

09.15.10 MS
06.12.14 MLS

Genotyping Protocol: **MMRRC 11840**

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

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 µl of Extraction Solution, 12.5 µl Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Primer Information:

WT:

- 1) Name: WT F M11840 Sequence: 5'-TCG GCT AGA AGC CAA GCT GGA TAA-3'
2) Name: WT R M11840 Sequence: 5'-ATA GTG GGA CAT TTA GCA GCC AGC-3'

KO:

- 3) Name: Neo F Sequence: 5'-CAT TCG ACC ACC AAG CGA AAC ATC-3'
4) Name: Neo R Sequence: 5'-ATA TCA CGG GTA GCC AAC GCT ATG-3'

Primer location: WT gene: Binds to the mouse *Lcat* gene. Forward primer binds to exon 2 and reverse binds to exon 3.

KO gene: primers both bind to the neomycin resistance gene. [*Lcat* gene exons 2-5 are deleted in this mouse model]

Assay Name: MMRRC 11840

PCR Master Mix Components:

Run separate reaction for KO gene and WT gene:

Master Mix for WT gene:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
WT F M11840	IDT	25µM	0.3
WT R M11840	IDT	25µM	0.3
sterile water			5.4

Master Mix for KO gene:

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:

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 Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 3 minutes
2) 94°C 1 minute
3) 68°C KO / 64°C WT 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:

For analysis 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 100bp-3kb (Cat# 929553)

Method: AH320

Injection: 20s at 2kV

Separation: 320s at 6kV

Expected Products:

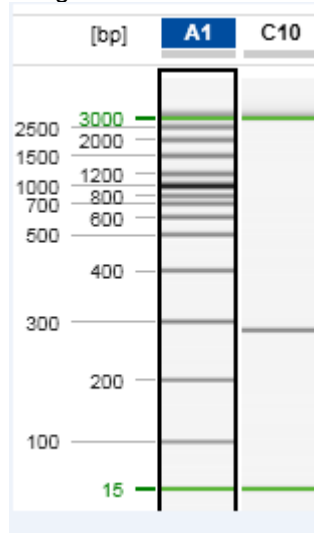
Wild type product: 185 bp

Knockout gene product: 289 bp

Heterozygous animals will have both the 185 and 289 bp products.

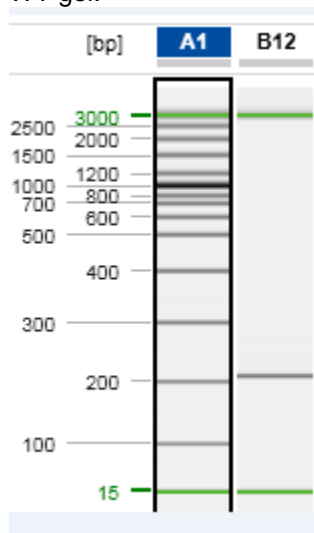
Example gels:

KO gel:



Lane A1 displays a 15bp-3kb size marker.
Lane C10 displays a sample positive for the
KO allele (289bp product).

WT gel:



Lane A1 displays a 15bp-3kb size marker.
Lane B12 displays a sample positive for the
WT allele (185bp product).