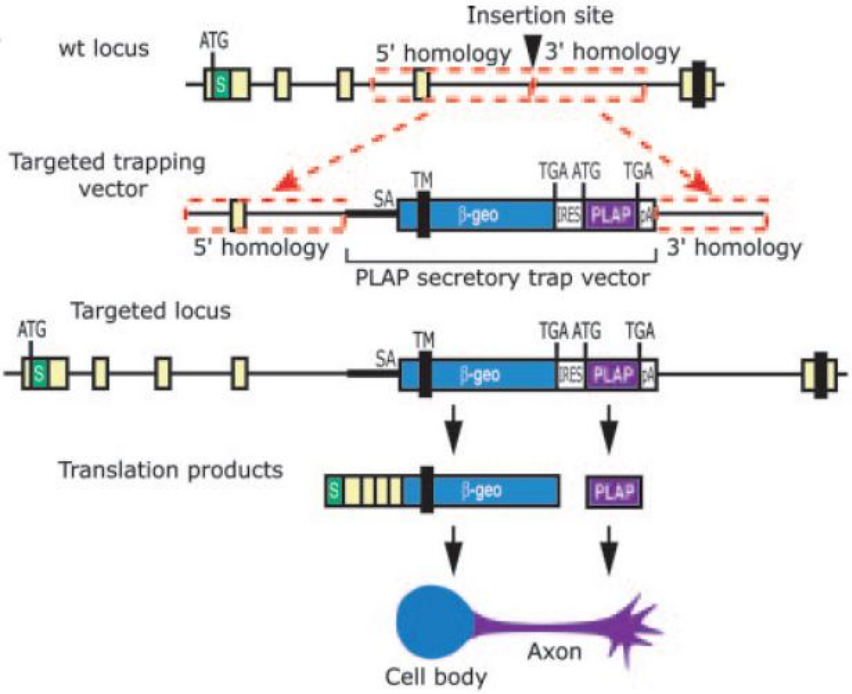
11.16.09 MS
08.02.10 HB updated

Genotyping Protocol: **MMRRC 30748**

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 200µl of AE buffer once.

Strain Description: This strain carries an insertion of a PLAP secretory trap cassette within the semaphorin 4b gene (*Sema4b*) on Chromosome 7. The PLAP secretory trap vector contains β-geo, PLAP(human placental alkaline phosphatase), and an internal ribosome entry site (IRES). Details can be found in Friedel et al (2005) Proc Natl Acad Sci U S A 102:13188-93.



Primer Information:

1) Name: M30748 Mut IRES Sequence: TGC ACA TGC TTT ACG TGT G
2) Name: M30748 Mut PLAP Sequence: TGC CGC GTG TCG TGT TGC AC
3) Name: M30748 WT F Sequence: AAT CTG GAT CAG TGG ACA GC
4) Name: M30748 WT R Sequence: CCA CAA GCT GCT GGA ATC CT

Primer location: M30748 WT F is located in intron 14 and M30748 WT R is located in exon 15 of Sema4b. M30748 Mut IRES and Mut PLAP are located in the inserted cassette.

Assay Name: Sema4b PCR

Mut PCR:

PCR Master Mix Components:

Component	manufacturer	concentration	µl/rxn
Buffer with MgCl2 (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M30748 Mut IRES	Sigma	25µM	0.3
M30748 Mut PLAP	Sigma	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.

Cycle Parameters:
1) 95°C 3 minutes
2) 94°C 30 seconds
3) 55°C 30 seconds
4) 72°C 30 seconds
5) Repeat steps 2-3 34 times for a total of 35 cycles
6) 72°C 10minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).
Alignment Marker: QX Alignment Marker 15bp/1000bp (Cat# 929521)
Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)
Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Expected Product: 510bp

WT PCR:

PCR Master Mix Components:

Component	manufacturer	concentration	µl/rxn
Buffer	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M30748 WT F	Sigma	25µM	0.3
M30748 WT R	Sigma	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.

Cycle Parameters:
1) 95°C 3 minutes
2) 94°C 30 seconds
3) 66°C 30 seconds
4) 72°C 30 seconds
5) Repeat steps 2-3 34 times for a total of 35 cycles
6) 72°C 10minutes
7) 4°C hold until refrigerate product

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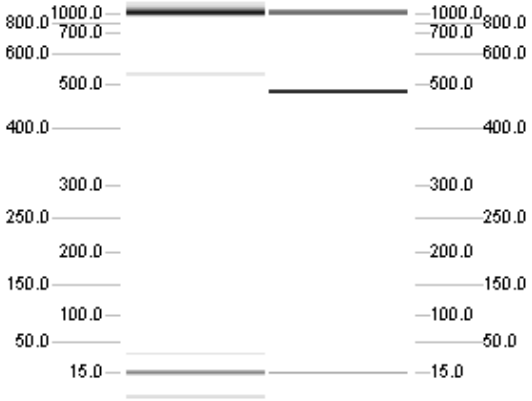
Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/1000bp (Cat# 929521)
Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)
Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Expected Product: 490bp

Genotype	Mut PCR	WT PCR
WT	no product	490bp
Heterozygous	510bp	490bp
Homozygous	510bp	no product

Example Gel:



Lane C02 displays a sample positive for the mutant allele (510bp band).
Lane C08 displays a sample positive for the WT allele (490bp band).

Please note: the bands at 15bp and 1000bp are reference bands specific to the QIAxcel method and do not represent expected products.