09.18.09 MS
07.31.10 HB updated
02.24.14 MLS

Genotyping Protocol: **MMRRC 30406**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200ul of AE buffer once.

A. PlexinA3 PCR

Strain Description: This strain has IRES-Tau-GFP-Neomycin inserted into exons 2-4 of the Plexin A3 gene (*Plxna3*) on Chromosome X. Details can be found in Yaron et al (2005) Neuron 45:513-523.

Primer Information:
1) Name: M30406 141 Sequence: 5'-CAC CTG CTT CTC ACT CAG GA-3'
2) Name: M30406 143 Sequence: 5'-GCC AAG TTC TAA TTC CAT CAG A-3'
3) Name: M30406 145 WT B Sequence: 5'-CCA CAT CCG GCG CCG AAT CCA AT-3'

Primer location: M30406 141 and 145 WT B are located within the *Plxna3* gene on Chromosome X. M30406 143 is located within the inserted IRES-Tau-GFP-Neomycin.

Assay Name: PlexinA3 PCR

PCR Master Mix Components:
PlexinA3 WT PCR:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30406 141	Sigma	25µM	0.3
M30406 145 WT B	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PlexinA3 Mut PCR:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30406 141	Sigma	25µM	0.3
M30406 143	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup(both Mut and WT):
Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

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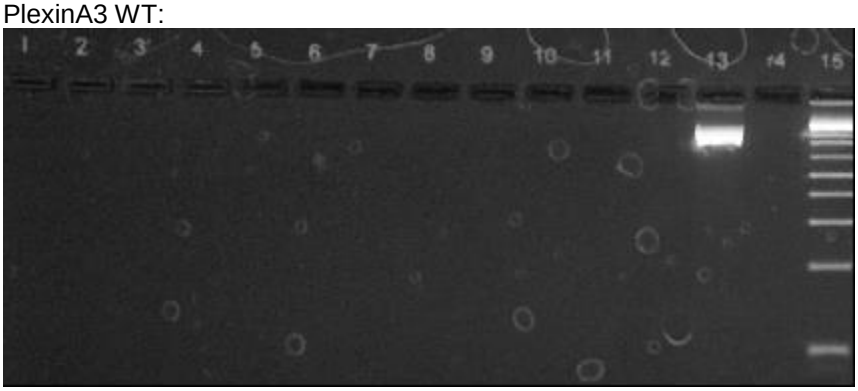
Cycle Parameters (both WT and Mut):

1)	95°C	5 minutes
2)	94°C	1 minute
3)	66°C	1 minute
4)	72°C	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	10 minutes
7)	4°C	hold until refrigerate product

Product Analysis:
Mut PCR products were analyzed on a 3% agarose gel with ethidium bromide staining. WT PCR products were analyzed on a 1% agarose gel with ethidium bromide staining.
Homozygous (female) or Hemizygous (male): 300bp
Heterozygous (female): 1013bp, 300bp
Wild Type: 1013bp

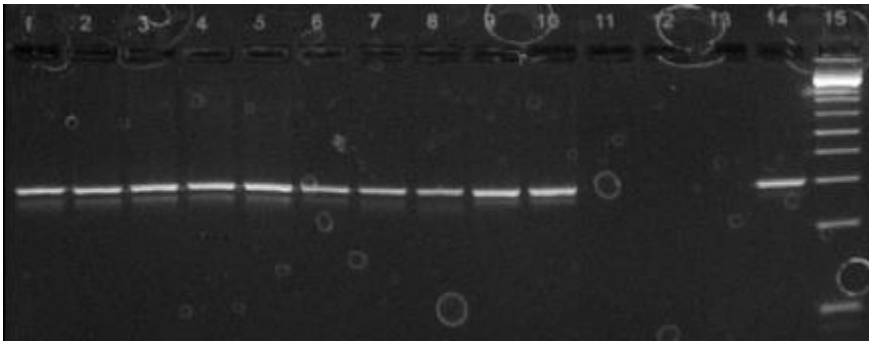
Example gels:
PlexinA3 WT:



< 1013bp

Wells 1-10 are negative for the WT allele. Wells 11 and 12 are blanks. Well 13 is a WT control and Well 14 is a homozygous control. Well 15 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

PlexinA3 Mut:



<300bp

Wells 1-10 are homozygous female and hemizygous male samples. Wells 11 and 12 are blanks. Well 13 is a WT control and Well 14 is a homozygous control. Well 15: 1Kb+ Ladder.

B. PlexinA4 PCR:

Strain Description: An ER retention signal and Neomycin cassette are inserted into exons 18 and 19 of the Plexin A4 gene (*Plxna4)* on Chromosome 6. Details can be found in Yaron et al (2005) Neuron 45:513-523.

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Primer Information:
PlexinA4 PCR:
1) Name: M30406 61 Sequence: 5'-CTT CAG CAC TGG CTG CTG TCA TCT-3'
2) Name: M30406 62 Sequence: 5'-CCA TGC TCT CCT TCA GCC TGC TCT-3'
3) Name: M30406 63 Sequence: 5'-GCT AAA GCG CAT GCT CCA GAC TGC-3'

Primer Location: M30406 61 and 62 are located in the PlexinA4 gene on Chromosome 6. M30406 63 is located in the insertion.

Assay name: PlexinA4 PCR

PCR Master Mix Components:
PlexinA4 PCR:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30406 61	Sigma	25µM	0.3
M30406 62	Sigma	25µM	0.3
M30406 63	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
PlexinA4 PCR:
1) 95°C 5 minutes
2) 94°C 1 minute
3) 68°C 1 minute
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining.

PlexinA4 PCR:
Heterozygous: 428bp, 676bp
Homozygous mutant: 428bp
Wild Type: 676bp

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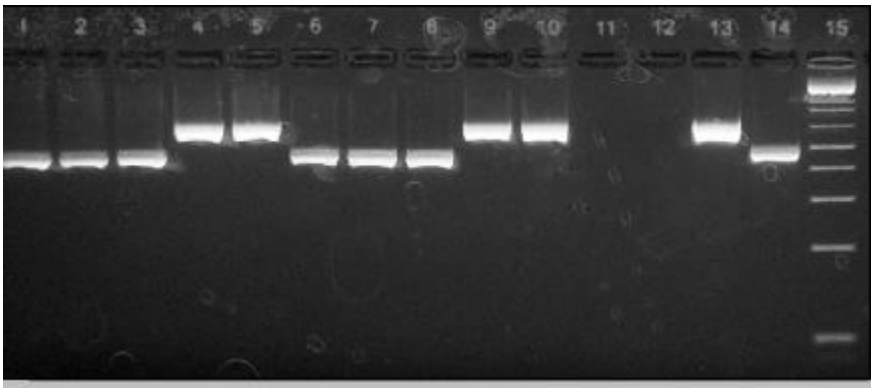
Example gel:
PlexinA4:



<676bp
< 428bp

Wells 1-3, 6-8 are homozygous mutant. Wells 4, 5, 9, 10 are WT. Wells 11 and 12 are blanks. Well 13 is a WT control and Well 14 is a homozygous mutant control. Well 15: 1Kb+ Ladder (Invitrogen Cat# 10787-018).