

## MU 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  $\mu$ l of Extraction Solution, 12.5  $\mu$ l Tissue Preparation Solution and 50  $\mu$ 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: GCT AAA GCG CAT GCT CCA GAC TG  
4) Name: M9979 R                      Sequence: CAT CTC AGG GCC TCT GGA CCA C

Primer location: WT gene: wild type primers amplify the mouse *Cacna1h* (calcium channel, voltage-dependent, T type, alpha 1H subunit) gene

KO allele: M9979 Neo R is designed to the Neo insert in the KO allele and in combination with M9979 WT R3 (wild type primer) amplifies the mutant allele.

**Assay Name:** A1H KO PCR

### PCR Master Mix Components:

Run separate reaction for KO gene and WT gene:

#### Master Mix for WT gene:

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

#### Master Mix for KO gene:

component	manufacturer	concentration	$\mu$ l/rxn
REDExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
M9979 Neo R	IDT	25 $\mu$ M	0.3
M9979 R	IDT	25 $\mu$ M	0.3
sterile water			5.4

### PCR Setup:

Final Reaction: 16 $\mu$ l master mix & 4 $\mu$ l DNA template

All reactions were performed in 200 $\mu$ 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:**

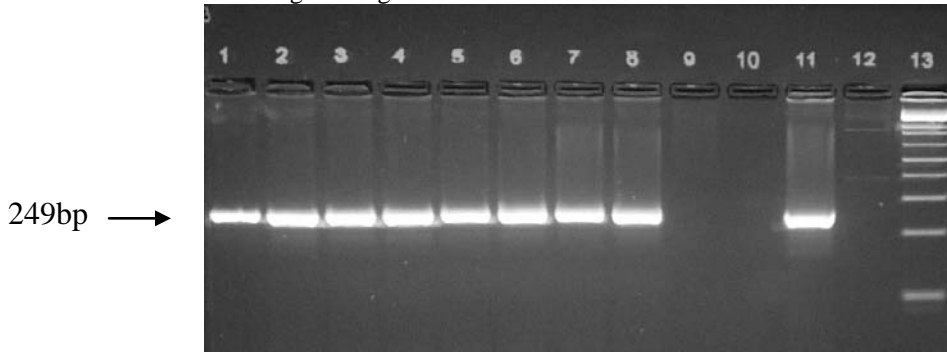
All products were analyzed on a 3% agarose gel with ethidium bromide staining

Wild type: 249 bp on WT assay

Homozygous: 330 bp on KO assay

Heterozygous : 249 bp on WT assay, 330 bp on KO assay

Gel image: WT gene



Heterozygous samples= lanes 1, 3, 5 and 7

Wild type samples= lanes 2, 4, 6, 8 and 11

Homozygous sample = lane 12

Lanes 9 & 10= no DNA control lanes.

Lane 13 contains 1Kb+ ladder (Invitrogen catalog #10787-018)

Gel image: KO gene

