

H/M Genotyping Protocol: MMRRC Line 21

Strain Characteristics: XIAP 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: ML 21: WT1 | Sequence: TGG AGA GTT TGT TGA ATT TGG G |
| 2) Name: ML 21: WT2 | Sequence: TGG GAA ATA GAA ATC CTT TTG C |
| 3) Name: ML 21: KO1 | Sequence: TTT GAA GTT CCT AAT GCA ATG TTC TC |
| 4) Name: ML 21: KO2 | Sequence: ATC GAG CGA GCA CGT ACT TCG GAT G |

Primer location: WT gene: ML21:WT1 and ML21:WT2
KO gene: ML21:KO1 and ML21:KO2

PCR Master Mix Components:

Run separate reaction for KO gene and WT gene:

Master Mix for WT gene:

component	manufacturer	concentration	ml/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	3.2
ML21 WT1	IDT	25µM	0.3
ML21 WT2	IDT	25µM	0.3
FastStart taq	Roche	5 U/µl	0.2
sterile water			13

Master Mix for KO gene:

component	manufacturer	concentration	ml/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	4
ML21 KO1	IDT	25µM	0.3
ML21 KO2	IDT	25µM	0.3
FastStart taq	Roche	5 U/µl	0.2
sterile water			13

PCR Setup:

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

KO Final Reaction: 19µl master mix & 1µl 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) 63°C KO / 63°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: 400 bp

Knockout gene product: 600 bp