

H/M Genotyping Protocol: MMRRC Line 255

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: M255 (Shh) F Sequence: 5'- **ATG TGT TCC GTT ACC AGC GAC C**-3'
Genomic location : chromosome 5, bases 28797790 to 28797811
- 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 product sequence:

ATGTGTTCCGTTACCAGCGACCGGCAGCCTGCCATCGCAGCCCCAGTCTGGGTGGGGATCGGA
GACAAGTCCCCTGCAGCAGCGGCAGGCAAGGTTATATAAAATCCCGGGATCCACCGGTCGCC
ACCATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCATCCTGGTCGAGCT
GGACGGCGACGTA AACCGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCC
ACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTG
GCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTA

Underlined area= Shh region, bold area = GFP transgene

PCR Master Mix Components:

Master Mix:

| component | manufacturer | concentration | µl/rxn |
|----------------|--------------|---------------|--------|
| buffer | Roche | 10X | 2 |
| dNTP | Roche | 1.25 mM | 3.2 |
| M255 (Shh) 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) 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 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: 352 bp