11.06.08 MS
08.02.10 HB updated
03.04.14 MLS

Genotyping Protocol: **MMRRC 30410**

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.

Strain Description: This strain has a secretory gene trap cassette containing beta-gal/neomycin phophotransferase fusion inserted into the unc-5 homolog B gene (*Unc5h2*) on Chromosome 10. This results in a 2kb deletion which includes exons 3 and 4 of *Unc5h2*. Details can be found in Lu et al (2004) Nature 432:179-186.

Primer Information:

1) Name: M30410 Unc5bfwd Sequence: 5'-ACT AGA ATG CTG TCC AGA C-3'
2) Name: M30410 Unc5brev Sequence: 5'-AGA GGA GAG CAA CGG ATG-3'
3) Name: M30400 Z2046 anti Sequence: 5'-TCG TCT GCT CAT CCA TGA CC-3'
4) Name: M30400 Z1924 Sequence: 5'-GTC TCG TTG CTG CAT AAA CC-3'

Primer location: M30410 Unc5bfwd and Unc5brev are both located between exons 2 and 3 of the *Unc5h2* gene on Chromosome 10. M30400 Z2046 and Z1924 are located on the inserted gene trap.

Assay name: Unc5h2 PCR

PCR Master Mix Components:

M30410 Mut :

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30400 Z2046 anti	Sigma or IDT	25µM	0.3
M30400 Z1924	Sigma or IDT	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

M30410 WT :

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30410 Unc5bfwd	Sigma or IDT	25µM	0.3
M30410 Unc5brev	Sigma or IDT	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

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.

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Cycle Parameters:

Mut 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

WT PCR:

1)	95°C	5 minutes
2)	94°C	1 minute
3)	63.5°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

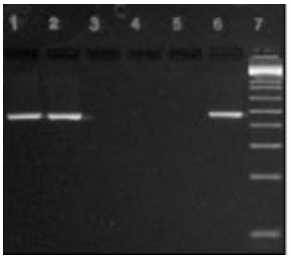
Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining.

Expected products:
WT: 400bp
Mutant: 500bp

Genotype:	**Mut PCR**	**WT PCR**
Heterozygous	500bp	400bp
Homozygous	500bp	no product
Wild Type	no product	400bp

Example gels:

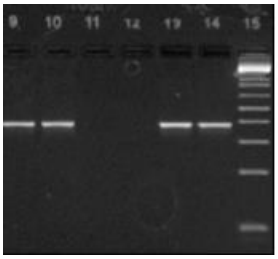
Mut Gel:



< 500bp

Wells 1 and 2 are positive for the mutant allele (500bp products). Wells 3 and 4 are negative controls. Well 5 is a WT control. Well 6 is a heterozygous control. Well 7 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

WT Gel:



<400 bp

Wells 9 and 10 are positive for the WT allele (400bp products). Wells 11 and 12 are negative controls. Well 13 is a WT control and Well 14 is a heterozygous Well 15 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).

Analysis:
Sample 1 (wells 1 and 9) and Sample 2 (wells 2 and 10) are both Heterozygous.