

H/M Genotyping Protocol: MMRRC Line 9979

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

DNA Extraction: DNA from tail snips was extracted using Sigma's RedExtract-N-Amp Tissue PCR Kit. Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 μ l of Extraction Solution, 12.5 μ l Tissue Preparation Solution and 50 μ l of Neutralization Solution B.

Primer Information:

WT:

- 1) Name: M9979 F Sequence: ATT CAA GGG CTT CCA CAG GGT A
2) Name: M9979 WT R3 Sequence: AGC GGC TTA GTG CAC AAG TAA CTG

KO:

- 3) Name: M9979 Neo R Sequence: AGC GGC TTA GTG CAC AAG TAA CTG
4) Name: M9979 WT R3 Sequence: GCT AAA GCG CAT GCT CCA GAC TG

Primer location: WT gene: Bind to the mouse Cacna alpha 1H

KO gene: M9979 Neo R binds to the Neo insert in the KO and acts uses M9979 WT R3 from the Cacna alpha 1H to form the KO product.

PCR Master Mix Components:

Run separate reaction for KO gene and WT gene:

Master Mix for WT gene:

component	manufacturer	concentration	μ l/rxn
REDExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
M9979 F	IDT	25 μ M	0.3
M9979 WT R3	IDT	25 μ M	0.3
sterile water			5.4

Master Mix for KO gene:

component	manufacturer	concentration	μ l/rxn
REDExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
M9979 Neo R	IDT	25 μ M	0.3
M9979 WT R3	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler

Cycle Parameters:

- 1) 94°C 3 minutes
- 2) 94°C 30 sec
- 3) 70°C KO / 60°C WT 30 sec
- 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

Wild type product: 249 bp

Knockout gene product: 330 bp

Heterozygous animals will have both the 249 and 330 bp products.