

## H/M Genotyping Protocol: MMRRC Line 37

### Assay Type: PCR

**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: GD3S 9692    | Sequence: CAC AGT TAC ATC TAC ATG CCT        |
| 2) Name: GD3S 9694    | Sequence: GCA AGA CGT TGT CAT AGT AGT        |
| 3) Name: RLP290 (Neo) | Sequence: TCG CCT TCT TGA CGA GTT CTT CTG AG |

Primer location: WT gene:GD3S 9692 and GD3S 9694  
KO gene: GD3S 9694 and RLP 290 (neo)

### PCR Master Mix Components:

component	manufacturer	concentration	ml/rxn
PCR buffer II	Applied Biosystems	10X	2
dNTP	Roche	1.25mM	2.2
MgCl <sub>2</sub>	Applied Biosystems	25mM	2
GD3S 9692	IDT	20µM	0.2
GD3S 9694	IDT	20µM	0.2
RLP 290 (Neo)	IDT	20µM	0.2
AmpliTaq Gold	Applied Biosystems	5 U/µl	0.2
sterile water			8

### PCR Setup:

Final Reaction: 15µl master mix & 5µl 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 10minutes
- 2) 94°C 30 seconds
- 3) 58°C 60 seconds
- 4) 72°C 60 seconds
- 5) Repeat steps 2-4 39 times for a total of 40 cycles
- 6) 72°C 7minutes
- 7) 4°C hold until refrigerate product

### Product Analysis:

All products were analyzed on a 3% agarose gel with ethidium bromide staining

Wild type GD3 gene product: 300 bp

Knockout GD3 gene product: 250 bp