

**Application of DNA metabarcoding and  
high-throughput sequencing for enhanced  
forensic soil DNA analysis**

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## THESIS ABSTRACT

The complex and variable soil matrix can support a wide range of biota that can provide information about local vegetation, soil conditions (e.g. soil acidity) and habitat type. As the combination of microbes, plants and animals within a soil is often specific to a given site, identification of the soil biota can narrow the likely source of a soil sample. DNA fingerprinting analysis of soil microbes has been used as forensic evidence in court to establish a link between a suspect and a site, victim or object. However, previous genetic analyses have relied on patterns of fragment length variation produced by amplification of unidentified taxa in the soil extract, particularly bacteria. In contrast, the development of advanced DNA sequencing technologies now provides the ability to generate a detailed picture of soil communities and the taxa present, allowing for improved discrimination between samples. This thesis examines the use of DNA metabarcoding combined with high-throughput sequencing (HTS) technology to distinguish between soils from different locations in a forensic context. Specifically, I review the DNA extraction protocols available for soils and recommend best practice for successful analysis (Chapter 2). Following this, I examine the reproducibility and discriminatory power of four different genetic markers for forensic soil discrimination using HTS (Chapter 3). Non-bacterial DNA, particularly fungi, were found to be the most promising target for soil discrimination and additionally showed consistent PCR amplification and low contamination risk. It is known that DNA extraction protocols can introduce discrepancies in soil community profiles, and the optimal sample size for an accurate and representative survey of soil diversity has been debated. Therefore I used various soil types to test the robustness of modified DNA extraction protocols (Chapter 4) and trace, or limited, amounts of soil (Chapter 5). I make recommendations about the optimal DNA extraction method and sample size given soil properties such as clay content, soil pH and texture. To assess the

application of this method in forensic casework, I then designed a mock case scenario. DNA profiles of six soil samples recovered from a suspect's belongings were compared to those collected from seven reference sites around Adelaide, South Australia. This study demonstrated that the soil from the suspect's belongings had eukaryote diversity more similar to those collected from the crime scene than to any other sample collected at random. This suggested the presence of the suspect at the crime scene. This result was compared to that from a soil analysis method currently accepted in court. In this case example, both methods successfully established a link between the suspect's belongings and the crime scene; however, DNA analysis improved resolution between reference locations. This thesis demonstrates the first practical application of DNA metabarcoding and high-throughput sequencing (HTS) to forensic soil analysis. I show that this approach is consistently able to distinguish between soil samples taken from different localities, and consequently may be employed as an additional line of evidence or investigation in forensic casework.



## THESIS DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Jennifer M. Young and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference has been made in the text. I certify that no part of this work will, in the future, be used in a submission 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.

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July 2014



## **Publications**

**Young, Jennifer M.**, et al. "Limitations and recommendations for successful DNA extraction from forensic soil samples: A review." *Science & Justice* 54.3 (2014): 238-244.

**Young, Jennifer M.**, Laura S. Weyrich, and Alan Cooper. "Forensic soil DNA analysis using high-throughput sequencing: A comparison of four molecular markers." *Forensic Science International: Genetics* 13 (2014): 176-184.

RAWLENCE, N. J., LOWE, D. J., WOOD, J. R., **YOUNG, J. M.**, CHURCHMAN, G., HUANG, Y. T., & COOPER, A. (2014). Using palaeoenvironmental DNA to reconstruct past environments: progress and prospects. *Journal of Quaternary Science*, 29(7), 610-626.

## **Oral Presentations**

**22nd International Symposium on the Forensic Sciences (ANZFSS)**, Adelaide, August 2014. High-throughput sequencing of soil eukaryotes links a suspect to a crime scene: a mock case scenario.

**Forensic Science South Australia (FSSA) Seminar**, Adelaide, July 2014, Overview of PhD Thesis

**25th World Congress of the International Society for Forensic Genetics (ISFG)**, Melbourne, September 2013. The use of NGS for enhanced forensic soil DNA analysis.

**Three minute thesis competition**, University of Adelaide, July 2013, Forensic soil analysis using metagenomic analysis.

**AFP-UC Forensic R&D Workshop**, Canberra, March 2012. DNA metabarcoding for forensic soil analysis.

**SMANZFL Meeting** (Senior Managers of Australian and New Zealand Forensic Labs), November 2011

**Post Graduate Initial Seminar**, University of Adelaide, November 2011,  
Environmental Genomics for soil Forensics.

Upcoming presentations

**Australian Federal Police Research and Development Team**, Canberra, December 2014, *Overview of PhD Thesis*

**Poster Presentations**

**22nd International Symposium on the Forensic Sciences (ANSFSS)**, Hobart, September 2012. *Forensic soil analysis: Using DNA metabarcoding and next generation sequencing to differentiate between habitat types.*

**2012 Post Graduate Poster Day**, University of Adelaide, July 2012. *DIGGING THE DIRT: validation of DNA metabarcoding to differentiate forensic soil samples from different habitat types.*

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