H/M Genotyping Protocol: **MMRRC Line 306**

**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: M306 (Sim1) F Sequence: 5'- TTG TAA GAA GAA AGG GAG CCC G -3'
Genomic location: chromosome 10, bases 51089615 to 51089636 (-11 to -32 position from the Sim1 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 |
| M306 (Sim1) 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) 65°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: 256 bp