08.24.10 MS
12.20.13 MLS

Genotyping Protocol: **MMRRC 352**

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:
1) Name: m352 F Sequence: 5'- AAC TAG TGC TTG CCC TGC TGA GAT-3'
2) Name: m352 WT R Sequence: 5'-TCA GAG ATT CAC CTG CCA CTG GTT-3'
2) Name: m352 KO R Sequence: 5'-CGC GAG CTC AAT TAA CCC TCA CTA-3'

Primer location: m352 F & WT R: *Mus musculus* LCAD gene intron 4
Primer location: m352 KO R: Insertion plasmid sequence

Assay Name: LCAD KO PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M352 F	IDT	25µM	0.3
M352 WT R	IDT	25µM	0.3
M352 KO R	IDT	25µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			12.7

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 94°C 5 minutes
2) 94°C 30 seconds
3) 66°C 30 seconds
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 7minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining
Homozygous mutant: 114bp product
Heterozygous: 114bp and 292bp products
Wild type: 292bp product

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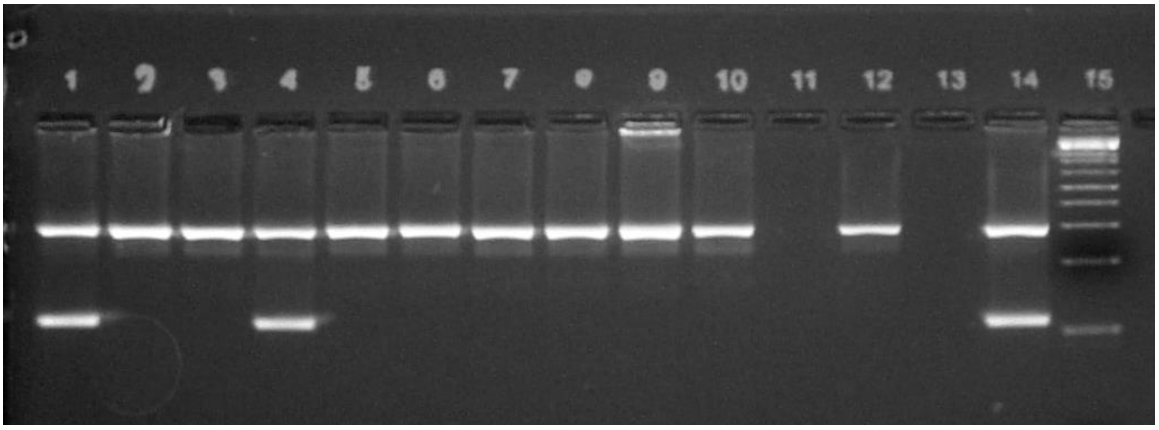
Example of Gel:



Lanes 1, 4 and 14 display heterozygous samples (114bp and 292bp products)
Lanes 2, 3, 5-10, and 12 display Wild-type samples (292bp product only)
Lane 15 displays 1Kb+ Ladder (Invitrogen Cat# 10787-018)