

H/M Genotyping Protocol: MMRRC Line 10317

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Sigma's REDExtract-N-Amp Tissue PCR Kit. Kit directions for fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Primer Information:

- | | |
|-------------------|---|
| 1) Name: 5armF2 | Sequence: GAC TTC TGT CAT AGA GAC CCG AG |
| 2) Name: GTR | Sequence: GCG GGC TCT CAG GCA CAG TAT CTG C |
| 3) Name: PCRscrR1 | Sequence: GCT GCA TCT AGA GCT TGC GGA ACC |

Primer location: WT gene: 5armF2 and GTR
KO gene: 5armF2 and PCRscrR1

PCR Master Mix Components:

Run separate reaction for KO gene and WT gene:

Master Mix for WT gene:

component	manufacturer	concentration	µl/rxn
REDExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
5armF2	IDT	25µM	0.3
GTR	IDT	25µM	0.3
sterile water			5.4

Master Mix for KO gene:

Component	manufacturer	concentration	µl/rxn
REDExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
5armF2	IDT	25µM	0.3
PCRscrR1	IDT	25µM	0.3
sterile water			5.4

PCR Setup:

WT Final Reaction: 16µl master mix & 4µl DNA template
KO Final Reaction: 16µl master mix & 4µl DNA template

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) 94°C 3 minutes
- 2) 94°C 1 minute
- 3) 59°C KO / 61°C WT 1 minute
- 4) 72°C 4 minute KO/ 3 minute WT
- 5) Repeat steps 2-4 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 a 1% agarose gel with ethidium bromide staining

Wild type gene product: 2,568 bp

Knockout gene product: ~2.8 Kbp