

H/M Genotyping Protocol: **MMRRC Line 36**

Strain Characteristics: GalNacT knockout, **homozygous males infertile!!!**, maintained by mating heterozygous males with heterozygous or homozygous females.

Assay Type: PCR- can 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: L36-exon7S | Sequence: ACA CGT GGA GCA CTA CTT CAT G |
| 2) Name: L36-exon8AS | Sequence: AGG TCC AGG GGC GTC TTC T |
| 3) Name: L36-neo AS | Sequence: TGC AAT CCA TCT TGT TCA ATG |

Primer location: WT gene: L36-exon7S and L36-exon8AS
KO gene: L36-exon8AS and L36-neo AS

PCR Master Mix Components:

Run separate reaction for KO gene and WT gene:

Master Mix for WT gene:

component	manufacturer	concentration	µl/rxn
PCR buffer II	Applied Biosystems	10X	5
dNTP	Roche	1.25mM	8
MgCl ₂	Applied Biosystems	25mM	3
L36-exon7S	IDT	20µM	1
L36-exon8AS	IDT	20µM	1
amplitaq gold	Applied Biosystems	5 U/µl	0.2
sterile water			26.8

Master Mix for KO gene:

component	manufacturer	concentration	µl/rxn
PCR buffer II	Applied Biosystems	10X	5
dNTP	Roche	1.25mM	8
MgCl ₂	Applied Biosystems	25mM	3
L36-exon8AS	IDT	20µM	1
L36-neo AS	IDT	20µM	1
amplitaq gold	Applied Biosystems	5 U/µl	0.2
sterile water			26.8

PCR Setup:

Final Reaction: 45µl 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 10 minutes
- 2) 94°C 1 minute
- 3) 64°C 30 seconds
- 4) 72°C 2 minute
- 5) Repeat steps 2-4 44 times for a total of 45 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 GalNAcT gene product: ~600 bp

Knockout gene product: ~300 bp