

H/M Genotyping Protocol: MMRRC Line 27

Strain Characteristics: Phospholamban knockout, maintained homozygous

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: MMRRC line 27 HLT7 | Sequence: TGT GGG TTG CAA AGT TAG GC |
| 2) Name: L27 NEO 3' | Sequence: TCC TCG TGC TTT ACG GTA TC |
| 3) Name: 27 WT F | Sequence: CAC GTC AGA ATC TCC AGA ACC |
| 4) Name: 27 WT R | Sequence: TCC CCC TTT AAC TCT CATAAG C |

Primer location: WT gene: 27 WT F and 27 WT R
KO gene: NEO 3' and HLT7

PCR Master Mix Components:

Run separate reaction for KO gene and WT gene:

Master Mix for WT gene:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	4
27 WT F	IDT	25µM	0.3
27 WT R	IDT	25µM	0.3
FastStart taq	Roche	5 U/µl	0.2
sterile water			13

Master Mix for KO gene:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	4
L27 NEO 3'	IDT	1 µg/µl	0.125
MMRRC line 27 HLT7	IDT	1 µg/µl	0.125
FastStart taq	Roche	5 U/µl	0.2
sterile water			8.55

PCR Setup:

WT Final Reaction: 19µl master mix & 1µl DNA template

KO 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 5 minutes
- 2) 94°C 1 minute
- 3) 59°C KO / 60°C WT 1 minute
- 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

Wild type Phospolamban gene product: 700 bp

Knockout gene product: 450 bp