09.14.10 MS
01.06.16.MLS

Genotyping Protocol: **MMRRC 11590**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals. Homozygous animals are not viable for this deletion so all results should be wildtype or heterozygous.

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). 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:
M11590 WT Assay
1) Name: CPT1 F Sequence: 5'- CAC GAG CCA GAC TCC TCA GCA GCA GGT -3'
2) Name: CPT1 R Sequence: 5'- GTA GGA AAC ACC ATA GCC GTC ATC AGC -3'
Primer Location: CPT1 F binds to sequence on Chromosome 19

M11590 KO Assay
1) Name: Neo F Sequence: 5'- CAT TCG ACC ACC AAG CGA AAC ATC -3'
2) Name: Neo R Sequence: 5'- ATA TCA CGG GTA GCC AAC GCT ATG -3'
Primer Location: Both F and R bind to the Neo insert

Assay Name: Cpt-1a KO PCR

PCR Master Mix Components:
Run separate reaction for each assay:

Master Mix for M11590 WT Assay:

Component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
CPT1 F	IDT	25µM	0.3
CPT1 R	IDT	25µM	0.3
sterile water			5.4

Master Mix for M11590 KO Assay:

Component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma	2X	10
Neo F	IDT	25µM	0.3
Neo R	IDT	25µM	0.3
sterile water			5.4

PCR Setup:
Both assays final reaction: 16µl master mix & 4µl DNA template (10-20 ng/µl)
All reactions were performed in 200µl thin walled PCR tubes and were run in an Applied Biosystems 2700 thermocycler.

Cycle Parameters (both assays):
1) 94°C 3 minutes
2) 94°C 1 minute
3) 67°C 1 minute
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

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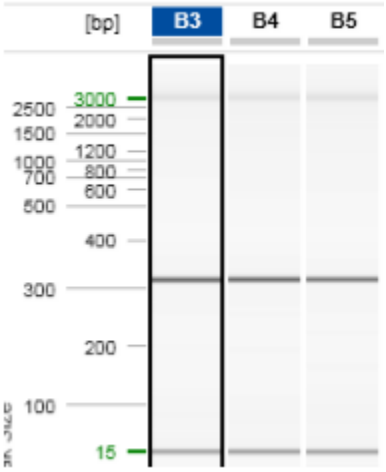
Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/3kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100-3kb (Cat# 929553)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected Products:
M11590 WT Assay product: 329 bp
M11590 KO Assay product: 289 bp

Example gels:

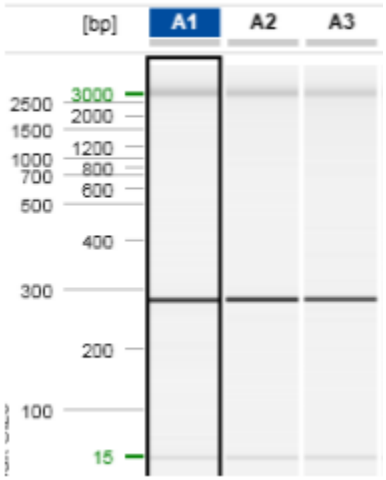
M11590 WT Assay gel:



Lanes B3, B4 and B5 display samples positive for the WT allele (329bp product)

Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products.

M11590 Mut Assay gel:



Lanes A1, A2, and A3 display samples positive for the Mut allele (289bp product)

Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected products.