

H/M Genotyping Protocol: MMRRC Line 316

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: M316 (Hes5) F Sequence: 5'- TAT ATA GGG CTG GCG TGC TGG -3'
 Genomic location: Chromosome 4, bases 153453255 to 153453275 (-104 to -84 of Hes5 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
M316 (Hes5) 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: 328 bp