H/M Genotyping Protocol: **MMRRC Line 253**

Assay Type: PCR (cannot distinguish heterozygous animals from homozygous animals)

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy kit. 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.

Primer Information:
1) Name:M253 (Sema3b) F Sequence: 5'- TTA CCC TCT CTG CTT TGC TGC G -3'
Genomic location: Chromosome 9, bases 107463273 to 107463294 (-116 to -95 of Sema3b start codon)
2) Name: Gensat GFP Rev Sequence: 5'- TAG CGG CTG AAG CAC TGC A -3'
Binds to bases 206-224 of the GFP transgene

PCR Master Mix Components:

Master Mix:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25 mM	3.2
M253 (Sema3b) F	IDT	20 µM	0.3
Gensat GFP Rev	IDT	20 µM	0.3
FastStart taq	Roche	5 U/µl	0.2
sterile water			13

PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template

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

Cycle Parameters:

1) 95°C 5 minutes
2) 94°C 1 minute
3) 63°C 1 minute
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 7 minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining
Expected product: 340 bp