

H/M Genotyping Protocol: MMRRC Line 32

Strain Characteristics: Kif C2 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:

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|-----------------|---|
| 1) Name: L32 P1 | Sequence: GGG GAT GTG CTG CAA GGC GA |
| 2) Name: L32 P2 | Sequence: GAG TCG TCC CGC AGC TCT CTT CTG CCC CCA A |
| 3) Name: L32 P3 | Sequence: CTC TCT GCT CAT CTA CAT CTT C |

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	3.2
L32 P1	IDT	20µM	0.2
L32 P2	IDT	20µM	0.2
L32 P3	IDT	20µM	0.2
FastStart taq	Roche	5 U/µl	0.2
sterile water			12.8

PCR Setup:

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) 95°C 15 minutes
- 2) 94°C 45 seconds
- 3) 60°C 45 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

Wild type Kif C2 gene product: ~800 bp

Knockout gene product: ~150 bp