

Genotyping Protocol: **MMRRC 37**

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

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat#69506). 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: 5'-CAC AGT TAC ATC TAC ATG CCT-3' |
| 2) Name: GD3S 9694 | Sequence: 5'-GCA AGA CGT TGT CAT AGT AGT-3' |
| 2) Name: RLP290 (Neo) | Sequence: 5'-TCG CCT TCT TGA CGA GTT CTT CTG AG-3' |

Primer location: GD3S 9692 & GD3S 9694: mouse GD3 gene

Primer location: RLP290 (Neo): neomycin resistance gene

Assay Name: GD3 PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
GD3S 9692	IDT	25µM	0.3
GD3S 9694	IDT	25µM	0.3
RLP290 (Neo)	IDT	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			12.7

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

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 5 minutes
- 2) 94°C 30 seconds
- 3) 58°C 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 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

Wildtype = ~320bp

Knockout = ~220bp

Heterozygous = ~320bp AND ~220bp

Example of Gel:



Lanes 1 and 2 display heterozygous samples.
Lanes 3 and 4 are extraction and PCR blanks, respectively.
Lane 5 is a homozygous control and Lane 6 is a WT control.
Lane 7 is 1Kb+ Ladder (Invitrogen Cat#10787-018)