

H/M Genotyping Protocol: MMRRC Line 10323

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

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

Primer Information:

- 1) Name: P58-Sma-del-5' Sequence: AGC CCG GCC TCC CCA GCC TCT TC
- 2) Name: P58-Sma-del-3' Sequence: CCC GTC CAC TCG CTC GCT CGC TC

This protocol checks for zygosity of the P58 allele and integration of the targeting construct. A 330 bp band represents the WT P58 allele and a 280 bp band represents the P58KO construct with a 50 bp deletion in the targeting arm of the construct.

PCR Master Mix Components:

component	manufacturer	concentration	μ l/rxn
REExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
P58-Sma-del-5'	IDT	25 μ M	0.3
P58-Sma-del-3'	IDT	25 μ M	0.3
sterile water			5.4

PCR Setup:

Final Reaction: 16 μ l master mix & 4 μ l DNA template

All reactions were performed in 200 μ l thin walled PCR tubes and were run in Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 3 minutes
- 2) 94°C 30 seconds
- 3) 65°C 30 seconds
- 4) 72°C 1 minute
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 10minutes
- 7) 4°C hold until refrigerate product

Product Analysis:

All products were analyzed on a 3% agarose gel with ethidium bromide staining; 30 minutes @100V.

Wild type gene product: ~330 bp

Homozygous Knockout gene product: ~280 bp

Heterozygous gene production: ~330 & 280 bp