Identification and characterisation of novel transcripts involved in the proliferation, differentiation and developmental networks of the mouse cerebral cortex

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Bachelor of Science (Hons.) (Biomedical Sciences)

Master of Science (Genetics)

A thesis submitted for the Degree of Doctor of Philosophy

School of Medicine

(Discipline of Medicine)

Faculty of Health Sciences

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APPENDIX B

Additional information for Chapter 4.

B-1. Authors' declaration.

B-2. Supplementary information 1. Original file is accessible at http://cercor.oxfordjournals.org/content/suppl/2010/07/14/bhq141.DC1/cercor-2010-00377-File009.pdf.

B-3. Supplementary information 2. Original file is accessible at http://cercor.oxfordjournals.org/content/suppl/2010/07/14/bhq141.DC1/cercor-2010-00377-File010.doc.

B-4. Supplementary information file 3. Original file is accessible at the publisher's website http://cercor.oxfordjournals.org/content/suppl/2010/07/14/bhq141.DC1/cercor-2010-00377-File011.tar.

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B-1 Authors' declaration

DECLARATION OF INDIVIDUAL CONTRIBUTIONS TO THE PUBLISHED OR SUBMITTED MANUSCRIPTS

Title:

Spatiotemporal regulation of multiple overlapping sense and novel antisense transcripts at the *Nrgn* and *Camk2n1* gene loci during mouse cerebral corticogenesis.

Authors:

King-Hwa Ling (KHL), Chelsee A Hewitt (CAH), Tim Beissbarth (TB), Lavinia Hyde (LH), Pike-See Cheah (PSC), Gordon K Smyth (GKS), Seong-Seng Tan (SST), Christopher N Hahn (CNH), Tim Thomas (TT), Paul Q Thomas (PQT) and Hamish S Scott (HSS).

Published in: Cerebral Cortex 2010, doi: 10.1093/cercor/bhq141.

Declaration:

KHL performed all the experiments in the manuscript. KHL, CAH, PSC and TT procured the mouse tissues used in the entire study. SST provided the SAGE datasets for analysis. KHL and CAH performed the SAGE validation analysis. TB, LH and GKS designed, performed and supervised the SAGE and genomic clustering analyses. KHL performed whereas GKS supervised the RT-qPCR analyses. KHL and TT performed all the radioactive ISH studies. KHL, PSC, CNH and PQT carried out the expression studies on *Nrgn* and *Camk2n1* transcripts. KHL and PSC performed the RNA FISH analysis. CAH, CNH, TT, PQT and HSS conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

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Chelsee A Hewitt		
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Lavinia Hyde		
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Christopher N Hahn		
Tim Thomas		
Paul Q Thomas		
Hamish S Scott		

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B-2 Supplementary information 1

NOTE:
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B-3 Supplementary information 2

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B-4 Supplementary information 3

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APPENDIX C

Additional information for Chapter 5.

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C-2. Supplementary information 1.

C-3. Supplementary information 2.

C-4. Supplementary information 3.

C-1 Authors' declaration

DECLARATION OF INDIVIDUAL CONTRIBUTIONS TO THE PUBLISHED OR SUBMITTED MANUSCRIPTS

Title:

Sense and overlapping natural antisense transcripts form double stranded RNA to produce a novel endogenous small interfering RNA during brain development

Authors:

King-Hwa Ling (KHL), Peter J Brautigan (PJB), Sarah Moore (SM), Rachel Fraser (RF), Pike-See Cheah (PSC), Joy M Raison (JMR), Milena Stankovic (MS), Tasman Daish (TD), Deidre M Mattiske (DMM), Jeffrey Mann (JRM), David L Adelson (DLA), Paul Q Thomas (PQT), Christopher N Hahn (CNH) and Hamish S Scott (HSS).

Submitted Manuscript.

Declaration:

KHL, PJB and CNH supervised, designed and carried out the overexpression studies and all qPCR analysis. KHL, SM and RF participated in the design and RNA FISH experiment. PQT supervised whereas KHL and PSC performed the LNA-ISH analysis. KHL, JMR and DLA participated in the small RNA sequence analysis, annotations and statistical analysis. KHL and MS performed the immunoblotting analysis. KHL and TD performed the small RNA northern analysis. DMM and JRM cultured and provided the mES cells with DICER1 conditional allele. KHL drafted the manuscript. PQT, CNH and HSS conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

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Sarah Moore		
Rachel Fraser		
Pike-See Cheah		
Joy M Raison		
Milena Stankovic		
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ID   Sox4\gene\locus\-\Supplementary\Information\1 standard; DNA;    ; 5859 BP.
DE   Sox4 gene locus of Mus musculus
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C-3 Supplementary information 2

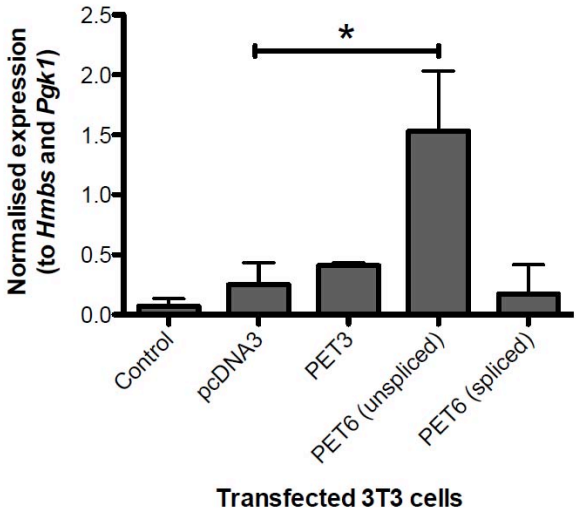

Figure S1 **Overexpression analysis of PET6-(unspliced) and PET6-(spliced) in NIH/3T3 cells.**
NIH/3T3 cells were transfected with different constructs to determine the effect of spliced and unspliced variants of PET6 on the *Sox4_sir3* expression level.

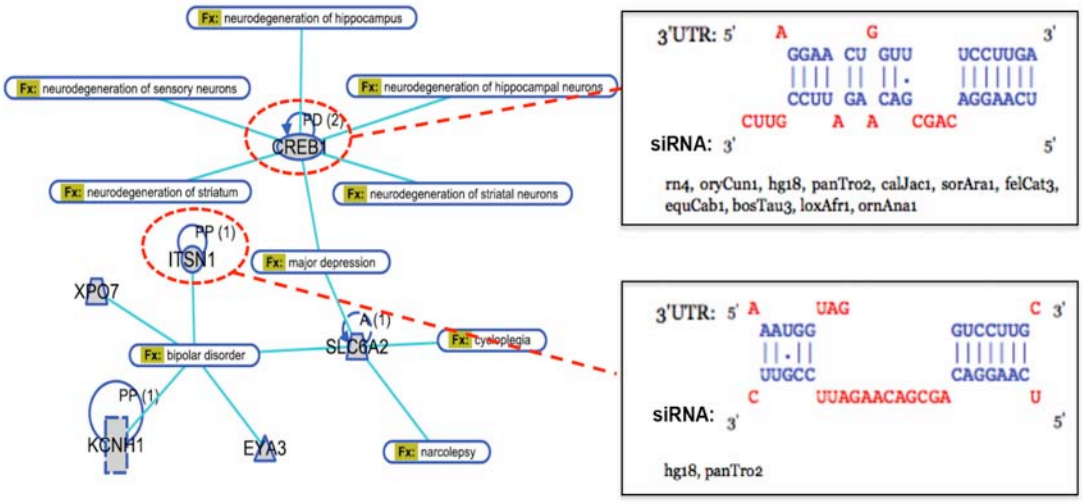

Figure S2 **Ingenuity Pathway Analysis and DIANA-microT v3.0 analyses.** The panel on the left shows the screenshot from the Ingenuity Pathway Analysis (IPA) analysis on associated known neurological disorders with *Sox4_sir3* downstream target genes as predicted by DIANA-microT 3.0 web-based program. The panel of the right shows the most conserved predicted target sites in *Creb1* and *Itsn1* for *Sox4_sir3*. Chromosomal position of the predicted target site was based on the mm9 mouse genome (July 2007). Conservation information of these sites was derived from the comparison of various genomes; bosTau3 = Cow genome (August 2006), calJac1 = Common marmoset (June 2007), dasNov1 = Armadillo genome (May 2005), equCab1 = Horse genome (January 2007), felCat3 = Cat genome (March 2006), hg18 = Human genome (May 2006), loxAfr1 = Elephant genome (May 2005), ornAna1 = platypus genome (March 2007), oryCun1 = Rabbit genome (May 2005), panTro2 = Chimpanzee (March 2006), rn4 = Rat genome (November 2004), sorAra1 = Shrew genome (January 2006), xenTro2 = Frog (*Xenopus tropicalis*) genome (August 2005).

C-4 Supplementary information 3

Table S1: List of predicted target sites for *Sox4_sir3* based on DIANA-microT v3.0 prediction.

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ENSMUSG00000022957	Itsn1	16	1	7mer(pos 2)	2692	2720	0	Not Conserved	16	91914947	91914975
ENSMUSG00000022957	Itsn1	16	1	9mer(pos 1)	3008	3036	0	Not Conserved	16	91915263	91915291
ENSMUSG00000022957	Itsn1	16	1	9mer(pos 1)	4827	4855	1	rn4	16	91917082	91917110
ENSMUSG00000022957	Itsn1	16	1	7mer(pos 2)	7938	7966	0	Not Conserved	16	91920193	91920221
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ENSMUSG00000034551	Hdx	15	1	7mer(pos 1)	3676	3704	1	xenTro2	X	108687177	108687205
ENSMUSG00000034551	Hdx	15	1	7mer(pos 1)	3927	3955	2	monDom4,galGal3	X	108686926	108686954
ENSMUSG00000034551	Hdx	15	1	8mer(pos 1)	4473	4501	1	ornAna1	X	108686380	108686408
ENSMUSG00000034551	Hdx	15	1	7mer(pos 1)	4623	4651	0	Not Conserved	X	108686230	108686258
ENSMUSG00000034551	Hdx	15	1	7mer(pos 1)	5627	5655	2	calJac1,ornAna1	X	108685226	108685254
ENSMUSG00000034551	Hdx	15	1	7mer(pos 1)	6060	6088	0	Not Conserved	X	108684793	108684821
ENSMUSG00000025958	Creb1	14	1	8mer(pos 2)	598	626	8	rn4,oryCun1,hg18,panTro2,calJac1,sorAra1,equCab1,bosTau3	1	64644551	64644579
ENSMUSG00000025958	Creb1	14	1	7mer(pos 1)	820	848	2	rn4,bosTau3	1	64644773	64644801
ENSMUSG00000025958	Creb1	14	1	8mer(pos 2)	864	892	2	rn4,dasNov1	1	64644817	64644845
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ENSMUSG00000032244	Fem1b	12	1	7mer(pos 2)	3931	3959	0	Not Conserved	9	62639940	62639968
ENSMUSG00000055368	Slc6a2	11	1	7mer(pos 1)	1247	1275	0	Not Conserved	8	95522796	95522824
ENSMUSG00000055368	Slc6a2	11	1	8mer(pos 2)	1447	1475	0	Not Conserved	8	95522996	95523024
ENSMUSG00000055368	Slc6a2	11	1	8mer(pos 1)	1667	1695	0	Not Conserved	8	95523216	95523244
ENSMUSG00000055368	Slc6a2	11	1	8mer(pos 2)	3809	3837	0	Not Conserved	8	95525358	95525386
ENSMUSG00000050592	A130092J06Rik	11	1	7mer(pos 1)	696	724	1	rn4	2	31924040	31924068
ENSMUSG00000050592	A130092J06Rik	11	1	9mer(pos 1)	1282	1310	0	Not Conserved	2	31923454	31923482
ENSMUSG00000050592	A130092J06Rik	11	1	7mer(pos 2)	1810	1838	0	Not Conserved	2	31922926	31922954
ENSMUSG00000050592	A130092J06Rik	11	1	8mer(pos 2)	2203	2231	1	dasNov1	2	31922533	31922561
ENSMUSG00000022043	Trim35	11	1	7mer(pos 2)	262	290	1	rn4	14	66928436	66928464
ENSMUSG00000022043	Trim35	11	1	9mer(pos 1)	443	471	0	Not Conserved	14	66928617	66928645
ENSMUSG00000022043	Trim35	11	1	7mer(pos 2)	782	810	1	rn4	14	66928956	66928984
ENSMUSG00000022043	Trim35	11	1	8mer(pos 2)	910	938	0	Not Conserved	14	66929084	66929112
ENSMUSG00000052271	Bhlhb8	10	1	7mer(pos 1)	315	343	0	Not Conserved	5	144952850	144952878
ENSMUSG00000052271	Bhlhb8	10	1	8mer(pos 2)	419	447	0	Not Conserved	5	144952954	144952982
ENSMUSG00000052271	Bhlhb8	10	1	8mer(pos 2)	1221	1249	1	rn4	5	144953756	144953784
ENSMUSG00000052271	Bhlhb8	10	1	7mer(pos 1)	1274	1302	0	Not Conserved	5	144953809	144953837
ENSMUSG00000038628	Polr3k	10	1	8mer(pos 1)	110	138	0	Not Conserved	2	181603147	181603175
ENSMUSG00000038628	Polr3k	10	1	7mer(pos 2)	135	163	2	rn4,oryCun1	2	181603172	181603200
ENSMUSG00000038628	Polr3k	10	1	7mer(pos 2)	277	305	1	rn4	2	181603314	181603342
ENSMUSG00000038628	Polr3k	10	1	8mer(pos 2)	286	314	0	Not Conserved	2	181603323	181603351
ENSMUSG00000037355	4933407H18Rik	10	1	9mer(pos 1)	4891	4919	0	Not Conserved	5	33762432	33762460
ENSMUSG00000037355	4933407H18Rik	10	1	7mer(pos 2)	5935	5963	0	Not Conserved	5	33763476	33763504
ENSMUSG00000037355	4933407H18Rik	10	1	9mer(pos 1)	7070	7098	0	Not Conserved	5	33764611	33764639
ENSMUSG00000079481	A2AMP5_MOUSE	9	1	7mer(pos 1)	388	416	0	Not Conserved	X	99278655	99278683
ENSMUSG00000079481	A2AMP5_MOUSE	9	1	7mer(pos 2)	3780	3808	1	rn4	X	99282047	99282075
ENSMUSG00000079481	A2AMP5_MOUSE	9	1	7mer(pos 1)	6437	6465	0	Not Conserved	X	99284704	99284732
ENSMUSG00000079481	A2AMP5_MOUSE	9	1	8mer(pos 2)	7504	7532	0	Not Conserved	X	99285771	99285799
ENSMUSG00000046897	Zfp740	9	1	8mer(pos 1)	213	241	0	Not Conserved	15	102043330	102043358
ENSMUSG00000046897	Zfp740	9	1	9mer(pos 1)	790	818	1	rn4	15	102043907	102043935
ENSMUSG00000046897	Zfp740	9	1	7mer(pos 2)	2483	2511	0	Not Conserved	15	102045600	102045628
ENSMUSG00000042873	Lhfpl4	9	1	7mer(pos 2)	336	364	2	rn4,cavPor2	6	113121487	113121515
ENSMUSG00000042873	Lhfpl4	9	1	7mer(pos 2)	1571	1599	1	rn4	6	113120252	113120280
ENSMUSG00000042873	Lhfpl4	9	1	8mer(pos 2)	1684	1712	1	rn4	6	113120139	113120167
ENSMUSG00000042873	Lhfpl4	9	1	7mer(pos 2)	3170	3198	1	rn4	6	113118653	113118681
ENSMUSG00000036022	4632404H22Rik	9	1	9mer(pos 1)	1021	1049	0	Not Conserved	X	50598273	50598301
ENSMUSG00000036022	4632404H22Rik	9	1	8mer(pos 2)	1099	1127	0	Not Conserved	X	50598195	50598223
ENSMUSG00000036022	4632404H22Rik	9	1	7mer(pos 2)	1505	1533	0	Not Conserved	X	50597789	50597817
ENSMUSG00000029486	Mrpl1	9	1	9mer(pos 1)	681	709	0	Not Conserved	5	96694038	96694066
ENSMUSG00000029486	Mrpl1	9	1	7mer(pos 1)	779	807	0	Not Conserved	5	96694136	96694164
ENSMUSG00000029486	Mrpl1	9	1	8mer(pos 2)	1250	1278	0	Not Conserved	5	96694607	96694635
ENSMUSG00000028649	Macf1	9	1	7mer(pos 1)	6126	6154	8	rn4,oryCun1,felCat3,equCab1,dasNov1,loxAfr1,monDom4,xenTro2	4	123056014	123056042
ENSMUSG00000028649	Macf1	9	1	9mer(pos 1)	6135	6163	11	rn4,oryCun1,hg18,panTro2,felCat3,equCab1,bosTau3,dasNov1,loxAfr1,monDom4,xenTro2	4	123056005	123056033
ENSMUSG00000028649	Macf1	9	1	8mer(pos 1)	9029	9057	4	rn4,oryCun1,bosTau3,loxAfr1	4	123038208	123038974
ENSMUSG00000021770	Samd8	9	1	7mer(pos 1)	2233	2261	0	Not Conserved	14	22614697	22614725
ENSMUSG00000021770	Samd8	9	1	9mer(pos 1)	3467	3495	0	Not Conserved	14	22615931	22615959
ENSMUSG00000021770	Samd8	9	1	8mer(pos 2)	3635	3663	1	galGal3	14	22616099	22616127
ENSMUSG00000021215	Net1	9	1	8mer(pos 2)	154	182	0	Not Conserved	13	3883133	3883161
ENSMUSG00000021215	Net1	9	1	7mer(pos 1)	510	538	0	Not Conserved	13	3882777	3882805
ENSMUSG00000021215	Net1	9	1	9mer(pos 1)	709	737	0	Not Conserved	13	3882578	3882606
ENSMUSG00000069814	E130309D14Rik	8	1	8mer(pos 2)	260	288	0	Not Conserved	11	74452334	74452362
ENSMUSG00000069814	E130309D14Rik	8	1	8mer(pos 1)	489	517	1	rn4	11	74452563	74452591
ENSMUSG00000069814	E130309D14Rik	8	1	7mer(pos 2)	900	928	1	rn4	11	74452974	74453002
ENSMUSG00000058690	Gcap14	8	1	7mer(pos 2)	294	322	4	rn4,hg18,panTro2,calJac1	14	37692531	37692559
ENSMUSG00000058690	Gcap14	8	1	7mer(pos 1)	2600	2628	0	Not Conserved	14	37690225	37690253
ENSMUSG00000058690	Gcap14	8	1	7mer(pos 1)	2841	2869	0	Not Conserved	14	37689984	37690012
ENSMUSG00000058690	Gcap14	8	1	7mer(pos 1)	3955	3983	0	Not Conserved	14	37688870	37688898
ENSMUSG00000058248	Kcnh1	8	1	7mer(pos 1)	1618	1646	1	rn4	1	194331622	194331650
ENSMUSG00000058248	Kcnh1	8	1	8mer(pos 1)	3210	3238	1	rn4	1	194333214	194333242
ENSMUSG00000058248	Kcnh1	8	1	8mer(pos 1)	3511	3539	1	rn4	1	194333515	194333543
ENSMUSG00000054675	Tmem119	8	1	8mer(pos 1)	12	40	0	Not Conserved	5	114244864	114244892
ENSMUSG00000054675	Tmem119	8	1	7mer(pos 1)	61	89	0	Not Conserved	5	114244815	114244843
ENSMUSG00000054675	Tmem119	8	1	8mer(pos 1)	1019	1047	0	Not Conserved	5	114243857	114243885
ENSMUSG00000047248	C2cd3	8	1	7mer(pos 1)	1182	1210	7	rn4,cavPor2,hg18,panTro2,sorAra1,bosTau3,dasNov1	7	107566703	107566731
ENSMUSG00000047248	C2cd3	8	1	7mer(pos 1)	3890	3918	3	rn4,oryCun1,sorAra1	7	107598505	107602234
ENSMUSG00000047248	C2cd3	8	1	9mer(pos 1)	5343	5371	4	hg18,panTro2,calJac1,equCab1	7	107618327	107618355

ENSMUSG00000042444	B230380D07Rik	8	1	7mer(pos 2)	1260	1288	0	Not Conserved	9	70451679	70451707
ENSMUSG00000042444	B230380D07Rik	8	1	9mer(pos 1)	2123	2151	3	rn4,panTro2,calJac1	9	70450816	70450844
ENSMUSG00000042444	B230380D07Rik	8	1	7mer(pos 1)	3029	3057	1	calJac1	9	70449910	70449938
ENSMUSG00000042104	Ugcgl2	8	1	9mer(pos 1)	250	278	1	rn4	14	119385834	119385862
ENSMUSG00000042104	Ugcgl2	8	1	7mer(pos 2)	270	298	1	oryCun1	14	119385814	119385842
ENSMUSG00000042104	Ugcgl2	8	1	7mer(pos 1)	946	974	0	Not Conserved	14	119385138	119385166
ENSMUSG00000040612		8	1	8mer(pos 1)	2502	2530	0	Not Conserved	1	168243429	168243457
ENSMUSG00000040612		8	1	7mer(pos 1)	5349	5377	1	rn4	1	168246276	168246304
ENSMUSG00000040612		8	1	8mer(pos 2)	5794	5822	0	Not Conserved	1	168246721	168246749
ENSMUSG00000037085	Trmt12	8	1	7mer(pos 1)	254	282	1	rn4	15	58705905	58705933
ENSMUSG00000037085	Trmt12	8	1	7mer(pos 1)	1241	1269	0	Not Conserved	15	58706892	58706920
ENSMUSG00000037085	Trmt12	8	1	7mer(pos 1)	2348	2376	0	Not Conserved	15	58707999	58708027
ENSMUSG00000037085	Trmt12	8	1	7mer(pos 1)	2618	2646	2	rn4,calJac1	15	58708269	58708297
ENSMUSG00000028886	Eya3	8	1	7mer(pos 2)	464	492	1	rn4	4	132262230	132262258
ENSMUSG00000028886	Eya3	8	1	8mer(pos 1)	2229	2257	2	felCat3,bosTau3	4	132278698	132278726
ENSMUSG00000028886	Eya3	8	1	8mer(pos 1)	4124	4152	6	rn4,sorAra1,felCat3,equCab1,bosTau3,loxAfr1	4	132280593	132280621
ENSMUSG00000026878	Rab14	8	1	7mer(pos 1)	290	318	11	rn4,oryCun1,felCat3,equCab1,bosTau3,loxAfr1,monDom4,ornAna1,galGal3,xenTro2,danRer5	2	35038928	35038956
ENSMUSG00000026878	Rab14	8	1	8mer(pos 2)	1686	1714	6	rn4,oryCun1,dasNov1,monDom4,ornAna1,galGal3	2	35037532	35037560
ENSMUSG00000026878	Rab14	8	1	8mer(pos 2)	3141	3169	1	rn4	2	35036077	35036105
ENSMUSG00000026667	Uhmk1	8	1	9mer(pos 1)	1392	1420	1	rn4	1	172128713	172128741
ENSMUSG00000026667	Uhmk1	8	1	7mer(pos 1)	2576	2604	2	cavPor2,equCab1	1	172127529	172127557
ENSMUSG00000026667	Uhmk1	8	1	7mer(pos 2)	3872	3900	1	rn4	1	172126233	172126261
ENSMUSG00000026413	Pkp1	8	1	8mer(pos 2)	1401	1429	0	Not Conserved	1	137768798	137768826
ENSMUSG00000026413	Pkp1	8	1	7mer(pos 1)	2086	2114	1	rn4	1	137768113	137768141
ENSMUSG00000026413	Pkp1	8	1	8mer(pos 2)	2141	2169	0	Not Conserved	1	137768058	137768086
ENSMUSG00000026201	Stk16	8	1	7mer(pos 2)	89	117	1	rn4	1	75210617	75210645
ENSMUSG00000026201	Stk16	8	1	7mer(pos 1)	484	512	0	Not Conserved	1	75211012	75211040
ENSMUSG00000026201	Stk16	8	1	7mer(pos 2)	1358	1386	1	rn4	1	75211886	75211914
ENSMUSG00000026201	Stk16	8	1	7mer(pos 2)	1406	1434	0	Not Conserved	1	75211934	75211962
ENSMUSG00000023032	Slc4a8	8	1	7mer(pos 2)	585	613	0	Not Conserved	15	100646648	100646676
ENSMUSG00000023032	Slc4a8	8	1	7mer(pos 1)	2694	2722	0	Not Conserved	15	100648757	100648785
ENSMUSG00000023032	Slc4a8	8	1	7mer(pos 1)	3105	3133	0	Not Conserved	15	100649168	100649196
ENSMUSG00000023032	Slc4a8	8	1	7mer(pos 1)	5126	5154	1	rn4	15	100651189	100651217
ENSMUSG00000022100	Xpo7	8	1	8mer(pos 1)	3064	3092	0	Not Conserved	14	71062715	71062743
ENSMUSG00000022100	Xpo7	8	1	7mer(pos 1)	7396	7424	0	Not Conserved	14	71058383	71058411
ENSMUSG00000022100	Xpo7	8	1	8mer(pos 1)	11283	11311	1	rn4	14	71054496	71054524
ENSMUSG00000021481	Zfp346	8	1	7mer(pos 1)	629	657	2	oryCun1,equCab1	13	55234385	55234413
ENSMUSG00000021481	Zfp346	8	1	7mer(pos 1)	1414	1442	0	Not Conserved	13	55235170	55235198
ENSMUSG00000021481	Zfp346	8	1	7mer(pos 2)	1441	1469	1	rn4	13	55235197	55235225
ENSMUSG00000021481	Zfp346	8	1	7mer(pos 2)	1544	1572	0	Not Conserved	13	55235300	55235328
ENSMUSG00000020926	Adam11	8	1	8mer(pos 2)	4	32	5	rn4,hg18,panTro2,calJac1,bosTau3	11	102638998	102639026
ENSMUSG00000020926	Adam11	8	1	7mer(pos 2)	1642	1670	1	rn4	11	102640636	102640664
ENSMUSG00000020926	Adam11	8	1	8mer(pos 2)	2297	2325	1	rn4	11	102641291	102641319
ENSMUSG00000018322	Tomm34	8	1	7mer(pos 2)	315	343	0	Not Conserved	2	163879795	163879823
ENSMUSG00000018322	Tomm34	8	1	9mer(pos 1)	511	539	0	Not Conserved	2	163879599	163879627
ENSMUSG00000018322	Tomm34	8	1	7mer(pos 1)	672	700	4	rn4,bosTau3,dasNov1,loxAfr1	2	163879438	163879466
ENSMUSG00000011263	Exoc3l2	8	1	8mer(pos 1)	341	369	1	rn4	7	20081078	20081106
ENSMUSG00000011263	Exoc3l2	8	1	8mer(pos 1)	621	649	1	equCab1	7	20081358	20081386
ENSMUSG00000011263	Exoc3l2	8	1	7mer(pos 1)	949	977	1	rn4	7	20081686	20081714
ENSMUSG00000009112	Bcl2l13	8	1	9mer(pos 1)	1615	1643	0	Not Conserved	6	120839025	120839053
ENSMUSG00000009112	Bcl2l13	8	1	7mer(pos 1)	4862	4890	0	Not Conserved	6	120842272	120842300
ENSMUSG00000009112	Bcl2l13	8	1	7mer(pos 1)	4895	4923	3	rn4,oryCun1,equCab1	6	120842305	120842333

APPENDIX D

Additional information for Chapter 6.

D-1. Authors' declaration.

D-2. Additional files 1-12 in an electronic form in a compact disc (CD). Original files are accessible at http://www.biomedcentral.com/1471-2164/12/176/additional/.

D-3. Additional file 13. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s13.xls.

D-4. Additional file 14. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s14.doc.

D-1 Authors' declaration

DECLARATION OF INDIVIDUAL CONTRIBUTIONS TO THE PUBLISHED OR SUBMITTED MANUSCRIPTS

Title:

Deep sequencing analysis reveals novel microRNAs in the developing mouse brain

Authors:

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Submitted Manuscript.

Declaration:

KHL, JRR, JMR and DLA participated in the small RNA sequence analysis and annotations. KHL, PJB and CNH supervised, designed and carried out the qPCR analysis. KHL and TD performed the small RNA northern analysis. PQT supervised whereas KHL and PSC performed the LNA-ISH analysis. SP performed the procedures for superovulation and mating of mice, cultured the morulae and provided the blastocysts for the expression analysis. DMM and JRM cultured and provided the mES cells with DICER1 conditional allele. KHL drafted the manuscript. CNH, PQT, DLA and HSS conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

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NOTE:
Statements of authorship appear in the print copy of the thesis held in the University of Adelaide Library.

D-2 Additional files 1-12

Additional files 1-12 are provided in the DVD presented at the back of the thesis. Following are the description for each file:

1. Additional file 1 - List of 413,494 unique tags. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s1.zip
2. Additional file 2 - List of unique tags mapped to repetitive elements and ncRNAs based on the 3' end sequences. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s2.txt
3. Additional file 3 - List of unique tags mapped to repetitive elements and ncRNAs based on the 5' end sequences. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s3.txt
4. Additional file 4 - List of unique tags mapped to miRNAs, pre-miRNAs or miRNA-star based on 3' end sequences. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s4.txt
5. Additional file 5: List of unique tags mapped to RefSeq sequences based on 3' end sequences. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s5.txt
6. Additional file 6 - List of unique tags mapped to RefSeq sequences based on 5' end sequences. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s6.txt
7. Additional file 7 - List of unique tags mapped to redundant mouse ESTs based on 3' end sequences. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s7.txt
8. Additional file 8 - List of unique tags mapped to redundant mouse ESTs based on 5' end sequences. Original file is accessible at

http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s8.txt

9. Additional file 9 - List of unique tags mapped to a single locus in the mouse genome based on 3' end sequences. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s9.txt
10. Additional file 10 - List of unique tags mapped to a single locus in the mouse genome based on 5' end sequences. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s10.txt).
11. Additional file 11 - List of unique tags mapped to multiple loci in the mouse genome based on 3' end sequences. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s11.txt

12. Additional file 12 - List of unique tags mapped to multiple loci in the mouse genome based on 5' end sequences. Original file is accessible at http://www.biomedcentral.com/content/supplementary/1471-2164-12-176-s12.txt

D-3 Additional file 13

Table S1: List of known miRNAs in the E15.5 developing mouse brain.

No	E15 mouse brain sRNA ID	Count	Count per 1,000,000	Accession ID	miRNA ID	Chr	Chr start	Chr stop	Strand
1	mm_br_e15_1	487654	129574.91	MI0000559	mmu-let-7c-1	16	77599901	77599995	+
2	mm_br_e15_1010	223955	59507.25	MI0000563	mmu-let-7f-2	X	148346888	148346971	+
3	mm_br_e15_1001	214459	56984.06	MI0000557	mmu-let-7a-2	9	41344798	41344894	+
4	mm_br_e15_10749	101834	27058.39	MI0000721	mmu-mir-9-3	7	86650149	86650239	+
5	mm_br_e15_103211	96012	25511.42	MI0000137	mmu-let-7g	9	106081170	106081258	+
6	mm_br_e15_10459	82138	21824.95	MI0000561	mmu-let-7e	17	17967315	17967408	+
7	mm_br_e15_1036	73096	19422.39	MI0000558	mmu-let-7b	15	85537748	85537833	+
8	mm_br_e15_10	62237	16537.04	MI0000588	mmu-mir-103-2	2	131113787	131113873	+
9	mm_br_e15_101787	48946	13005.48	MI0000138	mmu-let-7i	10	122422695	122422780	-
10	mm_br_e15_10133	42411	11269.06	MI0000157	mmu-mir-9-2	13	83878418	83878490	+
11	mm_br_e15_106	36331	9653.54	MI0000720	mmu-mir-9-1	3	88019519	88019608	+
12	mm_br_e15_10266	35592	9457.18	MI0000405	mmu-let-7d	13	48631380	48631483	-
13	mm_br_e15_10166	29346	7797.55	MI0000689	mmu-mir-25	5	138606548	138606632	-
14	mm_br_e15_10031	27486	7303.33	MI0000155	mmu-mir-128-1	1	130098937	130099007	+
15	mm_br_e15_1011	25261	6712.12	MI0000147	mmu-mir-99b	17	17967151	17967221	+
16	mm_br_e15_10023	21869	5810.83	MI0000152	mmu-mir-125b-2	16	77646517	77646588	+
17	mm_br_e15_1017	20954	5567.70	MI0000146	mmu-mir-99a	16	77599180	77599245	+
18	mm_br_e15_13198	14894	3957.50	MI0000150	mmu-mir-124-3	2	180628744	180628812	+
19	mm_br_e15_10339	14692	3903.82	MI0000144	mmu-mir-30a	1	23279107	23279178	+
20	mm_br_e15_10279	9897	2629.74	MI0000165	mmu-mir-140	8	110075143	110075213	+
21	mm_br_e15_1000	9231	2452.78	MI0005450	mmu-mir-181d	8	86702614	86702686	-
22	mm_br_e15_10303	8742	2322.84	MI0000697	mmu-mir-181a-1	1	139863031	139863118	+
23	mm_br_e15_11367	8420	2237.28	MI0000148	mmu-mir-101a	4	101019549	101019632	-
24	mm_br_e15_10306	8045	2137.64	MI0000704	mmu-mir-320	14	70843316	70843398	+
25	mm_br_e15_10234	7783	2068.03	MI0000684	mmu-mir-107	19	34895176	34895263	-
26	mm_br_e15_10302	6968	1851.47	MI0000723	mmu-mir-181b-1	1	139863215	139863295	+
27	mm_br_e15_11023	6913	1836.86	MI0000549	mmu-mir-30d	15	68172769	68172851	-
28	mm_br_e15_10013	6197	1646.61	MI0000154	mmu-mir-127	12	110831055	110831125	+
29	mm_br_e15_11551	6011	1597.19	MI0000729	mmu-mir-7a-2	7	86033162	86033259	+
30	mm_br_e15_100	5886	1563.97	MI0000719	mmu-mir-92a-1	14	115443648	115443728	+
31	mm_br_e15_11425	4990	1325.90	MI0000398	mmu-mir-298	2	174093004	174093086	-
32	mm_br_e15_10628	4683	1244.32	MI0000713	mmu-mir-199a-2	1	164147944	164148054	+
33	mm_br_e15_1014	4255	1130.60	MI0000151	mmu-mir-125a	17	17967775	17967843	+
34	mm_br_e15_10203	4193	1114.13	MI0001525	mmu-mir-433	12	110829924	110830048	+
35	mm_br_e15_10126	4116	1093.67	MI0000799	mmu-mir-382	12	110971980	110972056	+
36	mm_br_e15_11784	4038	1072.94	MI0000227	mmu-mir-185	16	18327493	18327558	-
37	mm_br_e15_1013	3850	1022.99	MI0000586	mmu-mir-98	X	148347756	148347864	+
38	mm_br_e15_13715	3629	964.26	MI0000692	mmu-mir-100	9	41339507	41339587	+
39	mm_br_e15_10307	3575	949.92	MI0003519	mmu-mir-543	12	110955467	110955543	+
40	mm_br_e15_1035	3522	935.83	MI0000724	mmu-mir-181c	8	86702771	86702860	-
41	mm_br_e15_10726	3353	890.93	MI0000592	mmu-mir-323	12	110950717	110950803	+
42	mm_br_e15_10134	3236	859.84	MI0000259	mmu-mir-30e	4	120445210	120445302	-
43	mm_br_e15_10223	3219	855.32	MI0004637	mmu-mir-423	11	76891565	76891674	-
44	mm_br_e15_10829	2938	780.66	MI0000233	mmu-mir-191	9	108470649	108470723	+
45	mm_br_e15_14307	2837	753.82	MI0000817	mmu-mir-335	6	30691298	30691396	+
46	mm_br_e15_11794	2705	718.75	MI0004124	mmu-mir-744	11	65548234	65548334	-
47	mm_br_e15_1005	2694	715.82	MI0003521	mmu-mir-541	12	110980618	110980708	+
48	mm_br_e15_10323	2419	642.75	MI0000573	mmu-mir-26a-1	9	118940913	118941003	+
49	mm_br_e15_10803	2230	592.53	MI0000156	mmu-mir-130a	2	84581271	84581335	-
50	mm_br_e15_10051	2227	591.74	MI0000649	mmu-mir-101b	19	29209768	29209865	+
51	mm_br_e15_10635	2208	586.69	MI0000716	mmu-mir-124-1	14	65209493	65209578	+
52	mm_br_e15_10310	2196	583.50	MI0004639	mmu-mir-495	12	110956963	110957026	+
53	mm_br_e15_10356	2153	572.08	MI0004258	mmu-mir-672	X	101311513	101311613	-
54	mm_br_e15_10224	1917	509.37	MI0000709	mmu-mir-221	X	18723419	18723514	-
55	mm_br_e15_1154	1833	487.05	MI0000548	mmu-mir-30c-2	1	23298539	23298623	+
56	mm_br_e15_13555	1741	462.60	MI0000403	mmu-mir-34c	9	50911138	50911215	-
57	mm_br_e15_10613	1624	431.51	MI0000569	mmu-mir-21	11	86397568	86397660	-
58	mm_br_e15_11786	1484	394.31	MI0000139	mmu-mir-1-1	2	180123752	180123829	+
59	mm_br_e15_1058	1424	378.37	MI0003535	mmu-mir-369	12	110981627	110981706	+
60	mm_br_e15_1170	1375	365.35	MI0000575	mmu-mir-26b	1	74440883	74440968	+
61	mm_br_e15_10598	1343	356.85	MI0000726	mmu-mir-128-2	9	112021139	112021215	-
62	mm_br_e15_10992	1339	355.79	MI0001526	mmu-mir-434	12	110832715	110832809	+
63	mm_br_e15_11355	1161	308.49	MI0003492	mmu-mir-485	12	110973111	110973184	+
64	mm_br_e15_1114	1116	296.53	MI0000551	mmu-mir-192	19	6264843	6264932	+
65	mm_br_e15_11539	1102	292.81	MI0000800	mmu-mir-383	8	39315186	39315256	-
66	mm_br_e15_1188	1047	278.20	MI0001160	mmu-mir-409	12	110981367	110981446	+
67	mm_br_e15_135971	958	254.55	MI0000617	mmu-mir-148b	15	103115555	103115652	+
68	mm_br_e15_10347	788	209.38	MI0000572	mmu-mir-24-2	8	86732713	86732820	+
69	mm_br_e15_11656	779	206.99	MI0000741	mmu-mir-219-2	2	29701150	29701247	-
70	mm_br_e15_10206	753	200.08	MI0000161	mmu-mir-135a-1	9	106056454	106056544	+
71	mm_br_e15_10027	744	197.69	MI0000550	mmu-mir-148a	6	51219810	51219909	-
72	mm_br_e15_10270	722	191.84	MI0000408	mmu-mir-130b	16	17124153	17124235	-
73	mm_br_e15_169268	722	191.84	MI0000793	mmu-mir-376a	12	110961990	110962058	+
74	mm_br_e15_10833	703	186.79	MI0000715	mmu-mir-135a-2	10	91534830	91534930	-

75	mm_br_e15_10350	696	184.93	MI0000730	mmu-mir-7b	17	56382410	56382521	+
76	mm_br_e15_10980	693	184.14	MI0000605	mmu-mir-329	12	110951690	110951787	+
77	mm_br_e15_11781	651	172.98	MI0001649	mmu-mir-449a	13	113827741	113827832	+
78	mm_br_e15_10207	605	160.76	MI0004673	mmu-mir-669c	2	10430922	10431031	+
79	mm_br_e15_10941	603	160.22	MI0000566	mmu-mir-16-2	3	68813823	68813918	+
80	mm_br_e15_10198	578	153.58	MI0000581	mmu-mir-93	5	138606750	138606838	-
81	mm_br_e15_10130	569	151.19	MI0000222	mmu-mir-129-1	6	28972618	28972691	+
82	mm_br_e15_13471	555	147.47	MI0003206	mmu-mir-532	X	6825527	6825623	-
83	mm_br_e15_11316	485	128.87	MI0003538	mmu-mir-503	X	50407160	50407231	-
84	mm_br_e15_1085	474	125.95	MI0000228	mmu-mir-186	3	157207242	157207313	+
85	mm_br_e15_1279	463	123.02	MI0000407	mmu-mir-106b	5	138606964	138607046	-
86	mm_br_e15_180198	445	118.24	MI0000717	mmu-mir-124-2	3	17695661	17695770	+
87	mm_br_e15_11514	430	114.26	MI0000725	mmu-mir-125b-1	9	41390008	41390085	+
88	mm_br_e15_1818	428	113.72	MI0000702	mmu-mir-219-1	17	34161927	34162037	-
89	mm_br_e15_10770	427	113.46	MI0000615	mmu-mir-337	12	110823998	110824095	+
90	mm_br_e15_11995	423	112.40	MI0004665	mmu-mir-146b	19	46417251	46417360	+
91	mm_br_e15_13672	414	110.00	MI0000223	mmu-mir-181a-2	2	38708254	38708330	+
92	mm_br_e15_14678	391	103.89	MI0000401	mmu-mir-301a	11	86926505	86926591	+
93	mm_br_e15_1163	378	100.44	MI0004703	mmu-mir-501	X	6818368	6818477	-
94	mm_br_e15_12686	366	97.25	MI0001638	mmu-mir-448	X	143592752	143592864	+
95	mm_br_e15_13174	365	96.98	MI0000576	mmu-mir-29a	6	31012659	31012747	-
96	mm_br_e15_10197	354	94.06	MI0000687	mmu-mir-17	14	115442892	115442976	+
97	mm_br_e15_10984	352	93.53	MI0000590	mmu-mir-322	X	50407431	50407526	-
98	mm_br_e15_1023	348	92.47	MI0001730	mmu-mir-451	11	77886671	77886743	+
99	mm_br_e15_10843	342	90.87	MI0001163	mmu-mir-411	12	110948384	110948466	+
100	mm_br_e15_1573	338	89.81	MI0000765	mmu-mir-363	X	50094869	50094944	-
101	mm_br_e15_10648	331	87.95	MI0000707	mmu-mir-33	15	82028551	82028620	+
102	mm_br_e15_13441	326	86.62	MI0005003	mmu-mir-676	X	97576435	97576524	+
103	mm_br_e15_13472	317	84.23	MI0000162	mmu-mir-136	12	110833536	110833598	+
104	mm_br_e15_11008	288	76.52	MI0000247	mmu-mir-204	19	22825094	22825162	+
105	mm_br_e15_10328	285	75.73	MI0003522	mmu-mir-542	X	50402579	50402664	-
106	mm_br_e15_10606	278	73.87	MI0000400	mmu-mir-300	12	110962522	110962601	+
107	mm_br_e15_11535	266	70.68	MI0001165	mmu-mir-370	12	110856467	110856546	+
108	mm_br_e15_10725	262	69.62	MI0004611	mmu-mir-674	2	117010862	117010962	+
109	mm_br_e15_10830	244	64.83	MI0000607	mmu-mir-330	7	19766813	19766911	+
110	mm_br_e15_16159	239	63.50	MI0003493	mmu-mir-486	8	24253026	24253154	+
111	mm_br_e15_11562	230	61.11	MI0003534	mmu-mir-487b	12	110965542	110965624	+
112	mm_br_e15_14793	229	60.85	MI0000164	mmu-mir-138-2	8	96848210	96848281	+
113	mm_br_e15_16052	223	59.25	MI0000394	mmu-mir-296	2	174092547	174092626	-
114	mm_br_e15_12494	222	58.99	MI0000710	mmu-mir-222	X	18724018	18724097	-
115	mm_br_e15_136124	217	57.66	MI0000246	mmu-mir-203	12	113369090	113369166	+
116	mm_br_e15_11570	208	55.27	MI0000731	mmu-mir-217	11	28663727	28663835	+
117	mm_br_e15_10669	200	53.14	MI0004706	mmu-mir-505	X	57647577	57647667	-
118	mm_br_e15_14602	194	51.55	MI0004203	mmu-mir-770	12	110801901	110801995	+
119	mm_br_e15_1366	190	50.49	MI0001161	mmu-mir-410	12	110981924	110982005	+
120	mm_br_e15_1847	182	48.36	MI0003484	mmu-mir-483	7	149840828	149840901	-
121	mm_br_e15_12324	181	48.09	MI0005521	mmu-mir-92b	3	89031037	89031120	-
122	mm_br_e15_11032	175	46.50	MI0000256	mmu-mir-122	18	65408514	65408580	+
123	mm_br_e15_16565	172	45.70	MI0000142	mmu-mir-27b	13	63402019	63402092	+
124	mm_br_e15_13166	162	43.05	MI0000176	mmu-mir-154	12	110976642	110976708	+
125	mm_br_e15_15759	157	41.72	MI0000399	mmu-mir-299	12	110948847	110948910	+
126	mm_br_e15_196187	153	40.65	MI0000160	mmu-mir-134	12	110972348	110972419	+
127	mm_br_e15_10277	140	37.20	MI0004171	mmu-mir-665	12	110824523	110824617	+
128	mm_br_e15_10828	138	36.67	MI0003518	mmu-mir-540	12	110824289	110824356	+
129	mm_br_e15_13848	137	36.40	MI0000630	mmu-mir-344-1	7	69022655	69022750	-
130	mm_br_e15_13362	137	36.40	MI0005515	mmu-mir-504	X	56350834	56350913	-
131	mm_br_e15_182859	135	35.87	MI0000798	mmu-mir-381	12	110965031	110965106	+
132	mm_br_e15_176778	133	35.34	MI0003520	mmu-mir-539	12	110966338	110966412	+
133	mm_br_e15_135805	126	33.48	MI0000632	mmu-mir-345	12	110075182	110075278	+
134	mm_br_e15_145094	125	33.21	MI0000171	mmu-mir-149	1	94746954	94747020	+
135	mm_br_e15_176074	120	31.89	MI0000565	mmu-mir-16-1	14	62250716	62250809	-
136	mm_br_e15_13135	115	30.56	MI0000174	mmu-mir-152	11	96711706	96711779	+
137	mm_br_e15_17717	113	30.03	MI0000797	mmu-mir-380	12	110950012	110950073	+
138	mm_br_e15_21815	111	29.49	MI0000792	mmu-mir-375	1	74947231	74947295	-
139	mm_br_e15_11796	110	29.23	MI0001524	mmu-mir-431	12	110828656	110828747	+
140	mm_br_e15_179180	109	28.96	MI0000141	mmu-mir-23b	13	63401791	63401865	+
141	mm_br_e15_16035	102	27.10	MI0001164	mmu-mir-412	12	110981498	110981578	+
142	mm_br_e15_10844	99	26.31	MI0000570	mmu-mir-22	11	75277217	75277312	+
143	mm_br_e15_13240	96	25.51	MI0000257	mmu-mir-143	18	61808849	61808912	-
144	mm_br_e15_181985	95	25.24	MI0000796	mmu-mir-379	12	110947269	110947335	+
145	mm_br_e15_136308	95	25.24	MI0003532	mmu-mir-494	12	110953527	110953612	+
146	mm_br_e15_169390	92	24.45	MI0000153	mmu-mir-126	2	26446876	26446949	+
147	mm_br_e15_13663	92	24.45	MI0000696	mmu-mir-212	11	74986889	74986980	+
148	mm_br_e15_17888	92	24.45	MI0004692	mmu-mir-708	7	103397933	103398042	+
149	mm_br_e15_176754	87	23.12	MI0001162	mmu-mir-376b	12	110961667	110961749	+
150	mm_br_e15_18113	83	22.05	MI0000145	mmu-mir-30b	15	68168976	68169072	-
151	mm_br_e15_11565	82	21.79	MI0000571	mmu-mir-23a	8	86732416	86732491	+
152	mm_br_e15_10841	82	21.79	MI0005496	mmu-mir-421	X	100768259	100768335	-
153	mm_br_e15_12031	81	21.52	MI0000693	mmu-mir-139	7	108623889	108623957	+

154	mm_br_e15_25669	75	19.93	MI0000585	mmu-mir-129-2	2	94081520	94081610	-
155	mm_br_e15_21564	75	19.93	MI0000763	mmu-mir-362	X	6819107	6819172	-
156	mm_br_e15_10996	74	19.66	MI0000175	mmu-mir-153	12	118489289	118489358	+
157	mm_br_e15_11745	72	19.13	MI0004633	mmu-mir-488	1	160435755	160435864	+
158	mm_br_e15_180795	71	18.87	MI0004705	mmu-mir-450b	X	50401173	50401255	-
159	mm_br_e15_260035	68	18.07	MI0000695	mmu-mir-210	7	148407282	148407392	-
160	mm_br_e15_16335	68	18.07	MI0001146	mmu-mir-384	X	102539620	102539708	-
161	mm_br_e15_184950	66	17.54	MI0000577	mmu-mir-29c	1	196863740	196863828	+
162	mm_br_e15_10604	65	17.27	MI0000646	mmu-mir-135b	1	134094664	134094761	+
163	mm_br_e15_14335	64	17.01	MI0000685	mmu-mir-10a	11	96178478	96178588	+
164	mm_br_e15_11432	63	16.74	MI0005550	mmu-mir-873	4	36615542	36615619	-
165	mm_br_e15_16411	61	16.21	MI0004645	mmu-mir-449c	13	113826190	113826299	+
166	mm_br_e15_180808	60	15.94	MI0000163	mmu-mir-137	3	118136774	118136847	+
167	mm_br_e15_10637	56	14.88	MI0000568	mmu-mir-20a	14	115443378	115443485	+
168	mm_br_e15_12170	50	13.29	MI0000711	mmu-mir-224	X	69506369	69506451	-
169	mm_br_e15_10137	48	12.75	MI0000603	mmu-mir-328	8	107832263	107832360	-
170	mm_br_e15_10584	48	12.75	MI0000643	mmu-mir-351	X	50406431	50406530	-
171	mm_br_e15_17676	48	12.75	MI0003491	mmu-mir-484	16	14159718	14159785	+
172	mm_br_e15_181142	48	12.75	MI0000580	mmu-mir-92a-2	X	50095014	50095105	-
173	mm_br_e15_204169	47	12.49	MI0005553	mmu-mir-877	17	36097674	36097759	-
174	mm_br_e15_135313	46	12.22	MI0000170	mmu-mir-146a	11	43187898	43187963	-
175	mm_br_e15_10486	46	12.22	MI0000794	mmu-mir-377	12	110978719	110978787	+
176	mm_br_e15_184912	44	11.69	MI0000564	mmu-mir-15a	14	62250863	62250947	-
177	mm_br_e15_315183	44	11.69	MI0000249	mmu-mir-206	1	20669090	20669163	+
178	mm_br_e15_12676	39	10.36	MI0000224	mmu-mir-182	6	30115917	30115992	-
179	mm_br_e15_15100	39	10.36	MI0000718	mmu-mir-19b-1	14	115443526	115443613	+
180	mm_br_e15_14264	39	10.36	MI0004605	mmu-mir-760	3	121996502	121996621	-
181	mm_br_e15_16652	38	10.10	MI0001734	mmu-mir-452	X	69507562	69507647	-
182	mm_br_e15_150434	36	9.57	MI0000229	mmu-mir-187	18	24587610	24587671	-
183	mm_br_e15_10180	36	9.57	MI0004636	mmu-mir-497	11	70048218	70048302	+
184	mm_br_e15_14673	35	9.30	MI0000230	mmu-mir-188	X	6825114	6825182	-
185	mm_br_e15_320479	35	9.30	MI0004125	mmu-mir-374	X	100768398	100768493	-
186	mm_br_e15_184168	34	9.03	MI0000404	mmu-mir-34b	9	50911666	50911750	-
187	mm_br_e15_197442	33	8.77	MI0003533	mmu-mir-376c	12	110960927	110961013	+
188	mm_br_e15_21813	33	8.77	MI0001653	mmu-mir-450a-1	X	50401330	50401421	-
189	mm_br_e15_10605	32	8.50	MI0000225	mmu-mir-183	6	30119667	30119737	-
190	mm_br_e15_10315	32	8.50	MI0005514	mmu-mir-493	12	110818442	110818525	+
191	mm_br_e15_10040	31	8.24	MI0000691	mmu-mir-32	4	56908100	56908170	-
192	mm_br_e15_176108	31	8.24	MI0000619	mmu-mir-338	11	119876078	119876176	-
193	mm_br_e15_24636	30	7.97	MI0000714	mmu-mir-199b	2	32173979	32174089	+
194	mm_br_e15_363929	30	7.97	MI0005472	mmu-mir-879	5	9375703	9375779	+
195	mm_br_e15_11016	29	7.71	MI0000733	mmu-mir-194-2	19	6264642	6264728	+
196	mm_br_e15_260979	29	7.71	MI0000547	mmu-mir-30c-1	4	120442138	120442227	-
197	mm_br_e15_32697	29	7.71	MI0000640	mmu-mir-350	1	178702455	178702554	-
198	mm_br_e15_17787	28	7.44	MI0000158	mmu-mir-132	11	74987183	74987249	+
199	mm_br_e15_10615	27	7.17	MI0000140	mmu-mir-15b	3	68813693	68813757	+
200	mm_br_e15_192058	27	7.17	MI0000554	mmu-mir-200a	4	155429004	155429094	-
201	mm_br_e15_11550	26	6.91	MI0005002	mmu-mir-490	6	36371741	36371825	+
202	mm_br_e15_18761	25	6.64	MI0001447	mmu-mir-425	9	108471107	108471192	+
203	mm_br_e15_403951	24	6.38	MI0004126	mmu-mir-216b	11	28646190	28646276	+
204	mm_br_e15_12684	24	6.38	MI0000700	mmu-mir-218-1	5	48615180	48615290	+
205	mm_br_e15_226834	24	6.38	MI0004553	mmu-mir-666	12	110955294	110955393	+
206	mm_br_e15_10711	23	6.11	MI0000168	mmu-mir-144	11	77886506	77886572	+
207	mm_br_e15_46161	23	6.11	MI0000226	mmu-mir-184	9	89697097	89697166	-
208	mm_br_e15_16412	23	6.11	MI0000579	mmu-mir-31	4	88556460	88556566	-
209	mm_br_e15_12379	23	6.11	MI0000595	mmu-mir-324	11	69825544	69825633	+
210	mm_br_e15_12149	22	5.85	MI0004679	mmu-mir-455	4	62917884	62917966	+
211	mm_br_e15_181959	21	5.58	MI0004134	mmu-mir-668	12	110972941	110973007	+
212	mm_br_e15_10620	19	5.05	MI0000221	mmu-mir-10b	2	74564126	74564194	+
213	mm_br_e15_11317	18	4.78	MI0000237	mmu-mir-195	11	70048543	70048637	+
214	mm_br_e15_14737	17	4.52	MI0000556	mmu-let-7a-1	13	48633547	48633641	-
215	mm_br_e15_18016	17	4.52	MI0004122	mmu-mir-301b	16	17124492	17124589	-
216	mm_br_e15_190311	15	3.99	MI0000567	mmu-mir-18a	14	115443072	115443168	+
217	mm_br_e15_22077	15	3.99	MI0004589	mmu-mir-496	12	110977328	110977407	+
218	mm_br_e15_198374	14	3.72	MI0004118	mmu-mir-1224	16	20604524	20604609	+
219	mm_br_e15_24635	14	3.72	MI0000167	mmu-mir-142	11	87570365	87570429	+
220	mm_br_e15_11318	14	3.72	MI0000712	mmu-mir-29b-2	1	196863233	196863314	+
221	mm_br_e15_183675	13	3.45	MI0006298	mmu-mir-1193	12	110953880	110954001	+
222	mm_br_e15_14943	13	3.45	MI0005554	mmu-mir-511	2	14182629	14182708	+
223	mm_br_e15_189790	12	3.19	MI0000698	mmu-mir-214	1	164153498	164153608	+
224	mm_br_e15_18369	12	3.19	MI0000728	mmu-mir-7a-1	13	58494139	58494247	-
225	mm_br_e15_190114	11	2.92	MI0000699	mmu-mir-216a	11	28657011	28657083	+
226	mm_br_e15_207099	11	2.92	MI0000701	mmu-mir-218-2	11	35430317	35430427	+
227	mm_br_e15_18794	11	2.92	MI0006127	mmu-mir-582	13	110114937	110115018	+
228	mm_br_e15_198332	10	2.66	MI0000560	mmu-let-7c-2	15	85537032	85537127	+
229	mm_br_e15_382664	10	2.66	MI0004702	mmu-mir-500	X	6814808	6814900	-
230	mm_br_e15_23410	10	2.66	MI0004601	mmu-mir-673	12	110810199	110810290	+
231	mm_br_e15_145880	9	2.39	MI0000172	mmu-mir-150	7	52377126	52377191	+
232	mm_br_e15_368959	9	2.39	MI0005478	mmu-mir-190b	3	89873941	89874021	+

233	mm_br_e15_18793	9	2.39	MI0000235	mmu-mir-193	11	79525470	79525536	+
234	mm_br_e15_182915	8	2.13	MI0000688	mmu-mir-19a	14	115443221	115443303	+
235	mm_br_e15_238861	8	2.13	MI0004131	mmu-mir-551b	3	29315744	29315842	+
236	mm_br_e15_173759	8	2.13	MI0004133	mmu-mir-671	5	24097931	24098029	+
237	mm_br_e15_134942	7	1.86	MI0000621	mmu-mir-339	5	139845603	139845699	-
238	mm_br_e15_23645	7	1.86	MI0000768	mmu-mir-365-1	16	13453932	13454019	+
239	mm_br_e15_190191	7	1.86	MI0002402	mmu-mir-467a-1	#N/A	#N/A	#N/A	#N/A
240	mm_br_e15_198296	7	1.86	MI0004686	mmu-mir-702	5	137467302	137467411	+
241	mm_br_e15_184329	6	1.59	MI0006305	mmu-mir-1197	12	110950526	110950646	+
242	mm_br_e15_272447	6	1.59	MI0005484	mmu-mir-193b	16	13449615	13449694	+
243	mm_br_e15_21848	6	1.59	MI0005557	mmu-mir-653	6	3671300	3671385	-
244	mm_br_e15_135853	6	1.59	MI0004196	mmu-mir-667	12	110958215	110958307	+
245	mm_br_e15_249528	6	1.59	MI0004310	mmu-mir-764	X	143436801	143436909	+
246	mm_br_e15_176011	5	1.33	MI0000169	mmu-mir-145	18	61807478	61807548	-
247	mm_br_e15_146250	5	1.33	MI0000609	mmu-mir-331	10	93426512	93426608	-
248	mm_br_e15_25998	5	1.33	MI0000583	mmu-mir-96	6	30119445	30119551	-
249	mm_br_e15_28841	4	1.06	MI0005483	mmu-mir-18b	X	50095507	50095590	-
250	mm_br_e15_190144	4	1.06	MI0000243	mmu-mir-200b	4	155429789	155429859	-
251	mm_br_e15_32494	4	1.06	MI0004127	mmu-mir-592	6	27886654	27886750	-
252	mm_br_e15_146338	3	0.80	MI0003536	mmu-mir-20b	X	50095289	50095369	-
253	mm_br_e15_180728	3	0.80	MI0000703	mmu-mir-223	X	93438155	93438265	+
254	mm_br_e15_41882	3	0.80	MI0000578	mmu-mir-27a	8	86732570	86732657	+
255	mm_br_e15_369434	3	0.80	MI0000388	mmu-mir-290	7	3218626	3218709	+
256	mm_br_e15_192617	3	0.80	MI0000402	mmu-mir-302a	3	127248413	127248482	+
257	mm_br_e15_333003	3	0.80	MI0000634	mmu-mir-346	14	35707794	35707892	+
258	mm_br_e15_32775	3	0.80	MI0004676	mmu-mir-499	2	155448615	155448694	+
259	mm_br_e15_232638	3	0.80	MI0004638	mmu-mir-679	12	110953786	110953860	+
260	mm_br_e15_261975	3	0.80	MI0005479	mmu-mir-874	13	58124485	58124561	-
261	mm_br_e15_248248	2	0.53	MI0000562	mmu-let-7f-1	13	48633197	48633286	-
262	mm_br_e15_68548	2	0.53	MI0000587	mmu-mir-103-1	11	35595897	35595983	+
263	mm_br_e15_190070	2	0.53	MI0000177	mmu-mir-155	16	84714384	84714449	+
264	mm_br_e15_313243	2	0.53	MI0000823	mmu-mir-181b-2	2	38709349	38709438	+
265	mm_br_e15_46418	2	0.53	MI0000232	mmu-mir-190	9	67084466	67084533	-
266	mm_br_e15_249032	2	0.53	MI0001645	mmu-mir-365-2	11	79539901	79540013	+
267	mm_br_e15_190141	2	0.53	MI0001642	mmu-mir-429	4	155428013	155428096	-
268	mm_br_e15_323935	2	0.53	MI0004680	mmu-mir-491	4	87767943	87768029	+
269	mm_br_e15_322261	2	0.53	MI0004523	mmu-mir-669a-1	2	10398479	10398576	+
270	mm_br_e15_76125	2	0.53	MI0004295	mmu-mir-670	2	94101456	94101556	-
271	mm_br_e15_243766	2	0.53	MI0004684	mmu-mir-700	4	134972469	134972548	-
272	mm_br_e15_187058	1	0.27	MI0006283	mmu-mir-1-2-as	18	10785443	10785565	+
273	mm_br_e15_311719	1	0.27	MI0000406	mmu-mir-106a	X	50095679	50095744	-
274	mm_br_e15_336164	1	0.27	MI0000820	mmu-mir-133a-2	2	180133083	180133187	+
275	mm_br_e15_274512	1	0.27	MI0000722	mmu-mir-138-1	9	122591993	122592092	+
276	mm_br_e15_196154	1	0.27	MI0005482	mmu-mir-147	2	122466538	122466617	+
277	mm_br_e15_300921	1	0.27	MI0008326	mmu-mir-1894	17	36054833	36054914	+
278	mm_br_e15_190148	1	0.27	MI0000694	mmu-mir-200c	6	124668339	124668408	-
279	mm_br_e15_173348	1	0.27	MI0000248	mmu-mir-205	1	195333656	195333724	-
280	mm_br_e15_316011	1	0.27	MI0000974	mmu-mir-215	1	187137459	187137571	+
281	mm_br_e15_96795	1	0.27	MI0000231	mmu-mir-24-1	13	63402515	63402583	+
282	mm_br_e15_284587	1	0.27	MI0000706	mmu-mir-26a-2	10	126432585	126432669	+
283	mm_br_e15_324085	1	0.27	MI0000391	mmu-mir-293	7	3220343	3220423	+
284	mm_br_e15_209891	1	0.27	MI0000598	mmu-mir-326	7	106700778	106700873	+
285	mm_br_e15_173720	1	0.27	MI0000623	mmu-mir-340	11	49883203	49883301	+
286	mm_br_e15_245654	1	0.27	MI0005498	mmu-mir-465b-1	X	64082376	64082455	-
287	mm_br_e15_180779	1	0.27	MI0005500	mmu-mir-465c-1	X	64079129	64079210	-
288	mm_br_e15_315640	1	0.27	MI0006289	mmu-mir-669h	2	10439781	10439906	+
289	mm_br_e15_81475	1	0.27	MI0004634	mmu-mir-677	10	127522341	127522450	+
290	mm_br_e15_256129	1	0.27	MI0004653	mmu-mir-688	15	102502222	102502297	-
291	mm_br_e15_84088	1	0.27	MI0004685	mmu-mir-701	5	111433162	111433271	+
292	mm_br_e15_233943	1	0.27	MI0004688	mmu-mir-704	6	47753574	47753651	-
293	mm_br_e15_90348	1	0.27	MI0004700	mmu-mir-715	17	39981080	39981190	+
294	mm_br_e15_75311	1	0.27	MI0004129	mmu-mir-758	12	110951019	110951100	+

D-4 Additional file 14

Supplementary figures and data for statistical analysis.

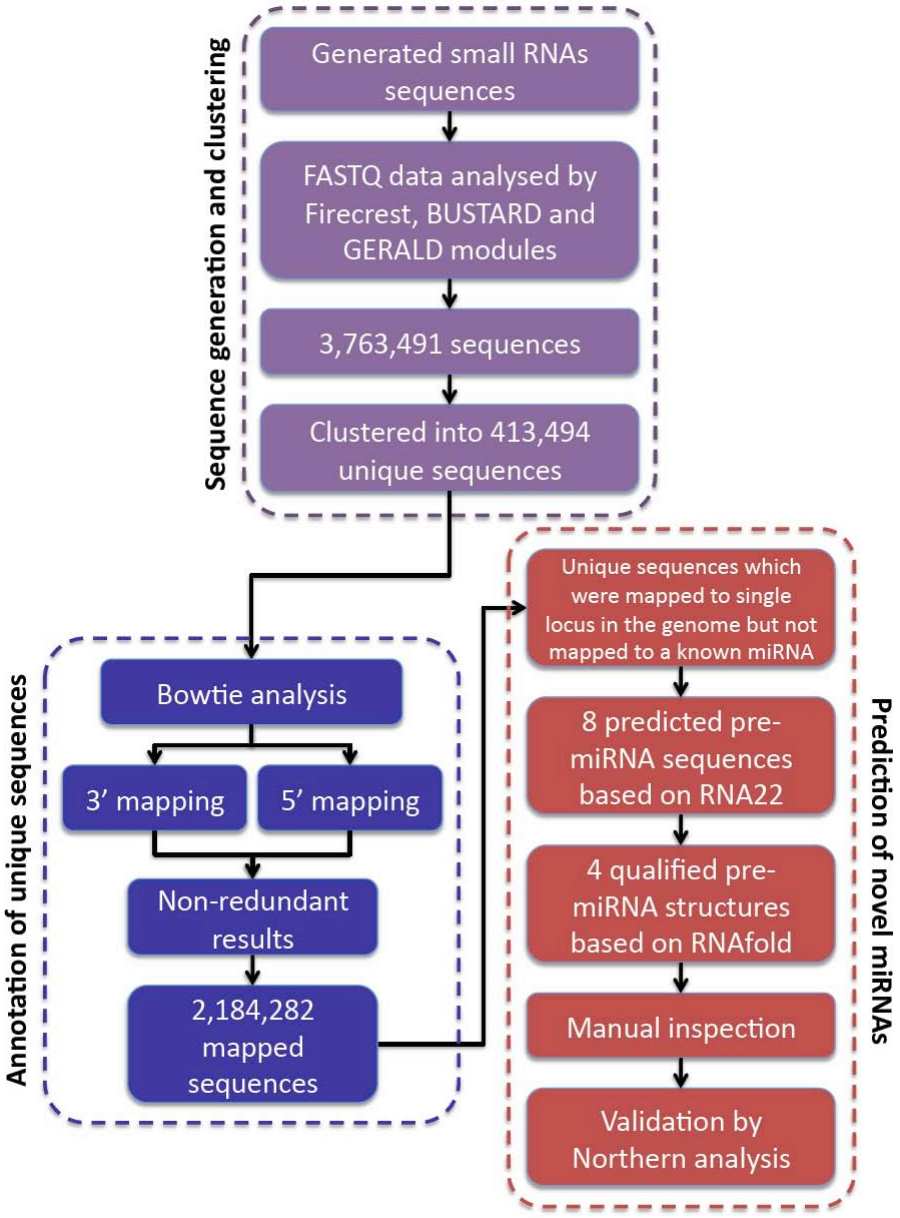
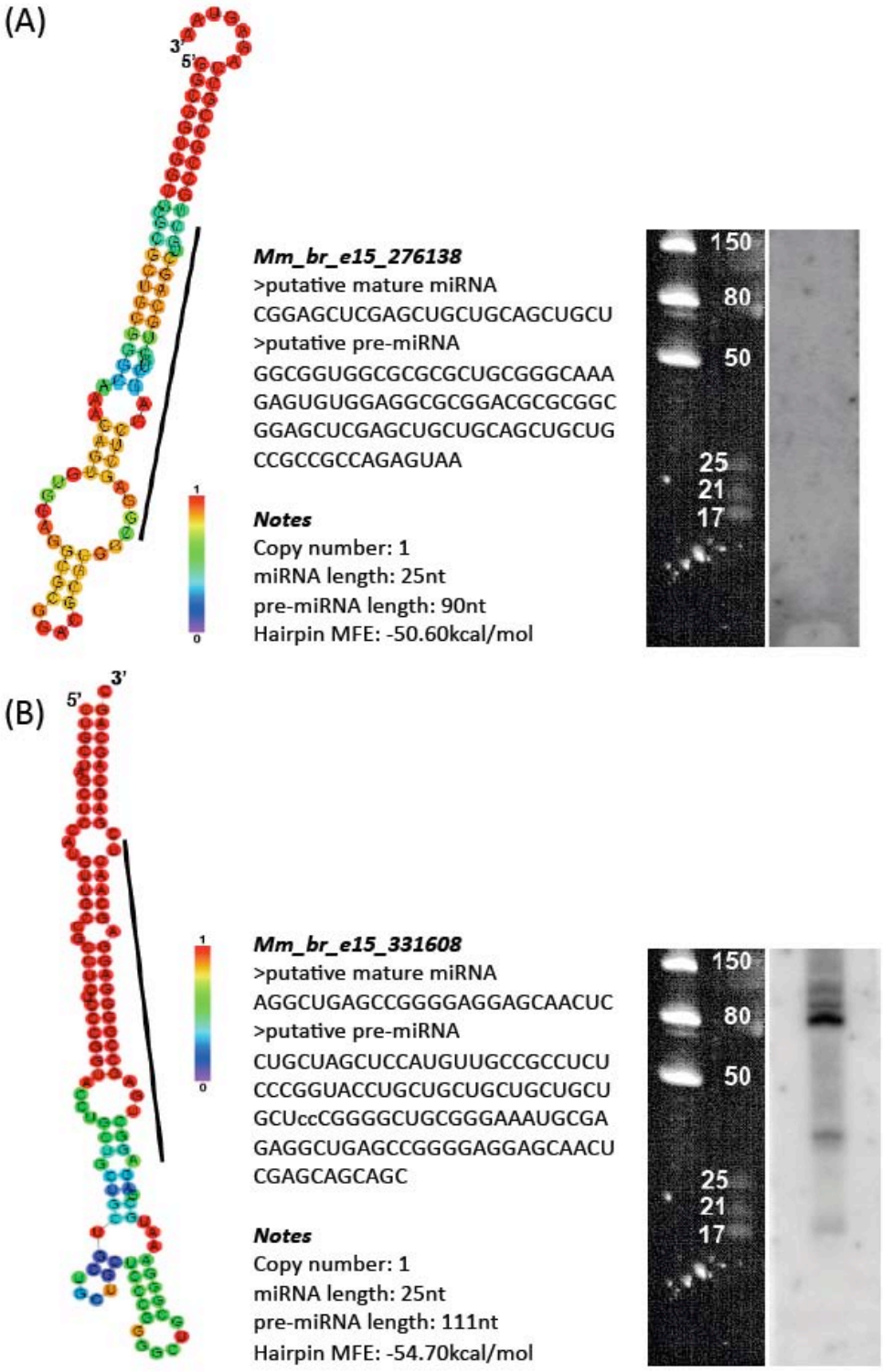


Figure S1 Pipeline for sequence annotations and discovery of novel miRNA. The pipeline involves sequence generation and clustering followed by annotation of unique sequences and prediction of novel miRNAs.



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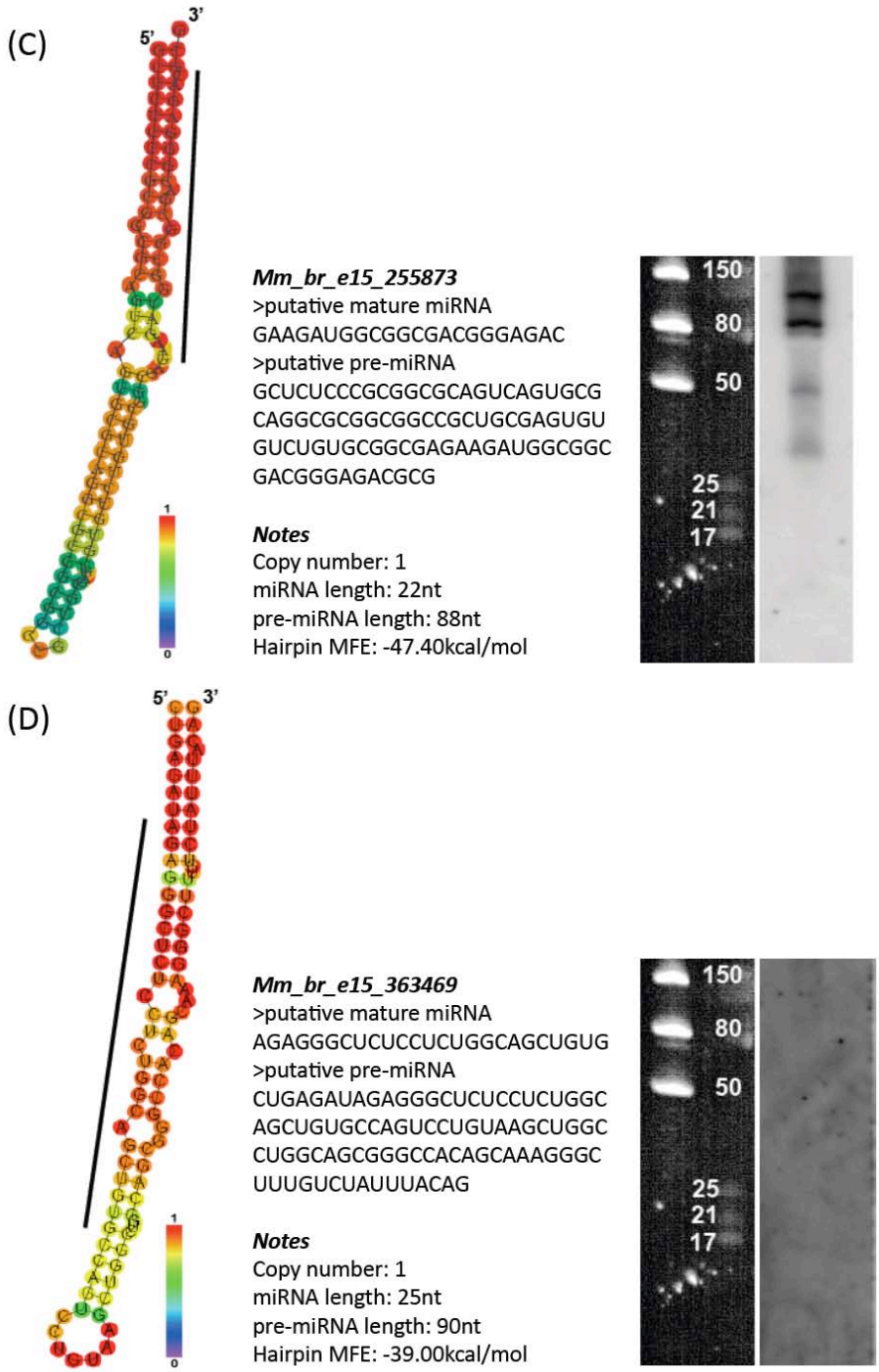

Figure S2 Non-validated predicted pre- and mature miRNAs for mm_br_e15_276138 (A), mm_br_e15_331608 (B), mm_br_e15_255873 (C) and mm_br_e15_363469. These sequences were rejected due to either one or combination of the following features: a large internal loop (A), branching stemloops (B) or an oversize precursor structure (A-D). Northern blot analysis for all predicted pre- and mature miRNAs did not validate the existence of these putative miRNAs.

Statistical information and analysis results of *miR-3099* expression

Experiment (A): Expression of *miR-3099* in mouse embryonic stem cells:

Purpose: To determine if DICER1 enzyme activity effects the expression levels of *miR-3099* in embryonic stem cells.

Experimental Treatments: Embryonic stem cells with (wild type) and without (knock out) DICER1 enzyme activity.

Statistical methods: One way Analysis of Variance.

Results: Based on three replicates in each group, there was a significant difference in the expression levels in the stem cells with and without DICER1 activity ($P<0.01$). Wild type cells had a higher expression level (-2.86 ± 0.52) than the knock out cells (-8.16 ± 0.52).

Experiment (B): Expression of *miR-3099* in embryogenesis:

Purpose: To determine if the expression levels of *miR-3099* vary throughout embryogenesis.

Experimental Treatments: Embryonic day 3 blastocysts, and day 7 embryos.

Statistical methods: One way Analysis of Variance.

Results: Based on three replicates in each group, there was a significant difference in the expression levels in the blastocysts and day 7 embryos ($P<0.001$). The blastocysts had a higher expression level (-3.45 ± 0.12) than 7-day old embryos (-6.69 ± 0.12).

Experiment (C): Expression of *miR-3099* in whole brains at different developmental stages:

Purpose: To determine if there were differences in the whole brain expression levels of *miR-3099* in the brain during development.

Experimental Treatments: Whole brains of mice were taken at embryonic age 11.5, 13.5, 15.5 and 17.5 days, and postnatal days 1.5 and 150.

Statistical Methods: One way Analysis of Variance.

Results: There was a significant difference in the *miR-3099* expression levels in whole brains obtained from different ages (P=0.02). Whole brains at embryonic day 11.5 had significantly less expression than at any other age. There were no significant differences among the expression levels at the other ages. The LSDs are: 1) 1.31 for comparing postnatal Day 1.5 with any other age, and 1.44 for comparing any other ages. The means and standard errors for the whole brain expression at each age are given in the table below.

AGE	NUMBER OF REPLICATES	MEAN	SE
Embryonic Day 11.5	2	-1.97	0.43
Embryonic Day 13.5	2	0.30	0.43
Embryonic Day 15.5	2	-0.39	0.43
Embryonic Day 17.5	2	0.27	0.43
Postnatal Day 1.5	3	0.86	0.35
Postnatal Day 150	2	-0.12	0.43

Experiment (D): Expression in different brain regions of mature mice:

Purpose: To determine if there are differences in expression levels of *miR-3099* among the different brain regions in adult mice.

Experimental Treatments: The following brain regions were excised from adult mice (postanatal day 150): cerebellum; olfactory bulb; cerebrum; medulla; thalamus; and hippocampus.

Statistical methods: One way Analysis of Variance.

Results: There was no significant difference in the expression levels among the brain regions (P=0.45). The mean log2 normalized expression level is given in the following table for each brain region. For each region there are 2 replicates, and the standard error is 0.79.

BRAIN REGION	MEAN
Cerebellum	-3.28
Olfactory bulb	-2.95
Cerebrum	-2.56
Medulla	-1.62
Thalamus	-1.44
Hippocampus	-1.38

Experiment (E): Expression in different organs in adult mice:

Purpose: To determine if there are differences in expression levels of *miR-3099* among the different organs in adult mice.

Experimental Treatments: The following organs were taken from adult mice (postnatal day 150): spleen; stomach; liver; skeletal muscle; skin; ovary; testes; brain; kidney; small intestine; heart; large intestine; thymus and pancreas.

Statistical methods: One way Analysis of Variance.

Results: There was a significant difference in expression levels among the organs ($P<0.001$). The mean log2 normalized expression level is given in the following table for each organ.

ORGAN	MEAN	GROUP
Spleen	-4.88	A
Stomach	-4.33	A
Liver	-3.57	B
Skeletal muscle	-2.69	A
Skin	-1.77	B
Ovary	-1.21	B
Testes	-0.13	B
Brain	-0.12	B
Kidney	-0.01	A
Small intestine	0.32	B
Heart	1.48	A
Large intestine	1.98	B
Thymus	2.69	B
Pancreas	7.03	B

Group A organs have three replicates, and their standard error is 0.88, whereas group B organs have two replicates, and their standard error is 1.08. The least significant differences (LSDs) are: 2.61 when comparing two organs from group A; 3.19 when comparing two organs from group B; and 2.91 when comparing a group A organ with a group B organ.

Experiment (F): Expression in different cell stages in P19 cells:

Purpose: To determine if there is a difference in expression levels of *miR-3099* between P19 neurodifferentiating and proliferating cells.

Experimental Treatments: P19 cells were treated to differentiate of proliferate.

Statistical methods: One way Analysis of Variance.

Results: There was a significant difference in the expression levels in the different cell types (P=0.04). Differentiating cells had a higher expression level than the proliferating cells. The mean log2 normalized expression level is given in the following table for each cell stage.

CELL TYPE	NUMBER OF REPLICATES	MEAN	SE
Differentiating	3	-2.72	0.21
Proliferating	2	-3.85	0.26