

H/M Genotyping Protocol: MMRRC Line 10773

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

DNA Extraction: DNA from tail snips was extracted using Sigma's REDEExtract-N-Amp Tissue PCR Kit. Kit directions for fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Primer Information:

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|---------------|--|
| 1) Name: BSU4 | Sequence: 5' - AGC GAC ATC GGG ATG CAG TT - 3' |
| 2) Name: BSL5 | Sequence: 5' - CTC CAC ATC TCC TCC CAT GT - 3' |
| 3) Name: neoU | Sequence: 5' - GCC TGA AGA ACG AGA TCA GC - 3' |
| 4) Name: BSLF | Sequence: 5' - CAG GAC AGT GCT CAG CAA GA - 3' |

PCR Master Mix Components:

Run separate reaction for KO gene and WT gene:

Master Mix for WT gene:

component	manufacturer	concentration	µl/rxn
REDEExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
BSU4	IDT	25µM	0.3
BSL5	IDT	25µM	0.3
sterile water			5.4

Master Mix for KO gene:

Component	manufacturer	concentration	µl/rxn
REDEExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
neoU	IDT	25µM	0.3
BSLF	IDT	25µM	0.3
sterile water			5.4

PCR Setup:

WT Final Reaction: 16µl master mix & 4µl DNA template

KO Final Reaction: 16µl master mix & 4µl 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 3 minutes
- 2) 94°C 30 seconds
- 3) 62°C WT & KO 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

All products were analyzed on a 1% agarose gel with ethidium bromide staining

Wild type gene product: ~500bp

Knockout gene product: ~350 bp