS	464	ACC,CGC -> AGT,TCC	Substitution
2804628	2804634	6	AAGACGG -> TCCAGCC	Substitution	92.6% -> 93.9%	QDG -> LQP	mprA CDS	500	CAA,GAC,GGT -> CTC,CAG,CCT	Substitution
2804513	2804519	7	TTACCAC -> CCCGATT	Substitution	91.8% -> 93.5%	LPP -> PDS	mprA CDS	385	TTA,CCA,CCG -> CCC,GAT,TCG	Substitution
2804210	2804212	3	CTT -> TCA	Substitution	91.7% -> 92.0%	L -> S	mprA CDS	82	CTT -> TCA	Substitution
2804162	2804167	6	CTAAAA -> AGTTCC	Substitution	91.5% -> 91.7%	LK -> SS	mprA CDS	34	CTA,AAA -> AGT,TCC	Substitution

2804291	2804298	7	GAGACGTT -> TGACCCCG	Substitution	91.4% -> 92.1%		mprA CDS	163		Truncation
2804135	2804139	5	AGTTC -> CCGGG	Substitution	90.9% -> 91.2%	SS -> PG	mprA CDS	7	AGT,TCG -> CCG,GGG	Substitution
2804228	2804240	12	ATGCACATGCAAA -> TCAGGGCGCAAGG	Substitution	90.6% -> 91.5%	MHM QS - > SGRKG	mprA CDS	100	ATG,CAC,ATG,CAA,AGC -> TCA,GGG,CGC,AAG,GGC	Substitution
2804141	2804146	5	TTTACG -> ATCCGT	Substitution	90.4% -> 92.1%	FT -> IR	mprA CDS	13	TTT,ACG -> ATC,CGT	Substitution
2804463	2804466	4	GCCT -> TGGC	Substitution	89.7% -> 90.9%	CL -> LA	mprA CDS	335	TGC,CTG -> TTG,GCG	Substitution
2804499	2804505	7	TTTTGCG -> ACGGTAT	Substitution	89.5% -> 89.9%	FLR -> YGI	mprA CDS	371	TTT,TTG,CGC -> TAC,GGT,ATC	Substitution
2804148	2804151	4	CCAT -> GACC	Substitution	89.3% -> 89.5%	PI -> RP	mprA CDS	20	CCC,ATT -> CGA,CCT	Substitution
2804468	2804479	11	CATCTGCAATTA -> GCGAATGGGCTG	Substitution	89.1% -> 91.7%	HLQL -> ANGL	mprA CDS	340	CAT,CTG,CAA,TTA -> GCG,AAT,GGG,CTG	Substitution
2804156	2804161	6	CAAATG -> GTTCGA	Substitution	88.9% -> 89.1%	QM -> VR	mprA CDS	28	CAA,ATG -> GTT,CGA	Substitution
2804255	2804259	5	AACCG -> CGGAA	Substitution	88.8% -> 89.5%	NR -> RN	mprA CDS	127	AAC,CGC -> CGG,AAC	Substitution
2804582	2804590	9	GAGCAAATC -> CTATTCCGA	Substitution	88.5% -> 88.9%	EQI -> LFR	mprA CDS	454	GAG,CAA,ATC -> CTA,TTC,CGA	Substitution
2804616	2804619	4	ACCA -> GAAC TTC	Insertion	87.9% -> 88.2%	DQ -> GTS	mprA CDS	488	GAC,CAG -> GGA,ACT,TCG	Insertion
2804282	2804289	8	GGGATTAA -> AAACGGTG	Substitution	87.5% -> 90.8%	GIN -> KRC	mprA CDS	154	GGG,ATT,AAC -> AAA,CGG,TGC	Substitution
2804307	2804316	10	CGTTGATTAC -> TCAGCTACTG	Substitution	83.9% -> 95.5%	ALIT -> VSYW	mprA CDS	179	GCG,TTG,ATT,ACG -> GTC,AGC,TAC,TGG	Substitution
2804171	2804185	14	CGCGCCAGCCGCCAC -> TCTCTAGAAAGTATA	Substitution	82.2% -> 83.4%	RASRH -> SLESI	mprA CDS	43	CGC,GCC,AGC,CGC,CAC -> TCT,CTA,GAA,AGT,ATA	Substitution
2804193	2804206	14	TTCCTTATCAGGAG - > CAGAGCGCTTTTGA	Substitution	79.3% -> 80.2%		mprA CDS	65		Truncation
2804484	2804497	14	AAAAAGGTCACGAG -> CTCCTCGTGCTT	Deletion	60.4% -> 74.4%		mprA CDS	356		Frame Shift
2804456	2804458	3	CGC -> AAG	Substitution	58.8% -> 62.5%	R -> K	mprA CDS	328	CGC -> AAG	Substitution
2804444	2804446	2	AGC -> CGT	Substitution	58.3% -> 77.8%	S -> R	mprA CDS	316	AGC -> CGT	Substitution
2804451	2804452	2	AC -> TT	Substitution	52.0% -> 54.2%	N -> I	mprA CDS	323	AAC -> ATT	Substitution
2804453	2804455	3	GAT -> CTG	Substitution	42.1% -> 48.5%	D -> L	mprA CDS	325	GAT -> CTG	Substitution
2804496	2804497	2	AG -> TT	Substitution	39.2% -> 39.6%	E -> V	mprA CDS	368	GAG -> GTT	Substitution

3790199	3790205	7	TGGTGGG -> GATCTTA	Substitution	38.3% -> 44.0%	IGG -> MIL	waaC CDS	861	ATT,GGT,GGG -> ATG,ATC,TTA	Substitution
3790191	3790194	4	GGAT -> ATCA	Substitution	38.3% -> 40.3%	GL -> II	waaC CDS	853	GGA,TTA -> ATC,ATA	Substitution
4576628	4576632	5	AACAT -> TCGCA	Substitution	37.0% -> 42.3%	ML -> CE	hsdR CDS	1	ATG,TTA -> TGC,GAA	Start Codon Loss
3790188	3790189	2	CC -> AG	Substitution	35.2% -> 35.7%	P -> R	waaC CDS	850	CCG -> AGG	Substitution
3790206	3790211	5	TATGGG -> CCCAGC	Substitution	31.2% -> 32.2%	YG -> PS	waaC CDS	868	TAT,GGG -> CCC,AGC	Substitution
4576624	4576626	3	CCA -> TTG	Substitution	30.7% -> 35.2%	W -> Q	hsdR CDS	7	TGG -> CAA	Substitution
2804450	2804452	3	AAC -> TTG	Substitution	28.0% -> 30.4%	N -> L	mprA CDS	322	AAC -> TTG	Substitution
2804456	2804458	3	CGC -> GAA	Substitution	25.5% -> 31.0%	R -> E	mprA CDS	328	CGC -> GAA	Substitution
3790218	3790219	2	CA -> GG	Substitution	25.1% -> 25.7%	Q -> G	waaC CDS	880	CAG -> GGG	Substitution
2804318	2804319	2	CT -> TA	Substitution	25.0% -> 100.0%		mprA CDS	190		Truncation
4576617	4576620	4	TTAA -> CAGG	Substitution	23.3% -> 28.2%	LN -> PD	hsdR CDS	13	TTA,AAT -> CCT,GAT	Substitution
2804562	2804562	1	C -> T	SNP (transition)	99.40%	T -> I	mprA CDS	434	ACA -> ATA	Substitution
2804511	2804511	1	T -> C	SNP (transition)	99.40%	V -> A	mprA CDS	383	GTT -> GCT	Substitution
2804534	2804537	3	CTGC -> TTCT	Substitution	99.20%	LH -> FY	mprA CDS	406	CTG,CAT -> TTC,TAT	Substitution
2804208	2804208	1	T -> G	SNP (transversion)	99.20%	I -> S	mprA CDS	80	ATC -> AGC	Substitution
2804169	2804169	1	T -> A	SNP (transversion)	99.10%	F -> Y	mprA CDS	41	TTT -> TAT	Substitution
2804508	2804509	2	AG -> CC	Substitution	98.80%	E -> A	mprA CDS	380	GAG -> GCC	Substitution
2701992	2701992	1	A -> C	SNP (transversion)	98.50%	L -> R	rseB CDS	122	CTG -> CGG	Substitution
3281162	3281162	1	G -> A	SNP (transition)	98.30%		yraH CDS	378	GTG -> GTA	None
2804579	2804579	1	C -> T	SNP (transition)	98.30%	L -> F	mprA CDS	451	CTC -> TTC	Substitution
2804189	2804190	2	GA -> AC	Substitution	98.00%	D -> T	mprA CDS	61	GAT -> ACT	Substitution
2804187	2804187	1	A -> G	SNP (transition)	98.00%	E -> G	mprA CDS	59	GAA -> GGA	Substitution
2804154	2804154	1	A -> C	SNP (transversion)	97.80%	E -> A	mprA CDS	26	GAA -> GCA	Substitution
2804601	2804602	2	TG -> CT	Substitution	97.60%	L -> S	mprA CDS	473	TTG -> TCT	Substitution
2804597	2804599	2	AAA -> TAT	Substitution	97.60%	K -> Y	mprA CDS	469	AAA -> TAT	Substitution
2804531	2804532	2	TG -> GC	Substitution	97.60%	C -> A	mprA CDS	403	TGC -> GCC	Substitution

2719426	2719426	1	T -> C	SNP (transition)	97.60%					
2804557	2804560	4	CAGC -> TCTA	Substitution	97.10%	LS -> LL	mprA CDS	429	CTC,AGC -> CTT,CTA	Substitution
2804252	2804252	1	G -> A	SNP (transition)	97.00%	E -> K	mprA CDS	124	GAG -> AAG	Substitution
2804636	2804638	3	GTG -> ACA	Substitution	96.70%	V -> T	mprA CDS	508	GTG -> ACA	Substitution
2804132	2804133	2	GA -> AT	Substitution	96.70%	D -> I	mprA CDS	4	GAT -> ATT	Substitution
2804304	2804305	2	TG -> AT	Substitution	95.70%	M -> N	mprA CDS	176	ATG -> AAT	Substitution
2804482	2804482	1	G -> C	SNP (transversion)	95.50%		mprA CDS	354	ACG -> ACC	None
2804622	2804622	1	T -> A	SNP (transversion)	95.30%	M -> K	mprA CDS	494	ATG -> AAG	Substitution
2804526	2804527	2	AT -> GC	Substitution	95.20%	H -> R	mprA CDS	398	CAT -> CGC	Substitution
2804553	2804554	2	CG -> TT	Substitution	95.10%	A -> V	mprA CDS	425	GCG -> GTT	Substitution
2804529	2804529	1	A -> T	SNP (transversion)	94.50%	N -> I	mprA CDS	401	AAC -> ATC	Substitution
2804318	2804318	1	C -> G	SNP (transversion)	75.00%	L -> V	mprA CDS	190	CTG -> GTG	Substitution
150047	150047	1	C -> A	SNP (transversion)	71.40%	A -> S	htrE CDS	1867	GCT -> TCT	Substitution
2804459	2804459	1	C -> A	SNP (transversion)	69.80%	R -> S	mprA CDS	331	CGC -> AGC	Substitution
2804443	2804443	1	A -> C	SNP (transversion)	66.70%	E -> D	mprA CDS	315	GAA -> GAC	Substitution
2804454	2804454	1	A -> C	SNP (transversion)	39.40%	D -> A	mprA CDS	326	GAT -> GCT	Substitution
4076057	4076057	1	A -> G	SNP (transition)	37.40%		fdoH CDS	63	CCT -> CCC	None
3790186	3790186	1	A -> G	SNP (transition)	36.50%	D -> G	waaC CDS	848	GAT -> GGT	Substitution
2804446	2804446	1	C -> T	SNP (transition)	33.30%		mprA CDS	318	AGC -> AGT	None
2804443	2804443	1	A -> G	SNP (transition)	33.30%		mprA CDS	315	GAA -> GAG	None
4076062	4076062	1	C -> G	SNP (transversion)	31.90%	A -> P	fdoH CDS	58	GCG -> CCG	Substitution
4576622	4576622	1	G -> A	SNP (transition)	30.90%	A -> V	hsdR CDS	11	GCC -> GTC	Substitution
4076055	4076055	1	T -> G	SNP (transversion)	30.70%	Q -> P	fdoH CDS	65	CAG -> CCG	Substitution

2804459	2804459	1	C -> G	SNP (transversion)	30.20%	R -> G	mprA CDS	331	CGC -> GGC	Substitution
3790216	3790216	1	A -> G	SNP (transition)	28.70%	N -> S	waaC CDS	878	AAT -> AGT	Substitution
1688395	1688395	1	A -> C	SNP (transversion)	27.20%		uidB CDS	126	GGT -> GGG	None
3790214	3790214	1	G -> T	SNP (transversion)	27.10%	K -> N	waaC CDS	876	AAG -> AAT	Substitution
3991413	3991413	1	A -> C	SNP (transversion)	26.50%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
4076055	4076055	1	T -> C	SNP (transition)	26.30%	Q -> R	fdoH CDS	65	CAG -> CGG	Substitution

**Appx. E.10: All mutations found in *ΔoppF::kan<sup>R</sup>* Keio mutant**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
360287	360292	6	GGAAT -> AATTC	Substitution	28.0% -> 29.2%					
2099562	2099564	3	AAG -> TGT	Substitution	25.9% -> 27.7%	L -> T	wbbH CDS	1144	CTT -> ACA	Substitution
1203239	1203240	2	GA -> AT	Substitution	25.1% -> 25.8%	E -> I	stfP CDS	283	GAA -> ATA	Substitution
360294	360298	5	AATTC -> GGCAG	Substitution	24.8% -> 27.3%					
3045862	3045862	1	T -> A	SNP (transversion)	56.30%	D -> V	ubiH CDS	1016	GAT -> GTT	Substitution
4076057	4076057	1	A -> G	SNP (transition)	46.80%		fdoH CDS	63	CCT -> CCC	None
1062305	1062305	1	C -> G	SNP (transversion)	36.30%					
123458	123458	1	T -> A	SNP (transversion)	35.00%	V -> D	aceF CDS	1277	GTT -> GAT	Substitution
4368798	4368798	1	C -> G	SNP (transversion)	33.10%					
1881093	1881093	1	A -> G	SNP (transition)	32.70%					
1881085	1881085	1	T -> G	SNP (transversion)	32.50%					
4076055	4076055	1	T -> G	SNP (transversion)	32.10%	Q -> P	fdoH CDS	65	CAG -> CCG	Substitution
2100724	2100724	1	T -> G	SNP (transversion)	32.00%	I -> L	glf CDS	1087	ATA -> CTA	Substitution
1881088	1881088	1	C -> G	SNP (transversion)	30.80%					
910661	910661	1	C -> G	SNP (transversion)	30.10%					
2222674	2222674	1	G -> C	SNP (transversion)	30.00%					
1203245	1203245	1	C -> G	SNP (transversion)	29.40%	L -> V	stfP CDS	289	CTT -> GTT	Substitution
3991413	3991413	1	A -> C	SNP (transversion)	29.10%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
1881082	1881082	1	A -> G	SNP (transition)	28.80%					
3469936	3469936	1	G -> C	SNP (transversion)	28.40%					
1076384	1076384	1	G -> C	SNP (transversion)	28.00%					
910664	910664	1	C -> G	SNP (transversion)	27.50%					
161082	161082	1	T -> C	SNP (transition)	27.50%					
2222677	2222677	1	G -> C	SNP (transversion)	27.10%					
161051	161051	1	A -> G	SNP (transition)	26.50%					



3498172	3498172	1	G -> C	SNP (transversion)	26.20%					
161088	161088	1	T -> C	SNP (transition)	26.00%					
4368790	4368790	1	G -> C	SNP (transversion)	25.90%					
360301	360301	1	G -> T	SNP (transversion)	25.90%					
161096	161096	1	A -> C	SNP (transversion)	25.90%					
1076387	1076387	1	G -> C	SNP (transversion)	25.80%					
4076052	4076052	1	G -> C	SNP (transversion)	25.60%	A -> G	fdoH CDS	68	GCG -> GGG	Substitution
4143602	4143602	1	A -> G	SNP (transition)	25.40%					
4076055	4076055	1	T -> C	SNP (transition)	25.40%	Q -> R	fdoH CDS	65	CAG -> CGG	Substitution
161084	161084	1	A -> C	SNP (transversion)	25.10%					

**Appx. E.11: All mutations found in *Adam::kan<sup>R</sup>* Keio mutant**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
3509231	3509232	2	CT -> AA	Substitution	98.5% -> 98.8%	K -> I	dam CDS	41	AAG -> ATT	Substitution
3509123	3509125	3	ATA -> CGG	Substitution	97.6% -> 98.8%	Y -> P	dam CDS	148	TAT -> CCG	Substitution
3509162	3509164	2	ACC -> CCT	Substitution	97.1% -> 97.6%	G -> R	dam CDS	109	GGT -> AGG	Substitution
3508490	3508492	3	CAC -> TTT	Substitution	97.1% -> 97.6%	V -> K	dam CDS	781	GTG -> AAA	Substitution
3509174	3509178	5	CTCAA -> AGTG	Deletion	96.9% -> 97.4%		dam CDS	95		Frame Shift
3509212	3509213	2	TA -> GT	Substitution	96.9% -> 97.2%	DI -> EL	dam CDS	60	GAT,ATT -> GAA,CTT	Substitution
3509144	3509145	2	GA -> CC	Substitution	96.8% -> 96.9%	L -> R	dam CDS	128	CTC -> CGG	Substitution
3509256	3509257	2	GC -> CG	Substitution	96.5% -> 96.6%	A -> R	dam CDS	16	GCT -> CGT	Substitution
3508538	3508543	4	GACGAC -> TAGAAA	Substitution	95.9% -> 96.2%	VV -> FL	dam CDS	730	GTC,GTC -> TTT,CTA	Substitution
3508523	3508528	5	ACTGCG -> TCAAGA	Substitution	95.4% -> 95.7%		dam CDS	745		Truncation
3508512	3508516	5	TTGCT -> GAATA	Substitution	94.9% -> 95.2%	SN -> YS	dam CDS	757	AGC,AAC -> TAT,TCC	Substitution
3508518	3508521	4	CTTA -> GAAC	Substitution	94.3% -> 94.4%	IS -> SS	dam CDS	752	ATA,AGC -> AGT,TCC	Substitution
3508551	3508557	6	TTTGCGC -> CAAGAAG	Substitution	94.2% -> 94.6%		dam CDS	716		Truncation
3508457	3508464	8	GACTCCTG -> TGTAGGCT	Substitution	93.2% -> 94.1%	PGV -> QPT	dam CDS	809	CCA,GGA,GTC -> CAG,CCT,ACA	Substitution
3509153	3509157	5	CGACC -> TTTAG	Substitution	93.0% -> 93.9%	GS -> AK	dam CDS	116	GGG,TCG -> GCT,AAA	Substitution
3508545	3508549	5	TGCAA -> ACTCG	Substitution	92.1% -> 92.6%	LH -> RV	dam CDS	724	TTG,CAT -> CGA,GTT	Substitution
3509168	3509172	5	AAAAG -> GCCCT	Substitution	91.9% -> 92.3%	PF -> QG	dam CDS	101	CCT,TTT -> CAG,GGC	Substitution
3509243	3509244	2	CC -> GA	Substitution	91.3% -> 91.4%	W -> F	dam CDS	29	TGG -> TTC	Substitution
3509234	3509238	5	GCCCC -> AGGAA	Substitution	90.7% -> 90.9%	GG -> VP	dam CDS	35	GGG,GGC -> GTT,CCT	Substitution
3508466	3508471	5	TTTGTA -> GAGCTG	Substitution	90.6% -> 91.6%	YK -> QL	dam CDS	802	TAC,AAA -> CAG,CTC	Substitution

3509251	3509254	4	AAAA -> GGTCG	Insertion	90.5% -> 90.7%		dam CDS	19		Frame Shift
3509205	3509209	3	CGTTT -> GCTCTG	Insertion	90.1% -> 91.1%		dam CDS	64		Frame Shift
3508500	3508506	6	CGTGTGC -> ATAGGAA	Substitution	89.8% -> 90.5%	GTR -> VPI	dam CDS	767	GGC,ACA,CGT -> GTT,CCT,ATT	Substitution
3508598	3508605	7	GATCAGCA -> CCGTAAAG	Substitution	87.7% -> 88.7%	VLI -> ALR	dam CDS	668	GTG,CTG,ATC -> GCT,TTA,CGG	Substitution
3508631	3508632	2	TT -> GC	Substitution	87.5% -> 87.7%	E -> G	dam CDS	641	GAA -> GGC	Substitution
3509151	3509152	2	AC -> C	Deletion	86.4% -> 87.6%		dam CDS	121		Frame Shift
3508481	3508488	7	CAGTTCGT -> TTCCTATA	Substitution	86.3% -> 87.7%		dam CDS	785		Truncation
3508560	3508570	11	TGATACCACTC -> GATAGAAGGCG	Substitution	85.4% -> 86.7%	EWYQ -> RLLS	dam CDS	703	GAG,TGG,TAT,CAG -> CGC,CTT,CTA,TCG	Substitution
3509114	3509122	9	GGCAAGGAT -> CCGTTTCTG	Substitution	84.5% -> 86.2%	ILA -> QKR	dam CDS	151	ATC,CTT,GCC -> CAG,AAA,CGG	Substitution
3508643	3508644	2	CG -> TT	Substitution	84.0% -> 85.7%	A -> E	dam CDS	629	GCG -> GAA	Substitution
3508592	3508596	5	ATTGG -> GCGAT	Substitution	83.9% -> 84.6%	SN -> YR	dam CDS	677	TCC,AAT -> TAT,CGC	Substitution
3508637	3508639	2	GAT -> AAG	Substitution	83.7% -> 86.5%	I -> L	dam CDS	634	ATC -> CTT	Substitution
3508646	3508648	3	CAG -> AGC	Substitution	82.4% -> 87.0%	L -> A	dam CDS	625	CTG -> GCT	Substitution
3509128	3509138	9	GAGAAAAGTCG -> TGGCTTTCTAC	Substitution	82.1% -> 91.2%		dam CDS	135		Truncation
3508578	3508588	10	AACATCGTATC -> GCGAATCGGGA	Substitution	81.6% -> 83.5%	DTML -> SRFA	dam CDS	685	GAT,ACG,ATG,TTA -> TCC,CGA,TTT,GCA	Substitution
3508473	3508478	5	AAAGCC -> TTCGA	Deletion	81.6% -> 82.3%		dam CDS	795		Frame Shift
3509259	3509268	10	CGATTTTCT -> ATCCCCGAA	Substitution	81.4% -> 84.0%	KKNR -> IPGI	dam CDS	5	AAG,AAA,AAT,CGC -> ATT,CCG,GGG,ATC	Substitution
3509216	3509230	13	ATCAAGCA GGGGATA -> CTATAC TTTCTAGAG	Substitution	80.5% -> 87.6%		dam CDS	43		Truncation
3508626	3508629	4	AGAC -> CATT	Substitution	79.7% -> 81.4%	GL -> EW	dam CDS	644	GGT,CTG -> GAA,TGG	Substitution

3509183	3509197	15	ACATTCGC CCTTGGG -> CGGCAGCG TGAGCTT	Substitution	78.5% -> 81.5%	PKGEC - > KLTLF	dam CDS	76	CCC,AAG,GGC,GAA,TGT -> AAG,CTC,ACG,CTG,CCG	Substitution
3508652	3508653	2	CG -> AT	Substitution	72.7% -> 75.0%	A -> D	dam CDS	620	GCG -> GAT	Substitution
3508650	3508651	2	TG -> AT	Substitution	71.4% -> 73.3%	H -> I	dam CDS	622	CAT -> ATT	Substitution
3509096	3509113	17	CAGGTCGC TATTGATATC -> ATTTCGTCC GGGGTCAGCA	Substitution	49.1% -> 81.3%		dam CDS	160		Truncation
3508655	3508656	2	TT -> AC	Substitution	20.0% -> 33.3%	Q -> R	dam CDS	617	CAA -> CGT	Substitution
3509095	3509095	1	T -> C	SNP (transition)	100.00%	I -> V	dam CDS	178	ATC -> GTC	Substitution
3509088	3509089	2	AG -> TA	Substitution	100.00%	L -> Y	dam CDS	184	CTC -> TAC	Substitution
3509084	3509085	2	AT -> CC	Substitution	100.00%	Y -> W	dam CDS	188	TAT -> TGG	Substitution
3508436	3508443	8	=TTATTTTT	Deletion	100.00%		dam CDS	830		Extension
3508536	3508536	1	T -> A	SNP (transversion)	99.20%	K -> I	dam CDS	737	AAA -> ATA	Substitution
3509180	3509181	2	CA -> TT	Substitution	99.00%	L -> Q	dam CDS	92	CTG -> CAA	Substitution
3508607	3508608	2	TG -> AC	Substitution	99.00%	P -> R	dam CDS	665	CCA -> CGT	Substitution
3508611	3508612	2	AT -> GG	Substitution	98.90%	I -> P	dam CDS	661	ATT -> CCT	Substitution
3508576	3508576	1	T -> C	SNP (transition)	98.80%	T -> A	dam CDS	697	ACG -> GCG	Substitution
3509248	3509248	1	T -> G	SNP (transversion)	98.60%	K -> Q	dam CDS	25	AAG -> CAG	Substitution
3508572	3508572	1	C -> T	SNP (transition)	98.30%	R -> H	dam CDS	701	CGT -> CAT	Substitution
3509159	3509159	1	G -> A	SNP (transition)	98.10%		dam CDS	114	GCC -> GCT	None
3509147	3509149	3	AAA -> CTT	Substitution	97.80%	F -> K	dam CDS	124	TTT -> AAG	Substitution
3509166	3509166	1	A -> G	SNP (transition)	97.20%	V -> A	dam CDS	107	GTA -> GCA	Substitution
3508531	3508534	4	GAAC -> CCCT	Substitution	96.90%	VR -> RG	dam CDS	739	GTT,CGA -> AGG,GGA	Substitution
3508614	3508614	1	T -> A	SNP (transversion)	96.50%	H -> L	dam CDS	659	CAT -> CTT	Substitution
3509241	3509241	1	G -> T	SNP (transversion)	95.70%	A -> E	dam CDS	32	GCA -> GAA	Substitution
3509202	3509202	1	T -> A	SNP (transversion)	95.70%	H -> L	dam CDS	71	CAT -> CTT	Substitution
3508508	3508509	2	GC -> TT	Substitution	95.70%	G -> E	dam CDS	764	GGC -> GAA	Substitution
3508635	3508635	1	G -> C	SNP (transversion)	95.60%	A -> G	dam CDS	638	GCC -> GGC	Substitution

3508590	3508590	1	T -> C	SNP (transition)	95.60%	H -> R	dam CDS	683	CAC -> CGC	Substitution
3508495	3508498	4	TTTT -> AGAG	Substitution	94.40%		dam CDS	775		Truncation
3508623	3508623	1	A -> G	SNP (transition)	89.00%	V -> A	dam CDS	650	GTT -> GCT	Substitution
3508619	3508619	1	C -> G	SNP (transversion)	87.30%	E -> D	dam CDS	654	GAG -> GAC	Substitution
3508616	3508616	1	=-G	Deletion	76.80%		dam CDS	657		Frame Shift
3509090	3509090	1	A -> G	SNP (transition)	75.00%		dam CDS	183	AGT -> AGC	None
3508655	3508655	1	T -> A	SNP (transversion)	60.00%	Q -> H	dam CDS	618	CAA -> CAT	Substitution
4094388	4094388	1	C -> T	SNP (transition)	35.40%	M -> I	cpxA CDS	516	ATG -> ATA	Substitution
406497	406497	1	C -> T	SNP (transition)	35.40%	P -> S	mak CDS	898	CCA -> TCA	Substitution
4076057	4076057	1	A -> G	SNP (transition)	35.00%		fdoH CDS	63	CCT -> CCC	None
312250	312250	1	T -> G	SNP (transversion)	35.00%	T -> P	ykgA CDS	631	ACG -> CCG	Substitution
4076046	4076046	1	T -> C	SNP (transition)	30.00%	D -> G	fdoH CDS	74	GAC -> GGC	Substitution
4076052	4076052	1	G -> C	SNP (transversion)	29.10%	A -> G	fdoH CDS	68	GCG -> GGG	Substitution
4076057	4076057	1	A -> C	SNP (transversion)	28.30%		fdoH CDS	63	CCT -> CCG	None
1688395	1688395	1	A -> C	SNP (transversion)	27.50%		uidB CDS	126	GGT -> GGG	None
4076055	4076055	1	T -> C	SNP (transition)	26.50%	Q -> R	fdoH CDS	65	CAG -> CGG	Substitution
3991413	3991413	1	A -> C	SNP (transversion)	25.80%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
3509090	3509090	1	A -> C	SNP (transversion)	25.00%	S -> R	dam CDS	183	AGT -> AGG	Substitution

**Appx. E.12: All mutations found in *Δcrp::kan<sup>R</sup>* Keio mutant**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
3479555	3479556	2	CC -> TG	Substitution	98.7% -> 98.8%	S -> L	crp CDS	77	TCC -> TTG	Substitution
3480089	3480090	2	TC -> CA	Substitution	97.8% -> 97.9%	I -> T	crp CDS	611	ATC -> ACA	Substitution
3480017	3480021	4	CTCGT -> AAGGG	Substitution	97.2% -> 97.3%		crp CDS	539		Truncation
3479486	3479487	2	TT -> CG	Substitution	97.2% -> 97.3%	L -> P	crp CDS	8	CTT -> CCG	Substitution
3480024	3480027	4	AACC -> TCTT	Substitution	96.7% -> 97.0%	ET -> DL	crp CDS	546	GAA,ACC -> GAT,CTT	Substitution
3480014	3480015	2	GT -> AA	Substitution	96.3% -> 96.4%		crp CDS	536		Truncation
3480029	3480030	2	TG -> AA	Substitution	96.1% -> 96.2%	V -> E	crp CDS	551	GTG -> GAA	Substitution
3480057	3480060	4	TCAG -> AAGT	Substitution	95.6% -> 96.2%	DQ -> ES	crp CDS	579	GAT,CAG -> GAA,AGT	Substitution
3479591	3479594	4	AAAC -> GGCT	Substitution	95.4% -> 95.8%	ET -> GL	crp CDS	113	GAA,ACG -> GGG,CTG	Substitution
3479534	3479538	5	GCCAC -> ATAGG	Substitution	95.3% -> 95.5%	CH -> YR	crp CDS	56	TGC,CAC -> TAT,AGG	Substitution
3479942	3479943	2	CA -> TC	Substitution	94.5% -> 94.9%	P -> L	crp CDS	464	CCA -> CTC	Substitution
3479959	3479961	2	CCG -> GCC	Substitution	94.5% -> 94.7%	P -> A	crp CDS	481	CCG -> GCC	Substitution
3479525	3479532	7	TGTCTCAT -> CTAGAAAG	Substitution	93.9% -> 94.3%	LSH -> SRK	crp CDS	47	TTG,TCT,CAT -> TCT,AGA,AAG	Substitution
3479546	3479550	5	AGTAC -> GAGCG	Substitution	93.9% -> 94.2%	KY -> RA	crp CDS	68	AAG,TAC -> AGA,GCG	Substitution
3479540	3479544	5	TTCAT -> ACTTC	Substitution	93.5% -> 93.8%	IH -> NF	crp CDS	62	ATT,CAT -> AAC,TTC	Substitution
3479982	3479983	2	TA -> CG	Substitution	93.2% -> 93.3%	IT -> IA	crp CDS	504	ATT,ACC -> ATC,GCC	Substitution
3479568	3479571	4	TATT -> GCCG	Substitution	92.7% -> 93.2%	LI -> LP	crp CDS	90	CTT,ATT -> CTG,CCG	Substitution
3480046	3480048	1	ATG -> TTCCTATT	Insertion	92.0% -> 92.5%		crp CDS	568		Frame Shift
3479552	3479553	2	CA -> TT	Substitution	91.9% -> 92.2%	P -> L	crp CDS	74	CCA -> CTT	Substitution

3479933	3479940	8	CAAAACAA -> ACCGCTTC	Substitution	91.3% -> 94.0%	AKQ -> DRF	crp CDS	455	GCA,AAA,CAA -> GAC,CGC,TTC	Substitution
3479974	3479979	6	ATCAAA -> CAGCGC	Substitution	90.5% -> 92.0%	IK -> QR	crp CDS	496	ATC,AAA -> CAG,CGC	Substitution
3479945	3479948	4	ACGC -> TGCT	Substitution	90.5% -> 92.0%	DA -> VL	crp CDS	467	GAC,GCT -> GTG,CTT	Substitution
3480062	3480066	5	ACCTG -> TAGGA	Substitution	90.1% -> 90.8%	NL -> IG	crp CDS	584	AAC,CTG -> ATA,GGA	Substitution
3479633	3479634	2	TC -> GA	Substitution	90.0% -> 92.9%	I -> R	crp CDS	155	ATC -> AGA	Substitution
3479574	3479589	16	CCAGGGTGAAAAAGCG -> AGCACTCAGGGCGCAA	Substitution	89.4% -> 91.2%	HQGEKA -> QALRAQ	crp CDS	96	CAC,CAG,GGT, GAA,AAA,GCG -> CAA,GCA,CTC, AGG,GCG,CAA	Substitution
3479950	3479954	5	ATGAC -> TACGG	Substitution	89.2% -> 90.2%	MT -> YG	crp CDS	472	ATG,ACT -> TAC,GGT	Substitution
3480078	3480087	8	CGGTAAAACC -> GCTCCAGCCT	Substitution	87.6% -> 88.7%	HGKT -> QLQP	crp CDS	600	CAC,GGT,AAA,ACC -> CAG,CTC,CAG,CCT	Substitution
3479630	3479631	2	TG -> GC	Substitution	86.7% -> 90.5%	L -> R	crp CDS	152	CTG -> CGC	Substitution
3479986	3479995	9	CGTCAGGAAA -> TTCTATCGCC	Substitution	86.4% -> 90.8%	RQEI -> FYRL	crp CDS	508	CGT,CAG,GAA,ATT -> TTC,TAT,CGC,CTT	Substitution
3480072	3480075	4	CGCA -> GAAG	Substitution	85.9% -> 86.2%	SA -> SK	crp CDS	594	TCC,GCA -> TCG,AAG	Substitution
3480001	3480007	6	CAGATTG -> GACGAGT	Substitution	84.9% -> 92.9%	QIV -> DEF	crp CDS	523	CAG,ATT,GTC -> GAC,GAG,TTC	Substitution
3479506	3479520	14	CCGACTCTCGAATGG -> AGTTCGAAGTTCCTA	Substitution	84.6% -> 86.0%	PTLEW -> SSKFL	crp CDS	28	CCG,ACT,CTC, GAA,TGG -> AGT,TCG,AAG, TTC,CTA	Substitution
3479912	3479913	2	CA -> AG	Substitution	80.0% -> 85.7%	A -> E	crp CDS	434	GCA -> GAG	Substitution
3479915	3479931	17	AGACTCTGCTGAATCTG -> TTGGCGGCGAATGGGCT	Substitution	72.7% -> 84.4%	QTLNL -> LGGEWA	crp CDS	437	CAG,ACT,CTG, CTG,AAT,CTG -> CTT,GGC,GGC, GAA,TGG,GCT	Substitution
3479963	3479973	11	ACGGTATGCAA -> CTCCGATTTCG	Substitution	71.6% -> 82.9%	DGMQ -> APDS	crp CDS	485	GAC,GGT,ATG,CAA -> GCT,CCC,GAT,TCG	Substitution

3479609	3479610	2	TT -> AA	Substitution	59.0% -> 59.8%	V -> E	crp CDS	131	GTT -> GAA	Substitution
3479598	3479604	5	GTACTAC -> AAAGGAA	Substitution	57.5% -> 67.6%	LYY -> LKE	crp CDS	120	CTG,TAC,TAC -> CTA,AAG,GAA	Substitution
3479617	3479621	5	TCTGT -> AGAAA	Substitution	54.9% -> 75.7%	SV -> RK	crp CDS	139	TCT,GTG -> AGA,AAG	Substitution
3479605	3479607	3	ATC -> GCG	Substitution	51.0% -> 59.5%	I -> A	crp CDS	127	ATC -> GCG	Substitution
3479611	3479613	2	AAA -> CAC	Substitution	49.4% -> 54.3%	K -> H	crp CDS	133	AAA -> CAC	Substitution
3479615	3479616	2	GC -> TA	Substitution	42.9% -> 45.8%	G -> V	crp CDS	137	GGC -> GTA	Substitution
3479614	3479616	2	GGC -> CGT	Substitution	40.9% -> 48.2%	G -> R	crp CDS	136	GGC -> CGT	Substitution
3479609	3479610	2	TT -> GA	Substitution	36.1% -> 36.6%	V -> G	crp CDS	131	GTT -> GGA	Substitution
3479617	3479620	4	TCTG -> GAAA	Substitution	26.8% -> 37.3%	SV -> EM	crp CDS	139	TCT,GTG -> GAA,ATG	Substitution
3479908	3479909	2	AT -> GA	Substitution	25.0% -> 50.0%	I -> D	crp CDS	430	ATT -> GAT	Substitution
3479598	3479604	5	GTACTAC -> AAAGGA	Deletion	24.2% -> 28.4%		crp CDS	120		Frame Shift
3479740	3479740	1	C -> T	SNP (transition)	100.00%	R -> C	crp CDS	262	CGT -> TGT	Substitution
3479504	3479504	1	--A	Deletion	99.80%		crp CDS	26		Frame Shift
2921291	2921291	1	C -> T	SNP (transition)	99.20%					
3480035	3480036	2	GC -> CT	Substitution	99.10%	R -> P	crp CDS	557	CGC -> CCT	Substitution
3480010	3480011	2	GG -> TT	Substitution	98.80%	G -> F	crp CDS	532	GGC -> TTC	Substitution
3479498	3479498	1	A -> G	SNP (transition)	98.80%	Q -> R	crp CDS	20	CAA -> CGA	Substitution
3479490	3479490	1	C -> G	SNP (transversion)	98.70%		crp CDS	12	GGC -> GGG	None
3480041	3480041	1	T -> C	SNP (transition)	98.60%	L -> P	crp CDS	563	CTG -> CCG	Substitution
3479500	3479502	2	ACA -> CCT	Substitution	98.60%	T -> P	crp CDS	22	ACA -> CCT	Substitution
2719426	2719426	1	T -> C	SNP (transition)	98.60%					
3480051	3480054	3	GGAA -> CTA	Deletion	98.20%		crp CDS	573		Truncation
3479998	3479999	2	GG -> CT	Substitution	97.60%	G -> L	crp CDS	520	GGT -> CTT	Substitution
3479482	3479484	2	GTG -> ATT	Substitution	97.50%	V -> I	crp CDS	4	GTG -> ATT	Substitution
3479560	3479561	2	AG -> CT	Substitution	97.40%	S -> L	crp CDS	82	AGC -> CTC	Substitution
3480068	3480069	2	TC -> CT	Substitution	97.10%	I -> T	crp CDS	590	ATC -> ACT	Substitution
3479492	3479493	2	AA -> TC	Substitution	96.50%	K -> I	crp CDS	14	AAA -> ATC	Substitution



3480032	3480033	2	GA -> TT	Substitution	96.30%	G -> V	crp CDS	554	GGA -> GTT	Substitution
3479495	3479496	2	CG -> GT	Substitution	94.50%	P -> R	crp CDS	17	CCG -> CGT	Substitution
3479628	3479628	1	G -> C	SNP (transversion)	92.30%		crp CDS	150	GTG -> GTC	None
3479956	3479957	2	CA -> AT	Substitution	91.50%	H -> I	crp CDS	478	CAC -> ATC	Substitution
3479623	3479623	1	G -> C	SNP (transversion)	90.90%	A -> P	crp CDS	145	GCA -> CCA	Substitution
3479637	3479637	1	A -> C	SNP (transversion)	87.50%	K -> N	crp CDS	159	AAA -> AAC	Substitution
3479909	3479910	2	TT -> AA	Substitution	50.00%	I -> K	crp CDS	431	ATT -> AAA	Substitution
4076057	4076057	1	A -> G	SNP (transition)	43.00%		fdoH CDS	63	CCT -> CCC	None
3479612	3479612	1	A -> C	SNP (transversion)	40.30%	K -> T	crp CDS	134	AAA -> ACA	Substitution
3479606	3479606	1	T -> G	SNP (transversion)	33.00%	I -> S	crp CDS	128	ATC -> AGC	Substitution
3991425	3991425	1	T -> G	SNP (transversion)	32.30%	L -> R	uvrD CDS	83	CTG -> CGG	Substitution
3991413	3991413	1	A -> C	SNP (transversion)	25.90%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
4076050	4076050	1	G -> C	SNP (transversion)	25.50%	R -> G	fdoH CDS	70	CGG -> GGG	Substitution
887901	887901	1	C -> G	SNP (transversion)	25.20%	A -> G	rimK CDS	479	GCG -> GGG	Substitution

**Appx. E.13: All mutations found in *Δwzc::kan<sup>R</sup>* Keio mutant**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
2127058	2127063	5	CCCTTT -> AAGATC	Substitution	96.4% -> 96.5%	KG -> DL	wzc CDS	2071	AAA,GGG -> GAT,CTT	Substitution
2127028	2127030	2	GGC -> AGA	Substitution	96.1% -> 96.4%	A -> S	wzc CDS	2104	GCC -> TCT	Substitution
2127023	2127025	3	GCG -> TTT	Substitution	95.9% -> 96.1%	SA -> RK	wzc CDS	2109	AGC,GCG -> AGA,AAG	Substitution
2127077	2127082	5	TTTTGC -> AACTCG	Substitution	95.3% -> 95.5%	EQN -> DEF	wzc CDS	2052	GAG,CAA,AAC -> GAC,GAG,TTT	Substitution
2129011	2129019	8	GAATACGGT -> AGCCCTTGC	Substitution	95.0% -> 95.3%	TVF -> ARA	wzc CDS	115	ACC,GTA,TTT -> GCA,AGG,GCT	Substitution
2129086	2129088	3	ATC -> TAG	Substitution	94.9% -> 95.1%	D -> L	wzc CDS	46	GAT -> CTA	Substitution
2127148	2127150	3	CGT -> GCG	Substitution	94.7% -> 94.9%	T -> R	wzc CDS	1984	ACG -> CGC	Substitution
2127142	2127145	3	CATT -> GAGG	Substitution	94.5% -> 94.7%	LM -> FL	wzc CDS	1989	TTA,ATG -> TTC,CTC	Substitution
2127014	2127015	1	TC -> GTT	Insertion	94.0% -> 94.3%		wzc CDS	2119		Frame Shift
2127043	2127047	4	GGAGT -> CGGAA	Substitution	93.9% -> 94.1%	NS -> IP	wzc CDS	2087	AAC,TCC -> ATT,CCG	Substitution
2127171	2127174	4	CAAT -> GCTC	Substitution	93.8% -> 95.2%	IV -> EL	wzc CDS	1960	ATT,GTT -> GAG,CTT	Substitution
2127093	2127099	7	TCAGACT -> GATAGAA	Substitution	93.8% -> 94.0%	SLS -> FYR	wzc CDS	2035	AGT,CTG,AGC -> TTC,TAT,CGC	Substitution
2129035	2129040	6	CCACCA -> GGCAGC	Substitution	93.2% -> 93.4%	WW -> AA	wzc CDS	94	TGG,TGG -> GCT,GCC	Substitution
2127017	2127018	2	TG -> CT	Substitution	93.1% -> 93.3%	Q -> R	wzc CDS	2116	CAG -> AGG	Substitution
2129052	2129054	3	TGA -> AAG	Substitution	93.0% -> 93.2%	VI -> AF	wzc CDS	80	GTC,ATT -> GCT,TTT	Substitution
2127032	2127035	4	CGGC -> AATA	Substitution	92.9% -> 93.1%	RR -> LF	wzc CDS	2099	CGC,CGC -> CTA,TTT	Substitution
2127067	2127075	9	CGGAATACC -> TTATTAGAA	Substitution	92.0% -> 93.0%		wzc CDS	2059		Truncation
2127131	2127138	8	TAACGCGC -> CCGTAAAG	Substitution	91.5% -> 92.0%	ARY -> LYG	wzc CDS	1996	GCG,CGT,TAT -> CTT,TAC,GGT	Substitution
2127127	2127129	3	CGC -> GAT	Substitution	90.7% -> 91.0%	A -> I	wzc CDS	2005	GCG -> ATC	Substitution
2127157	2127166	8	GACATGACGA -> CCATTCGCCG	Substitution	90.4% -> 92.2%	GRHV -> GGEW	wzc CDS	1968	GGT,CGT,CAT,GTC -> GGC,GGC,GAA,TGG	Substitution
2128949	2128956	7	ACCAGTGC -> CATCCGGG	Substitution	89.8% -> 91.3%		wzc CDS	178		Truncation
2128942	2128947	6	GATTTG -> TGACAT	Substitution	88.8% -> 90.5%	QI -> MS	wzc CDS	187	CAA,ATC -> ATG,TCA	Substitution
2129021	2129030	10	GTGATGCCAA -> CCCTGAGTGC	Substitution	88.8% -> 89.3%	IGIT -> STQG	wzc CDS	104	ATT,GGC,ATC,ACC -> AGC,ACT,CAG,GGC	Substitution

2129000	2129008	8	GCACAGAGG -> CTTCCTTTA	Substitution	88.6% -> 91.0%	ALCA -> AKGS	wzc CDS	126	GCC,CTC,TGT,GCC -> GCT,AAA,GGA,AGC	Substitution
2128990	2128997	8	GTAAACCA -> ACGTGTTT	Substitution	88.1% -> 89.3%	VVY -> GTR	wzc CDS	137	GTG,GTT,TAC -> GGA,ACA,CGT	Substitution
2128981	2128988	8	GAAGAAGG -> TGGCTTTC	Substitution	88.0% -> 88.4%	TFF -> RKP	wzc CDS	146	ACC,TTC,TTC -> AGA,AAG,CCA	Substitution
2129065	2129071	7	CAGGCGA -> TGAAGTT	Substitution	86.1% -> 86.7%	GRL -> GTS	wzc CDS	63	GGT,CGC,CTG -> GGA,ACT,TCA	Substitution
2127152	2127155	4	GTTC -> TCAG	Substitution	86.0% -> 86.6%	GT -> AD	wzc CDS	1979	GGA,ACC -> GCT,GAC	Substitution
2127121	2127123	3	GTT -> AGC	Substitution	85.2% -> 85.6%	N -> A	wzc CDS	2011	AAC -> GCT	Substitution
2127190	2127193	3	TGCC -> GGGT	Substitution	84.6% -> 88.2%	LA -> LP	wzc CDS	1941	CTG,GCA -> CTA,CCC	Substitution
2126993	2127000	7	TCGTATTC -> GTAGGCTG	Substitution	84.1% -> 86.1%	EYE -> QPT	wzc CDS	2134	GAA,TAC,GAA -> CAG,CCT,ACA	Substitution
2129090	2129102	12	CTGCCCGTTACCG -> GAATAGGAACTTC	Substitution	83.1% -> 84.9%	PVTGS -> RSSYS	wzc CDS	32	CCG,GTA,ACG, GGC,AGT -> CGA,AGT,TCC, TAT,TCT	Substitution
2128965	2128971	6	TATAAAT -> CGTTT	Deletion	82.9% -> 92.3%		wzc CDS	163		Frame Shift
2128933	2128940	8	GTTTTGCT -> CCCAGTAG	Substitution	82.8% -> 86.8%	EQN -> ATG	wzc CDS	194	GAG,CAA,AAC -> GCT,ACT,GGG	Substitution
2129116	2129129	13	TTTTACTTTTTCTG -> ACGGATCCCCGGAA	Substitution	80.9% -> 85.1%	TEKVK - > IPGIR	wzc CDS	5	ACA,GAA,AAA, GTA,AAA -> ATT,CCG,GGG, ATC,CGT	Substitution
2127102	2127120	17	TTTCCACTTCTTTCAATGT - > CGATGCGCTGCGAATCGGG	Substitution	79.5% -> 81.2%	TLKEVET -> PDSQRIA	wzc CDS	2014	ACA,TTG,AAA,GAA ,GTG,GAA,ACC -> CCC,GAT,TCG,CAG, CGC,ATC,GCC	Substitution
2127002	2127011	9	TAATAGCCAT -> AGCTGCTTCG	Substitution	74.8% -> 79.6%	YGY -> SKQL	wzc CDS	2123	TAT,GGC,TAT,TAC -> TCG,AAG,CAG,CTC	Substitution
2127182	2127185	4	TCGG -> ATA	Deletion	51.4% -> 66.7%		wzc CDS	1949		Frame Shift
2128920	2128932	13	ACGAATTGCCGCT -> CTTGTCAGATAG	Substitution	50.0% -> 78.6%	SGNSL -> LSGQV	wzc CDS	202	AGC,GGC,AAT, TCG,TTA -> CTA,TCT,GGA, CAA,GTA	Substitution
2127182	2127185	4	TCGG -> ATAT	Substitution	25.9% -> 42.9%	TD -> NI	wzc CDS	1949	ACC,GAT -> AAT,ATT	Substitution
2129059	2129063	5	--GCCGA	Deletion	100.00%		wzc CDS	71		Frame Shift
2128979	2128979	1	G -> A	SNP (transition)	100.00%	A -> V	wzc CDS	155	GCC -> GTC	Substitution
2128977	2128977	1	--T	Deletion	100.00%		wzc CDS	157		Frame Shift

2127196	2127200	5	AATCG -> CCAAC	Substitution	100.00%	PI -> RW	wzc CDS	1934	CCG,ATT -> CGT,TGG	Substitution
2127176	2127176	1	G -> T	SNP (transversion)	100.00%	A -> E	wzc CDS	1958	GCA -> GAA	Substitution
219535	219535	1	-C	Deletion	99.60%		gmhB CDS	216		Frame Shift
2127125	2127125	1	A -> G	SNP (transition)	99.50%	V -> A	wzc CDS	2009	GTC -> GCC	Substitution
2128960	2128960	1	G -> A	SNP (transition)	99.30%		wzc CDS	174	GCC -> GCT	None
2127087	2127089	3	AGC -> GAA	Substitution	99.30%	RF -> LL	wzc CDS	2045	CGC,TTT -> CTT,CTT	Substitution
2127065	2127065	1	A -> C	SNP (transversion)	99.20%	V -> G	wzc CDS	2069	GTG -> GGG	Substitution
2129111	2129111	1	T -> G	SNP (transversion)	98.70%	H -> P	wzc CDS	23	CAT -> CCT	Substitution
2129107	2129107	1	G -> T	SNP (transversion)	98.70%		wzc CDS	27	GCC -> GCA	None
2129105	2129105	1	G -> A	SNP (transition)	98.70%	A -> V	wzc CDS	29	GCT -> GTT	Substitution
2127049	2127050	2	CA -> AG	Substitution	98.70%	L -> P	wzc CDS	2084	CTG -> CCT	Substitution
2129078	2129081	4	TCGA -> C	Deletion	98.60%	ID -> S	wzc CDS	53	ATC,GAT -> AGT	Deletion
2129042	2129042	1	C -> T	SNP (transition)	98.60%	R -> H	wzc CDS	92	CGC -> CAC	Substitution
2129057	2129057	1	G -> C	SNP (transversion)	98.50%	T -> S	wzc CDS	77	ACC -> AGC	Substitution
2129074	2129074	1	A -> T	SNP (transversion)	98.20%		wzc CDS	60	ATT -> ATA	None
2129114	2129114	1	T -> C	SNP (transition)	98.10%	Q -> R	wzc CDS	20	CAA -> CGA	Substitution
2128973	2128973	1	G -> T	SNP (transversion)	98.10%	P -> Q	wzc CDS	161	CCG -> CAG	Substitution
2129032	2129033	2	CA -> TG	Substitution	97.90%	V -> A	wzc CDS	101	GTG -> GCA	Substitution
2127178	2127178	1	C -> A	SNP (transversion)	97.90%		wzc CDS	1956	GCG -> GCT	None
2719426	2719426	1	T -> C	SNP (transition)	97.80%					
2127040	2127041	2	GA -> CT	Substitution	97.70%	I -> K	wzc CDS	2093	ATC -> AAG	Substitution
2127054	2127056	3	TCA -> CTT	Substitution	93.90%	VI -> EV	wzc CDS	2078	GTG,ATT -> GAA,GTT	Substitution
2129044	2129044	1	C -> A	SNP (transversion)	93.70%		wzc CDS	90	GCG -> GCT	None
3991413	3991413	1	A -> C	SNP (transversion)	31.80%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
4076057	4076057	1	A -> G	SNP (transition)	31.70%		fdoH CDS	63	CCT -> CCC	None
1688395	1688395	1	A -> C	SNP (transversion)	28.90%		uidB CDS	126	GGT -> GGG	None
4076055	4076055	1	T -> G	SNP (transversion)	25.90%	Q -> P	fdoH CDS	65	CAG -> CCG	Substitution
3991428	3991428	1	C -> G	SNP (transversion)	25.80%	A -> G	uvrD CDS	86	GCG -> GGG	Substitution

**Appx E.14: All mutations found in *E. coli* BW25113 parent strain**

Start	End	Length	Change	Polymorphism Type	Variant Frequency	Amino Acid Change	CDS	CDS Position	Codon Change	Protein Effect
4368790	4368790	1	G -> C	SNP (transversion)	26.80%					
4143602	4143602	1	A -> G	SNP (transition)	26.50%					
4076057	4076057	1	A -> G	SNP (transition)	39.00%		fdoH CDS	63	CCT -> CCC	None
4076055	4076055	1	T -> C	SNP (transition)	25.20%	Q -> R	fdoH CDS	65	CAG -> CGG	Substitution
4076052	4076052	1	G -> C	SNP (transversion)	26.80%	A -> G	fdoH CDS	68	GCG -> GGG	Substitution
3991425	3991425	1	T -> G	SNP (transversion)	29.40%	L -> R	uvrD CDS	83	CTG -> CGG	Substitution
3991413	3991413	1	A -> C	SNP (transversion)	25.50%	N -> T	uvrD CDS	71	AAC -> ACC	Substitution
3469953	3469953	1	C -> G	SNP (transversion)	28.10%					
3469936	3469936	1	G -> C	SNP (transversion)	33.30%					
2761028	2761028	1	G -> C	SNP (transversion)	25.30%					
2761026	2761026	1	T -> C	SNP (transition)	25.20%					
2450350	2450350	1	G -> A	SNP (transition)	26.20%					
2450346	2450346	1	G -> A	SNP (transition)	29.40%					
1881102	1881102	1	A -> C	SNP (transversion)	25.40%					
1881093	1881093	1	A -> G	SNP (transition)	32.40%					
1881088	1881088	1	C -> G	SNP (transversion)	27.80%					
1881085	1881085	1	T -> G	SNP (transversion)	34.20%					
1881082	1881082	1	A -> G	SNP (transition)	33.80%					
1714635	1714635	1	G -> C	SNP (transversion)	29.60%					
1714612	1714612	1	T -> G	SNP (transversion)	28.70%					
1714610	1714610	1	C -> G	SNP (transversion)	28.00%					
1076387	1076387	1	G -> C	SNP (transversion)	30.60%					
1076384	1076384	1	G -> C	SNP (transversion)	32.40%					
1062305	1062305	1	C -> G	SNP (transversion)	37.80%					
910654	910654	1	C -> G	SNP (transversion)	26.90%					

910652	910652	1	T -> G	SNP (transversion)	29.10%					
677168	677168	1	G -> C	SNP (transversion)	29.50%					
360294	360298	5	AATTC -> GGCAG	Substitution	23.8% -> 26.2%					
360287	360292	6	GGGAAT -> AATTCC	Substitution	28.7% -> 30.3%					
360140	360140	1	A -> C	SNP (transversion)	25.80%					
284974	284974	1	A -> G	SNP (transition)	27.80%					
161090	161090	1	A -> C	SNP (transversion)	28.10%					
161088	161088	1	T -> C	SNP (transition)	29.90%					
161082	161082	1	T -> C	SNP (transition)	30.20%					
161051	161051	1	A -> G	SNP (transition)	28.60%					

---

## Appendix F – Thesis bibliography

---

Alaniz, RC, Deatherage, BL, Lara, JC & Cookson, BT 2007, 'Membrane vesicles are immunogenic facsimiles of *Salmonella typhimurium* that potently activate dendritic cells, prime B and T cell responses, and stimulate protective immunity *in vivo*', *J Immunol*, vol. 179, no. 11, Dec 1, pp. 7692-7701.

Andrews, GP, Hromockyj, AE, Coker, C & Maurelli, AT 1991, 'Two novel virulence loci, *mxiA* and *mxiB*, in *Shigella flexneri* 2a facilitate excretion of invasion plasmid antigens', *Infection and Immunity*, vol. 59, no. 6, Jun, pp. 1997-2005.

Antonoaea, R, Furst, M, Nishiyama, K & Muller, M 2008, 'The periplasmic chaperone PpiD interacts with secretory proteins exiting from the SecYEG translocon', *Biochemistry*, vol. 47, no. 20, May 20, pp. 5649-5656.

Aoki, SK, Malinverni, JC, Jacoby, K, Thomas, B, Pamma, R, Trinh, BN, Remers, S, Webb, J, Braaten, BA, Silhavy, TJ & Low, DA 2008, 'Contact-dependent growth inhibition requires the essential outer membrane protein BamA (YaeT) as the receptor and the inner membrane transport protein AcrB', *Mol Microbiol*, vol. 70, no. 2, Oct, pp. 323-340.

Arner, ES, Sarioglu, H, Lottspeich, F, Holmgren, A & Bock, A 1999, 'High-level expression in *Escherichia coli* of selenocysteine-containing rat thioredoxin reductase utilizing gene fusions with engineered bacterial-type SECIS elements and co-expression with the *selA*, *selB* and *selC* genes', *J Mol Biol*, vol. 292, no. 5, Oct 8, pp. 1003-1016.

Baba, T, Ara, T, Hasegawa, M, Takai, Y, Okumura, Y, Baba, M, Datsenko, KA, Tomita, M, Wanner, BL & Mori, H 2006, 'Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection', *Mol Syst Biol*, vol. 2, no. 2006.0008, pp. 1-11.

Barr, K, Klena, J & Rick, PD 1999a, 'The modality of enterobacterial common antigen polysaccharide chain lengths is regulated by *o349* of the *wec* gene cluster of *Escherichia coli* K-12', *Journal of bacteriology*, vol. 181, no. 20, Oct, pp. 6564-6568.

Barr, K, Klena, J & Rick, PD 1999b, 'The modality of enterobacterial common antigen polysaccharide chain lengths is regulated by *o349* of the *wec* gene cluster of *Escherichia coli* K-12', *J Bacteriol*, vol. 181, no. 20, Oct, pp. 6564-6568.

Bastin, DA, Stevenson, G, Brown, PK, Haase, A & Reeves, PR 1993, 'Repeat unit polysaccharides of bacteria: a model for polymerization resembling that of ribosomes and fatty acid synthetase, with a novel mechanism for determining chain length.', *Mol Microbiol*, vol. 7, no. 5, Mar, pp. 725-734.

Beck, CM, Willett, JL, Cunningham, DA, Kim, JJ, Low, DA & Hayes, CS 2016, 'CdiA effectors from uropathogenic *Escherichia coli* use heterotrimeric osmoporins as receptors to recognize target bacteria', *PLoS Pathog*, vol. 12, no. 10, Oct, p. e1005925.

Bennish, ML & Khan, WA 2012, 'Shigellosis', *Hunter's Tropical Medicine and Emerging Infectious Disease: Ninth Edition*, pp. 454-461.

Bernardini, ML, Mounier, J, D'Hauteville, H, Coquis-Rondon, M & Sansonetti, PJ 1989, 'Identification of *icsA*, a plasmid locus of *Shigella flexneri* that governs bacterial intra- and intercellular spread through interaction with F-actin', *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 10, pp. 3867-3871.

Blocker, A, Gounon, P, Larquet, E, Niebuhr, K, Cabiaux, V, Parsot, C & Sansonetti, P 1999, 'The tripartite type III secretin of *Shigella flexneri* inserts IpaB and IpaC into host membranes', *Journal of Cell Biology*, vol. 147, no. 3, Nov 1, pp. 683-693.

Camacho, AI, de Souza, J, Sanchez-Gomez, S, Pardo-Ros, M, Irache, JM & Gamazo, C 2011, 'Mucosal immunization with *Shigella flexneri* outer membrane vesicles induced protection in mice', *Vaccine*, vol. 29, no. 46, Oct 26, pp. 8222-8229.

Carter, JA, Jimenez, JC, Zaldivar, M, Alvarez, SA, Marolda, CL, Valvano, MA & Contreras, I 2009, 'The cellular level of O-antigen polymerase Wzy determines chain length regulation by WzzB and WzzpHS-2 in *Shigella flexneri* 2a', *Microbiology*, vol. 155, no. Pt 10, Oct, pp. 3260-3269.

Cascales, E, Buchanan, SK, Duche, D, Kleanthous, C, Lloubes, R, Postle, K, Riley, M, Slatin, S & Cavard, D 2007, 'Colicin biology', *Microbiol Mol Biol Rev*, vol. 71, no. 1, Mar, pp. 158-229.

Castelli, ME & Vescovi, EG 2011, 'The Rcs signal transduction pathway is triggered by enterobacterial common antigen structure alterations in *Serratia marcescens*', *J Bacteriol*, vol. 193, no. 1, Jan, pp. 63-74.

Chen, YJ, Smith, MR, Thirumalai, K & Zychlinsky, A 1996, 'A bacterial invasin induces macrophage apoptosis by binding directly to ICE', *Embo Journal*, vol. 15, no. 15, Aug 1, pp. 3853-3860.

Christensen, SK, Maenhaut-Michel, G, Mine, N, Gottesman, S, Gerdes, K & Van Melderen, L 2004, 'Overproduction of the Lon protease triggers inhibition of translation in *Escherichia coli*: involvement of the yefM-yoeB toxin-antitoxin system', *Mol Microbiol*, vol. 51, no. 6, Mar, pp. 1705-1717.

Clark, CA, Beltrame, J & Manning, PA 1991, 'The *oac* gene encoding a lipopolysaccharide O-antigen acetylase maps adjacent to the integrase-encoding gene on the genome of *Shigella flexneri* bacteriophage Sf6', *Gene*, vol. 107, no. 1, Oct 30, pp. 43-52.



- Clarke, DJ, Joyce, SA, Toutain, CM, Jacq, A & Holland, IB 2002, 'Genetic analysis of the RcsC sensor kinase from *Escherichia coli* K-12', *J Bacteriol*, vol. 184, no. 4, Feb, pp. 1204-1208.
- Collins, RF, Kargas, V, Clarke, BR, Siebert, CA, Clare, DK, Bond, PJ, Whitfield, C & Ford, RC 2017, 'Full-length, oligomeric structure of Wzz determined by cryoelectron microscopy reveals insights into membrane-bound states.', *Structure*, vol. 25, no. 5, May 02, pp. 806-815 e803.
- Daniels, C & Morona, R 1999, 'Analysis of *Shigella flexneri* wzz (Rol) function by mutagenesis and cross-linking: Wzz is able to oligomerize', *Mol Microbiol*, vol. 34, no. 1, Oct, pp. 181-194.
- Daniels, C, Vindurampulle, C & Morona, R 1998, 'Overexpression and topology of the *Shigella flexneri* O-antigen polymerase (Rfc/Wzy)', *Molecular Microbiology*, vol. 28, no. 6, Jun, pp. 1211-1222.
- Datsenko, KA & Wanner, BL 2000, 'One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products', *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 12, Jun 6, pp. 6640-6645.
- De Las Penas, A, Connolly, L & Gross, CA 1997, 'The sigmaE-mediated response to extracytoplasmic stress in *Escherichia coli* is transduced by RseA and RseB, two negative regulators of sigmaE', *Mol Microbiol*, vol. 24, no. 2, Apr, pp. 373-385.
- DeLeo, FR 2004, 'Modulation of phagocyte apoptosis by bacterial pathogens', *Apoptosis*, vol. 9, no. 4, Jul, pp. 399-413.
- Delgado, MA, Mouslim, C & Groisman, EA 2006, 'The PmrA/PmrB and RcsC/YojN/RcsB systems control expression of the *Salmonella* O-antigen chain length determinant', *Molecular Microbiology*, vol. 60, no. 1, Apr, pp. 39-50.
- Dell, A, Oates, J, Lugowski, C, Romanowska, E, Kenne, L & Lindberg, B 1984, 'The enterobacterial common-antigen, a cyclic polysaccharide', *Carbohydr Res*, vol. 133, no. 1, Oct 1, pp. 95-104.
- Doberenz, C, Zorn, M, Falke, D, Nannemann, D, Hunger, D, Beyer, L, Ihling, CH, Meiler, J, Sinz, A & Sawers, RG 2014, 'Pyruvate formate-lyase interacts directly with the formate channel FocA to regulate formate translocation', *J Mol Biol*, vol. 426, no. 15, Jul 29, pp. 2827-2839.
- Ellis, TN, Leiman, SA & Kuehn, MJ 2010, 'Naturally produced outer membrane vesicles from *Pseudomonas aeruginosa* elicit a potent innate immune response via combined sensing of both lipopolysaccharide and protein components', *Infect Immun*, vol. 78, no. 9, Sep, pp. 3822-3831.
- Espina, M, Olive, AJ, Kenjale, R, Moore, DS, Ausar, SF, Kaminski, RW, Oaks, EV, Middaugh, CR, Picking, WD & Picking, WL 2006, 'IpaD localizes to the tip of the type III secretion system needle of *Shigella flexneri*', *Infection and Immunity*, vol. 74, no. 8, Aug, pp. 4391-4400.

Fasano, A, Noriega, FR, Maneval, DR, Chanasongcram, S, Russell, R, Guandalini, S & Levine, MM 1995, '*Shigella* enterotoxin-1 - an enterotoxin of *Shigella flexneri* 2a active in rabbit small-intestine in-vivo and in-vitro', *Journal of Clinical Investigation*, vol. 95, no. 6, Jun, pp. 2853-2861.

Fernandez-Prada, CM, Hoover, DL, Tall, BD, Hartman, AB, Kopelowitz, J & Venkatesan, MM 2000, '*Shigella flexneri* IpaH(7.8) facilitates escape of virulent bacteria from the endocytic vacuoles of mouse and human macrophages', *Infection and Immunity*, vol. 68, no. 6, Jun, pp. 3608-3619.

Fic, E, Bonarek, P, Gorecki, A, Kedracka-Krok, S, Mikolajczak, J, Polit, A, Tworzydło, M, Dziedzicka-Wasylewska, M & Wasylewski, Z 2009, 'cAMP receptor protein from *Escherichia coli* as a model of signal transduction in proteins--a review', *J Mol Microbiol Biotechnol*, vol. 17, no. 1, pp. 1-11.

Forchhammer, K, Boesmiller, K & Bock, A 1991, 'The function of selenocysteine synthase and SELB in the synthesis and incorporation of selenocysteine', *Biochimie*, vol. 73, no. 12, Dec, pp. 1481-1486.

Furst, M, Zhou, Y, Merfort, J & Müller, M 2018, 'Involvement of PpiD in Sec-dependent protein translocation', *Biochim Biophys Acta Mol Cell Res*, vol. 1865, no. 2, Feb, pp. 273-280.

Gaudet, P, Livstone, MS, Lewis, SE & Thomas, PD 2011, 'Phylogenetic-based propagation of functional annotations within the Gene Ontology consortium', *Brief Bioinform*, vol. 12, no. 5, Sep, pp. 449-462.

Gonzalez-Fernandez, A, Faro, J & Fernandez, C 2008, 'Immune responses to polysaccharides: lessons from humans and mice', *Vaccine*, vol. 26, no. 3, Jan 17, pp. 292-300.

Gozdziewicz, TK, Lugowski, C & Lukasiewicz, J 2014, 'First evidence for a covalent linkage between enterobacterial common antigen and lipopolysaccharide in *Shigella sonnei* phase II ECALPS', *J Biol Chem*, vol. 289, no. 5, Jan 31, pp. 2745-2754.

Guest, JR & Stephens, PE 1980, 'Molecular cloning of the pyruvate dehydrogenase complex genes of *Escherichia coli*', *Journal of General Microbiology*, vol. 121, no. 2, Dec, pp. 277-292.

Guest, RL, Same Guerra, D, Wissler, M, Grimm, J & Silhavy, TJ 2020, 'YejM modulates activity of the YciM/FtsH protease complex to prevent lethal accumulation of lipopolysaccharide', *MBio*, vol. 11, no. 2, Apr 14.

Hagan, CL, Kim, S & Kahne, D 2010, 'Reconstitution of outer membrane protein assembly from purified components', *Science*, vol. 328, no. 5980, May 14, pp. 890-892.

Han, W, Li, L, Pettit, N, Yi, W, Woodward, R, Liu, X, Guan, W, Bhatt, V, Song, JK & Wang, PG 2010, 'In vitro reconstitution of *Escherichia coli* O86 O antigen repeating unit', *Methods Mol Biol*, vol. 600, pp. 93-110.

Heinrichs, DE, Yethon, JA, Amor, PA & Whitfield, C 1998, 'The assembly system for the outer core portion of R1- and R4-type lipopolysaccharides of *Escherichia coli*. The R1 core-specific beta-glucosyltransferase provides a novel attachment site for O-polysaccharides', *J Biol Chem*, vol. 273, no. 45, Nov 6, pp. 29497-29505.

Hitchcock, PJ & Brown, TM 1983, 'Morphological heterogeneity among *Salmonella* lipopolysaccharide chemotypes in silver-stained polyacrylamide gels', *J Bacteriol*, vol. 154, no. 1, Apr, pp. 269-277.

Hong, M & Payne, SM 1997, 'Effect of mutations in *Shigella flexneri* chromosomal and plasmid-encoded lipopolysaccharide genes on invasion and serum resistance', *Mol Microbiol*, vol. 24, no. 4, May, pp. 779-791.

Inoue, H, Nojima, H & Okayama, H 1990, 'High efficiency transformation of *Escherichia coli* with plasmids', *Gene*, vol. 96, no. 1, Nov 30, pp. 23-28.

Islam, ST, Gold, AC, Taylor, VL, Anderson, EM, Ford, RC & Lam, JS 2011, 'Dual conserved periplasmic loops possess essential charge characteristics that support a catch-and-release mechanism of O-antigen polymerization by Wzy in *Pseudomonas aeruginosa* PAO1', *J Biol Chem*, vol. 286, no. 23, Jun 10, pp. 20600-20605.

Islam, ST, Huszczyński, SM, Nugent, T, Gold, AC & Lam, JS 2013, 'Conserved-residue mutations in Wzy affect O-antigen polymerization and Wzz-mediated chain-length regulation in *Pseudomonas aeruginosa* PAO1', *Sci Rep*, vol. 3, Dec 06, p. 3441.

Islam, ST & Lam, JS 2014, 'Synthesis of bacterial polysaccharides via the Wzx/Wzy-dependent pathway', *Can J Microbiol*, vol. 60, no. 11, Nov, pp. 697-716.

Islam, ST, Taylor, VL, Qi, M & Lam, JS 2010, 'Membrane topology mapping of the O-antigen flippase (Wzx), polymerase (Wzy), and ligase (WaaL) from *Pseudomonas aeruginosa* PAO1 reveals novel domain architectures', *MBio*, vol. 1, no. 3, Aug 24.

Jacobson, KH, Gunsolus, IL, Kuech, TR, Troiano, JM, Melby, ES, Lohse, SE, Hu, D, Chrisler, WB, Murphy, CJ, Orr, G, Geiger, FM, Haynes, CL & Pedersen, JA 2015, 'Lipopolysaccharide density and structure govern the extent and distance of nanoparticle interaction with actual and model bacterial outer membranes.', *Environ Sci Technol*, vol. 49, no. 17, Sep 01, pp. 10642-10650.

Jakhetia, R, Marri, A, Stahle, J, Widmalm, G & Verma, NK 2014, 'Serotype-conversion in *Shigella flexneri*: identification of a novel bacteriophage, Sf101, from a serotype 7a strain', *BMC Genomics*, vol. 15, Aug 30, p. 742.

Kabir, MM, Ho, PY & Shimizu, K 2005, 'Effect of *ldhA* gene deletion on the metabolism of *Escherichia coli* based on gene expression, enzyme activities, intracellular metabolite concentrations, and metabolic flux distribution', *Biochemical Engineering Journal*, vol. 26, no. 1, Nov 1, pp. 1-11.

- Kajimura, J, Rahman, A & Rick, PD 2005, 'Assembly of cyclic enterobacterial common antigen in *Escherichia coli* K-12', *J Bacteriol*, vol. 187, no. 20, Oct, pp. 6917-6927.
- Kalynych, S, Cherney, M, Bostina, M, Rouiller, I & Cygler, M 2015, 'Quaternary structure of WzzB and WzzE polysaccharide co-polymerases', *Protein Sci*, vol. 24, no. 1, Jan, pp. 58-69.
- Kalynych, S, Morona, R & Cygler, M 2014, 'Progress in understanding the assembly process of bacterial O-antigen', *FEMS Microbiol Rev*, vol. 38, no. 5, Sep, pp. 1048-1065.
- Kalynych, S, Ruan, X, Valvano, MA & Cygler, M 2011, 'Structure-guided investigation of lipopolysaccharide O-antigen chain length regulators reveals regions critical for modal length control', *J Bacteriol*, vol. 193, no. 15, Aug, pp. 3710-3721.
- Kalynych, S, Valvano, MA & Cygler, M 2012, 'Polysaccharide co-polymerases: the enigmatic conductors of the O-antigen assembly orchestra', *Protein Eng Des Sel*, vol. 25, no. 11, Nov, pp. 797-802.
- Kaniuk, NA, Vinogradov, E, Li, J, Monteiro, MA & Whitfield, C 2004, 'Chromosomal and plasmid-encoded enzymes are required for assembly of the R3-type core oligosaccharide in the lipopolysaccharide of *Escherichia coli* O157:H7', *J Biol Chem*, vol. 279, no. 30, Jul 23, pp. 31237-31250.
- Katz, C & Ron, EZ 2008, 'Dual role of FtsH in regulating lipopolysaccharide biosynthesis in *Escherichia coli*', *J Bacteriol*, vol. 190, no. 21, Nov, pp. 7117-7122.
- Kenne, L, Lindberg, B & Petersson, K 1977, 'Basic structure of the oligosaccharide repeating-unit of the *Shigella flexneri* O-antigens', *Carbohydr Res*, vol. 56, no. 2, Jul, pp. 363-370.
- Kihara, A, Akiyama, Y & Ito, K 1996, 'A protease complex in the *Escherichia coli* plasma membrane: HflKC (HflA) forms a complex with FtsH (HflB), regulating its proteolytic activity against SecY', *Embo Journal*, vol. 15, no. 22, Nov 15, pp. 6122-6131.
- Kim, MJ, Moon, YH, Kim, H, Rho, S, Shin, YK, Song, M, Walker, R, Czerkinsky, C, Kim, DW & Kim, JO 2018, 'Cross-protective *Shigella* whole-cell vaccine with a truncated O-polysaccharide chain', *Front Microbiol*, vol. 9, p. 2609.
- Kintz, EN & Goldberg, JB 2011, 'Site-directed mutagenesis reveals key residue for O antigen chain length regulation and protein stability in *Pseudomonas aeruginosa* Wzz2', *J Biol Chem*, vol. 286, no. 51, Dec 23, pp. 44277-44284.
- Klein, G & Raina, S 2019, 'Regulated assembly of LPS, its structural alterations and cellular response to LPS defects', *Int J Mol Sci*, vol. 20, no. 2, Jan 16.

Kneidinger, B, Marolda, C, Graninger, M, Zamyatina, A, McArthur, F, Kosma, P, Valvano, MA & Messner, P 2002, 'Biosynthesis pathway of ADP-L-glycero-beta-D-manno-heptose in *Escherichia coli*', *J Bacteriol*, vol. 184, no. 2, Jan, pp. 363-369.

Knirel, YA, Kondakova, AN, Vinogradov, E, Lindner, B, Perepelov, AV & Shashkov, AS 2011, 'Lipopolysaccharide core structures and their correlation with genetic groupings of *Shigella* strains. A novel core variant in *Shigella boydii* type 16', *Glycobiology*, vol. 21, no. 10, Oct, pp. 1362-1372.

Knirel, YA, Sun, Q, Senchenkova, SN, Perepelov, AV, Shashkov, AS & Xu, J 2015, 'O-antigen modifications providing antigenic diversity of *Shigella flexneri* and underlying genetic mechanisms', *Biochemistry (Mosc)*, vol. 80, no. 7, Jul, pp. 901-914.

Korea, CG, Badouraly, R, Prevost, MC, Ghigo, JM & Beloin, C 2010, '*Escherichia coli* K-12 possesses multiple cryptic but functional chaperone-usher fimbriae with distinct surface specificities', *Environ Microbiol*, vol. 12, no. 7, Jul, pp. 1957-1977.

Kotloff, KL, Riddle, MS, Platts-Mills, JA, Pavlinac, P & Zaidi, AKM 2018, 'Shigellosis', *Lancet*, vol. 391, no. 10122, Feb 24, pp. 801-812.

Kuhn, HM, Neter, E & Mayer, H 1983, 'Modification of the lipid moiety of the enterobacterial common antigen by the "Pseudomonas factor"', *Infect Immun*, vol. 40, no. 2, May, pp. 696-700.

Kuk, AC, Hao, A, Guan, Z & Lee, SY 2019, 'Visualizing conformation transitions of the Lipid II flippase MurJ', *Nat Commun*, vol. 10, no. 1, Apr 15, p. 1736.

Kuk, AC, Mashalidis, EH & Lee, SY 2017, 'Crystal structure of the MOP flippase MurJ in an inward-facing conformation', *Nat Struct Mol Biol*, vol. 24, no. 2, Feb, pp. 171-176.

Kumar, S, Rubino, FA, Mendoza, AG & Ruiz, N 2019, 'The bacterial lipid II flippase MurJ functions by an alternating-access mechanism', *J Biol Chem*, vol. 294, no. 3, Jan 18, pp. 981-990.

Lan, R & Reeves, PR 2002, '*Escherichia coli* in disguise: molecular origins of *Shigella*', *Microbes and Infection*, vol. 4, no. 11, pp. 1125-1132.

Langklotz, S, Baumann, U & Narberhaus, F 2012, 'Structure and function of the bacterial AAA protease FtsH', *Biochim Biophys Acta*, vol. 1823, no. 1, Jan, pp. 40-48.

Larue, K, Kimber, MS, Ford, R & Whitfield, C 2009, 'Biochemical and structural analysis of bacterial O-antigen chain length regulator proteins reveals a conserved quaternary structure', *Journal of Biological Chemistry*, vol. 284, no. 11, Mar 13, pp. 7395-7403.

Leinonen, M, Sakkinen, A, Kalliokoski, R, Luotonen, J, Timonen, M & Makela, PH 1986, 'Antibody response to 14-valent pneumococcal capsular polysaccharide vaccine in pre-school age children', *Pediatr Infect Dis*, vol. 5, no. 1, Jan-Feb, pp. 39-44.

Leo, V 2016, 'Screening for novel genes affecting Lipopolysaccharide in *Escherichia coli* K-12 as a model for discovering novel LPS genes in *Shigella flexneri*', Biological Sciences, Honours Thesis thesis, Bachelor of Science (Honours) thesis, The University of Adelaide.

Leo, V, Tran, E & Morona, R 2020, 'Polysaccharide co-polymerase WzzB/WzzE chimeras reveal transmembrane 2 region of WzzB is important for interaction with WzyB', *J Bacteriol*, Dec 23.

Lesinski, GB & Westerink, MA 2001, 'Vaccines against polysaccharide antigens', *Curr Drug Targets Infect Disord*, vol. 1, no. 3, Nov, pp. 325-334.

Levenson, VI, Chernokhvostova, EV, Lyubinskaya, MM, Salamatova, SA, Dzhikidze, EK & Stasilevitch, ZK 1988, 'Parenteral immunization with *Shigella* ribosomal vaccine elicits local IgA response and primes for mucosal memory', *Int Arch Allergy Appl Immunol*, vol. 87, no. 1, pp. 25-31.

Lewis, S, Sadarangani, M, Hoe, JC & Pollard, AJ 2009, 'Challenges and progress in the development of a serogroup B meningococcal vaccine', *Expert Rev Vaccines*, vol. 8, no. 6, Jun, pp. 729-745.

Lima, S, Guo, MS, Chaba, R, Gross, CA & Sauer, RT 2013, 'Dual molecular signals mediate the bacterial response to outer-membrane stress', *Science*, vol. 340, no. 6134, May 17, pp. 837-841.

Liu, Morris, P & Reeves, PR 2019, 'Wzx flippases exhibiting complex O-unit preferences require a new model for Wzx-substrate interactions', *Microbiologyopen*, vol. 8, no. 3, Mar, p. e00655.

Liu, B, Knirel, YA, Feng, L, Perepelov, AV, Senchenkova, SN, Reeves, PR & Wang, L 2014, 'Structural diversity in *Salmonella* O antigens and its genetic basis', *FEMS Microbiol Rev*, vol. 38, no. 1, Jan, pp. 56-89.

Lugowski, C, Romanowska, E, Kenne, L & Lindberg, B 1983, 'Identification of a trisaccharide repeating-unit in the enterobacterial common antigen', *Carbohydrate Research*, vol. 118, no. Jul, pp. 173-181.

Lugtenberg, B, Meijers, J, Peters, R, van der Hoek, P & van Alphen, L 1975, 'Electrophoretic resolution of the "major outer membrane protein" of *Escherichia coli* K12 into four bands', *FEBS Lett*, vol. 58, no. 1, Oct 15, pp. 254-258.

Macpherson, DF, Manning, PA & Morona, R 1994, 'Characterization of the dTDP-rhamnose biosynthetic genes encoded in the *rfb* locus of *Shigella flexneri*', *Mol Microbiol*, vol. 11, no. 2, Jan, pp. 281-292.

Macpherson, DF, Morona, R, Beger, DW, Cheah, KC & Manning, PA 1991, 'Genetic analysis of the *rfb* region of *Shigella flexneri* encoding the Y serotype O-antigen specificity', *Mol Microbiol*, vol. 5, no. 6, Jun, pp. 1491-1499.

Maes, M, Dyson, ZA, Smith, SE, Goulding, DA, Ludden, C, Baker, S, Kellam, P, Reece, ST, Dougan, G & Bartholdson Scott, J 2020, 'A novel therapeutic antibody screening method using bacterial high-content imaging reveals functional antibody binding phenotypes of *Escherichia coli* ST131', *Sci Rep*, vol. 10, no. 1, Jul 24, p. 12414.

Mannel, D & Mayer, H 1978, 'Isolation and chemical characterization of the enterobacterial common antigen', *Eur J Biochem*, vol. 86, no. 2, May 16, pp. 361-370.

Marczak, M, Dzwierzynska, M & Skorupska, A 2013, 'Homo- and heterotypic interactions between Pss proteins involved in the exopolysaccharide transport system in *Rhizobium leguminosarum* bv. trifolii', *Biol Chem*, vol. 394, no. 4, Apr, pp. 541-559.

Margaritis, A & Zajic, JE 1978, 'Mixing, mass-transfer, and scale-up of polysaccharide fermentations', *Biotechnology and Bioengineering*, vol. 20, no. 7, pp. 939-1001.

Marinus, MG & Lobner-Olesen, A 2014, 'DNA Methylation', *EcoSal Plus*, vol. 6, no. 1, May.

Marolda, CL, Tatar, LD, Alaimo, C, Aebi, M & Valvano, MA 2006, 'Interplay of the Wzx translocase and the corresponding polymerase and chain length regulator proteins in the translocation and periplasmic assembly of lipopolysaccharide O antigen', *J Bacteriol*, vol. 188, no. 14, Jul, pp. 5124-5135.

Matos, J, Nardi, M, Kumura, H & Monnet, V 1998, 'Genetic characterization of *pepP*, which encodes an aminopeptidase P whose deficiency does not affect *Lactococcus lactis* growth in milk, unlike deficiency of the X-prolyl dipeptidyl aminopeptidase', *Appl Environ Microbiol*, vol. 64, no. 11, Nov, pp. 4591-4595.

McConnell, MJ, Rumbo, C, Bou, G & Pachon, J 2011, 'Outer membrane vesicles as an acellular vaccine against *Acinetobacter baumannii*', *Vaccine*, vol. 29, no. 34, Aug 5, pp. 5705-5710.

Mitchell, AM, Srikumar, T & Silhavy, TJ 2018, 'Cyclic enterobacterial common antigen maintains the outer membrane permeability barrier of *Escherichia coli* in a manner controlled by YhdP', *MBio*, vol. 9, no. 4, Aug 7.

Morona, R, Mavris, M, Fallarino, A & Manning, PA 1994, 'Characterization of the *rfc* region of *Shigella flexneri*', *J Bacteriol*, vol. 176, no. 3, Feb, pp. 733-747.

Morona, R, Purins, L, Tocilj, A, Matte, A & Cygler, M 2009, 'Sequence-structure relationships in polysaccharide co-polymerase (PCP) proteins', *Trends Biochem Sci*, vol. 34, no. 2, Feb, pp. 78-84.

Morona, R, van den Bosch, L & Manning, PA 1995, 'Molecular, genetic, and topological characterization of O-antigen chain length regulation in *Shigella flexneri*', *J Bacteriol*, vol. 177, no. 4, Feb, pp. 1059-1068.

- Murray, GL, Attridge, SR & Morona, R 2003, 'Regulation of *Salmonella typhimurium* lipopolysaccharide O antigen chain length is required for virulence; identification of FepE as a second Wzz', *Mol Microbiol*, vol. 47, no. 5, Mar, pp. 1395-1406.
- Narberhaus, F, Obrist, M, Fuhrer, F & Langklotz, S 2009, 'Degradation of cytoplasmic substrates by FtsH, a membrane-anchored protease with many talents', *Res Microbiol*, vol. 160, no. 9, Nov, pp. 652-659.
- Nath 2015, 'Charaterisation of the *Shigella flexneri* O antigen polymerase Wzy', School of Biological science, Doctor of Philosophy thesis, The University of Adelaide
- Nath & Morona 2015, 'Detection of Wzy/Wzz interaction in *Shigella flexneri*', *Microbiology*, vol. 161, no. 9, pp. 1797-1805.
- Nath & Morona, R 2015, 'Mutational analysis of the major periplasmic loops of *Shigella flexneri* Wzy: identification of the residues affecting O-antigen modal chain length control, and Wzz-dependent polymerization activity', *Microbiology*, vol. 161, no. Pt 4, Apr, pp. 774-785.
- Nath, Tran, EN & Morona, R 2015, 'Mutational analysis of the *Shigella flexneri* O-antigen polymerase Wzy: identification of Wzz-dependent Wzy mutants', *J Bacteriol*, vol. 197, no. 1, Jan 1, pp. 108-119.
- Nwodo, UU, Green, E & Okoh, AI 2012, 'Bacterial exopolysaccharides: functionality and prospects', *Int J Mol Sci*, vol. 13, no. 11, Oct 30, pp. 14002-14015.
- Oaks, EV & Turbyfill, KR 2006, 'Development and evaluation of a *Shigella flexneri* 2a and *S. sonnei* bivalent invasin complex (Invaplex) vaccine', *Vaccine*, vol. 24, no. 13, Mar 20, pp. 2290-2301.
- Ogura, T, Inoue, K, Tatsuta, T, Suzaki, T, Karata, K, Young, K, Su, LH, Fierke, CA, Jackman, JE, Raetz, CR, Coleman, J, Tomoyasu, T & Matsuzawa, H 1999, 'Balanced biosynthesis of major membrane components through regulated degradation of the committed enzyme of lipid A biosynthesis by the AAA protease FtsH (HflB) in *Escherichia coli*', *Mol Microbiol*, vol. 31, no. 3, Feb, pp. 833-844.
- Okuda, J, Toyotome, T, Kataoka, N, Ohno, M, Abe, H, Shimura, Y, Seyedarabi, A, Pickersgill, R & Sasakawa, C 2005, '*Shigella* effector IpaH9.8 binds to a splicing factor U2AF(35) to modulate host immune responses', *Biochemical and Biophysical Research Communications*, vol. 333, no. 2, Jul 29, pp. 531-539.
- Pang, E, Tien-Lin, C, Selvaraj, M, Chang, J & Kwang, J 2011, 'Deletion of the aceE gene (encoding a component of pyruvate dehydrogenase) attenuates *Salmonella enterica* serovar *Enteritidis*', *FEMS Immunol Med Microbiol*, vol. 63, no. 1, Oct, pp. 108-118.



Papadopoulos, M & Morona, R 2010, 'Mutagenesis and chemical cross-linking suggest that Wzz dimer stability and oligomerization affect lipopolysaccharide O-antigen modal chain length control', *Journal of bacteriology*, vol. 192, no. 13, Jul, pp. 3385-3393.

Papadopoulos, M, Tran, EN, Murray, GL & Morona, R 2016, 'Conserved transmembrane glycine residues in the *Shigella flexneri* polysaccharide co-polymerase protein WzzB influence protein-protein interactions', *Microbiology*, vol. 162, no. 6, Jun, pp. 921-929.

Parker, DJ, Demetci, P & Li, GW 2019, 'Rapid accumulation of motility-activating mutations in resting liquid culture of *Escherichia coli*', *J Bacteriol*, vol. 201, no. 19, Oct 1.

Passwell, JH, Ashkenazi, S, Banet-Levi, Y, Ramon-Saraf, R, Farzam, N, Lerner-Geva, L, Even-Nir, H, Yerushalmi, B, Chu, C, Shiloach, J, Robbins, JB, Schneerson, R & Israeli Shigella Study, G 2010, 'Age-related efficacy of *Shigella* O-specific polysaccharide conjugates in 1-4-year-old Israeli children', *Vaccine*, vol. 28, no. 10, Mar 2, pp. 2231-2235.

Passwell, JH, Ashkenazi, S, Harlev, E, Miron, D, Ramon, R, Farzam, N, Lerner-Geva, L, Levi, Y, Chu, C, Shiloach, J, Robbins, JB, Schneerson, R & Israel Shigella Study, G 2003, 'Safety and immunogenicity of *Shigella sonnei*-CRM9 and *Shigella flexneri* type 2a-rEPAsucc conjugate vaccines in one- to four-year-old children', *Pediatr Infect Dis J*, vol. 22, no. 8, Aug, pp. 701-706.

Passwell, JH, Harlev, E, Ashkenazi, S, Chu, C, Miron, D, Ramon, R, Farzan, N, Shiloach, J, Bryla, DA, Majadly, F, Roberson, R, Robbins, JB & Schneerson, R 2001, 'Safety and immunogenicity of improved *Shigella* O-specific polysaccharide-protein conjugate vaccines in adults in Israel', *Infect Immun*, vol. 69, no. 3, Mar, pp. 1351-1357.

Peltola, H, Kilpi, T & Anttila, M 1992, 'Rapid disappearance of *Haemophilus influenzae* type b meningitis after routine childhood immunisation with conjugate vaccines', *Lancet*, vol. 340, no. 8819, Sep 05, pp. 592-594.

Perepelov, AV, Shekht, ME, Liu, B, Shevelev, SD, Ledov, VA, Senchenkova, SN, L'Vov V, L, Shashkov, AS, Feng, L, Aparin, PG, Wang, L & Knirel, YA 2012, '*Shigella flexneri* O-antigens revisited: final elucidation of the O-acetylation profiles and a survey of the O-antigen structure diversity', *FEMS Immunol Med Microbiol*, vol. 66, no. 2, Nov, pp. 201-210.

Phan, MD, Peters, KM, Sarkar, S, Lukowski, SW, Allsopp, LP, Gomes Moriel, D, Achard, ME, Totsika, M, Marshall, VM, Upton, M, Beatson, SA & Schembri, MA 2013, 'The serum resistome of a globally disseminated multidrug resistant uropathogenic *Escherichia coli* clone', *PLoS Genet*, vol. 9, no. 10, p. e1003834.

Philippe, JS 2004, 'War and peace at mucosal surfaces', *Nature Reviews Immunology*, vol. 4, no. 12, pp. 953-964.

Pines, O & Gutnick, D 1986, 'Role for Emulsan in Growth of *Acinetobacter calcoaceticus* RAG-1 on Crude Oil', *Appl Environ Microbiol*, vol. 51, no. 3, Mar, pp. 661-663.

Polissi, A & Sperandio, P 2014, 'The lipopolysaccharide export pathway in *Escherichia coli*: structure, organization and regulated assembly of the Lpt machinery', *Mar Drugs*, vol. 12, no. 2, Feb 17, pp. 1023-1042.

Poltorak, A, He, X, Smirnova, I, Liu, MY, Van Huffel, C, Du, X, Birdwell, D, Alejos, E, Silva, M, Galanos, C, Freudenberg, M, Ricciardi-Castagnoli, P, Layton, B & Beutler, B 1998, 'Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene', *Science*, vol. 282, no. 5396, Dec 11, pp. 2085-2088.

Purins, L, Van Den Bosch, L, Richardson, V & Morona, R 2008, 'Coiled-coil regions play a role in the function of the *Shigella flexneri* O-antigen chain length regulator Wzz<sub>PHS2</sub>', *Microbiology*, vol. 154, no. Pt 4, Apr, pp. 1104-1116.

Putker, F, Bos, MP, Tommassen, J & Filloux, A 2015, 'Transport of lipopolysaccharide to the Gram negative bacterial cell surface', vol. 39, no. 6, pp. 985-1002.

Raetz, CR, Reynolds, CM, Trent, MS & Bishop, RE 2007, 'Lipid A modification systems in Gram-negative bacteria', *Annu Rev Biochem*, vol. 76, pp. 295-329.

Raetz, CR & Whitfield, C 2002, 'Lipopolysaccharide endotoxins', *Annu Rev Biochem*, vol. 71, pp. 635-700.

Rai, AK & Mitchell, AM 2020, 'Enterobacterial common antigen: synthesis and function of an enigmatic molecule', *MBio*, vol. 11, no. 4, Aug 11.

Ranallo, RT, Kaminski, RW, George, T, Kordis, AA, Chen, Q, Szabo, K & Venkatesan, MM 2010, 'Virulence, inflammatory potential, and adaptive immunity induced by *Shigella flexneri* msbB mutants', *Infection and Immunity*, vol. 78, no. 1, pp. 400-412.

Rosenfeld, Y & Shai, Y 2006, 'Lipopolysaccharide (Endotoxin)-host defense antibacterial peptides interactions: role in bacterial resistance and prevention of sepsis', *Biochim Biophys Acta*, vol. 1758, no. 9, Sep, pp. 1513-1522.

Sakaguchi, T, Köhler, H, Gu, X, McCormick, BA & Reinecker, Hc 2002, '*Shigella flexneri* regulates tight junction-associated proteins in human intestinal epithelial cells', *Cellular Microbiology*, vol. 4, no. 6, pp. 367-381.

Sakai, T, Sasakawa, C & Yoshikawa, M 1988, 'Expression of 4 virulence antigens of *Shigella flexneri* is positively regulated at the transcriptional level by the 30 kilodalton Virf protein', *Molecular Microbiology*, vol. 2, no. 5, Sep, pp. 589-597.

Sakai, Y, Kimura, S & Suzuki, T 2019, 'Dual pathways of tRNA hydroxylation ensure efficient translation by expanding decoding capability', *Nat Commun*, vol. 10, no. 1, Jun 28, p. 2858.

Sansonetti, PJ, Phalipon, A, Arondel, J, Thirumalai, K, Banerjee, S, Akira, S, Takeda, K & Zychlinsky, A 2000, 'caspase-1 Activation of IL-1 $\beta$  and IL-18 are essential for *Shigella flexneri*-induced inflammation', *Immunity*, vol. 12, no. 5, pp. 581-590.

Satpute, SK, Banpurkar, AG, Dhakephalkar, PK, Banat, IM & Chopade, BA 2010, 'Methods for investigating biosurfactants and bioemulsifiers: a review', *Crit Rev Biotechnol*, vol. 30, no. 2, Jun, pp. 127-144.

Schild, S, Nelson, EJ, Bishop, AL & Camilli, A 2009, 'Characterization of *Vibrio cholerae* outer membrane vesicles as a candidate vaccine for cholera', *Infect Immun*, vol. 77, no. 1, Jan, pp. 472-484.

Schmidt, G, Mannel, D, Mayer, H, Whang, HY & Neter, E 1976, 'Role of a lipopolysaccharide gene for immunogenicity of the enterobacterial common antigen', *J Bacteriol*, vol. 126, no. 2, May, pp. 579-586.

Sham, LT, Zheng, S, Yakhnina, AA, Kruse, AC & Bernhardt, TG 2018, 'Loss of specificity variants of WzxC suggest that substrate recognition is coupled with transporter opening in MOP-family flippases', *Mol Microbiol*, vol. 109, no. 5, Sep, pp. 633-641.

Shim, DH, Chang, SY, Park, SM, Jang, H, Carbis, R, Czerkinsky, C, Uematsu, S, Akira, S & Kweon, MN 2007, 'Immunogenicity and protective efficacy offered by a ribosomal-based vaccine from *Shigella flexneri* 2a', *Vaccine*, vol. 25, no. 25, Jun 15, pp. 4828-4836.

Sperandeo, P, Villa, R, Martorana, AM, Samalikova, M, Grandori, R, Deho, G & Polissi, A 2011, 'New insights into the Lpt machinery for lipopolysaccharide transport to the cell surface: LptA-LptC interaction and LptA stability as sensors of a properly assembled transenvelope complex', *The Journal of Bacteriology*, vol. 193, no. 5, pp. 1042-1053.

Steimle, A, Autenrieth, IB & Frick, JS 2016, 'Structure and function: Lipid A modifications in commensals and pathogens', *Int J Med Microbiol*, vol. 306, no. 5, Aug, pp. 290-301.

Stenutz, R, Weintraub, A & Widmalm, G 2006, 'The structures of *Escherichia coli* O-polysaccharide antigens', *FEMS Microbiol Rev*, vol. 30, no. 3, May, pp. 382-403.

Stevenson, G, Kessler, A & Reeves, PR 1995, 'A plasmid-borne O-antigen chain length determinant and its relationship to other chain length determinants', *FEMS Microbiol Lett*, vol. 125, no. 1, Jan 1, pp. 23-30.

Taylor, PL, Sugiman-Marangos, S, Zhang, K, Valvano, MA, Wright, GD & Junop, MS 2010, 'Structural and kinetic characterization of the LPS biosynthetic enzyme D-alpha,beta-D-heptose-1,7-bisphosphate phosphatase (GmhB) from *Escherichia coli*', *Biochemistry*, vol. 49, no. 5, Feb 9, pp. 1033-1041.

Teh, Tran, ENH & Morona, R 2012, 'Absence of O antigen suppresses *Shigella flexneri* IcsA autochaperone region mutations', *Microbiology (United Kingdom)*, vol. 158, no. 11, pp. 2835-2850.

Teh, MY, Furevi, A, Widmalm, G & Morona, R 2020, 'Influence of *Shigella flexneri* 2a O Antigen acetylation on its bacteriophage Sf6 receptor activity and bacterial interaction with human cells', *J Bacteriol*, vol. 202, no. 24, Nov 19.

Tocilj, A, Munger, C, Proteau, A, Morona, R, Purins, L, Ajamian, E, Wagner, J, Papadopoulos, M, Van Den Bosch, L, Rubinstein, JL, Fethiere, J, Matte, A & Cygler, M 2008, 'Bacterial polysaccharide co-polymerases share a common framework for control of polymer length', *Nat Struct Mol Biol*, vol. 15, no. 2, Feb, pp. 130-138.

Tran, Day, CJ, McCartney, E, Poole, J, Tse, E, Jennings, MP & Morona, R 2020, '*Shigella flexneri* targets human colonic goblet cells by O Antigen binding to sialyl-Tn and Tn antigens via glycan-glycan interactions', *ACS Infect Dis*, vol. 6, no. 10, Oct 9, pp. 2604-2615.

Tran & Morona, R 2013, 'Residues located inside the *Escherichia coli* FepE protein oligomer are essential for lipopolysaccharide O-antigen modal chain length regulation', *Microbiology*, vol. 159, no. Pt 4, Apr, pp. 701-714.

Tran, Papadopoulos, M & Morona, R 2014, 'Relationship between O-antigen chain length and resistance to colicin E2 in *Shigella flexneri*', *Microbiology-Sgm*, vol. 160, no. 3, Mar, pp. 589-601.

Tsai, CM & Frasch, CE 1982, 'A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels', *Anal Biochem*, vol. 119, no. 1, Jan 1, pp. 115-119.

Tsukazaki, T, Mori, H, Echizen, Y, Ishitani, R, Fukai, S, Tanaka, T, Perederina, A, Vassylyev, DG, Kohno, T, Maturana, AD, Ito, K & Nureki, O 2011, 'Structure and function of a membrane component SecDF that enhances protein export', *Nature*, vol. 474, no. 7350, May 11, pp. 235-238.

Van den Bosch, L & Morona, R 2003, 'The actin-based motility defect of a *Shigella flexneri* *rmlD* rough LPS mutant is not due to loss of IcsA polarity', *Microb Pathog*, vol. 35, no. 1, Jul, pp. 11-18.

Whitfield & Trent 2014, 'Biosynthesis and export of bacterial lipopolysaccharides', *Annu Rev Biochem*, vol. 83, pp. 99-128.

Whitfield, C, Wear, SS & Sande, C 2020, 'Assembly of bacterial capsular polysaccharides and exopolysaccharides', *Annu Rev Microbiol*, Jul 17.

Whitfield, C, Williams, DM & Kelly, SD 2020, 'Lipopolysaccharide O-antigens-bacterial glycans made to measure', *J Biol Chem*, vol. 295, no. 31, Jul 31, pp. 10593-10609.

Wilce, MC, Bond, CS, Dixon, NE, Freeman, HC, Guss, JM, Lilley, PE & Wilce, JA 1998, 'Structure and mechanism of a proline-specific aminopeptidase from *Escherichia coli*', *Proc Natl Acad Sci U S A*, vol. 95, no. 7, Mar 31, pp. 3472-3477.

Willett, JL, Gucinski, GC, Fatherree, JP, Low, DA & Hayes, CS 2015, 'Contact-dependent growth inhibition toxins exploit multiple independent cell-entry pathways', *Proc Natl Acad Sci U S A*, vol. 112, no. 36, Sep 8, pp. 11341-11346.

Wiseman, B, Nitharwal, RG, Widmalm, G & Hogbom, M 2021, 'Structure of a full-length bacterial polysaccharide co-polymerase', *Nat Commun*, vol. 12, no. 1, Jan 14, p. 369.

Woodward, R, Yi, W, Li, L, Zhao, G, Eguchi, H, Sridhar, PR, Guo, H, Song, JK, Motari, E, Cai, L, Kelleher, P, Liu, X, Han, W, Zhang, W, Ding, Y, Li, M & Wang, PG 2010, '*In vitro* bacterial polysaccharide biosynthesis: defining the functions of Wzy and Wzz', *Nat Chem Biol*, vol. 6, no. 6, Jun, pp. 418-423.

Yamamoto, N, Nakahigashi, K, Nakamichi, T, Yoshino, M, Takai, Y, Touda, Y, Furubayashi, A, Kinjyo, S, Dose, H, Hasegawa, M, Datsenko, KA, Nakayashiki, T, Tomita, M, Wanner, BL & Mori, H 2009, 'Update on the Keio collection of *Escherichia coli* single-gene deletion mutants', *Mol Syst Biol*, vol. 5, p. 335.

Yoshida, S, Handa, Y, Suzuki, T, Ogawa, M, Suzuki, M, Tamai, A, Abe, A, Katayama, E & Sasakawa, C 2006, 'Microtubule-severing activity of *Shigella* is pivotal for intercellular spreading', *Science*, vol. 314, no. 5801, Nov 10, pp. 985-989.

Yoshida, S, Katayama, E, Kuwae, A, Mimuro, H, Suzuki, T & Sasakawa, C 2002, '*Shigella* deliver an effector protein to trigger host microtubule destabilization, which promotes Rac1 activity and efficient bacterial internalization', *Embo Journal*, vol. 21, no. 12, Jun 17, pp. 2923-2935.

Zakharov, SD, Sharma, O, Zhalnina, M, Yamashita, E & Cramer, WA 2012, 'Pathways of colicin import: utilization of BtuB, OmpF porin and the TolC drug-export protein', *Biochemical Society Transactions*, vol. 40, Dec, pp. 1463-1468.

Zumsteg, AB, Goosmann, C, Brinkmann, V, Morona, R & Zychlinsky, A 2014, 'IcsA is a *Shigella flexneri* adhesion regulated by the type III secretion system and required for pathogenesis', *Cell Host & Microbe*, vol. 15, no. 4, Apr 9, pp. 435-445.