03.02.12 MS

Genotyping Protocol: MMRRC 29592

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

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once

Mutation Information: This strain carries a knocked allele of the low density lipoprotein receptor-related protein 2 (*Lrp2*) gene on Chromosome 2.

Primer Information:
1) Name: UB761 Sequence: 5'- AGG GAA AGC AGC CTA AAA TTG -3'
2) Name: UB764 Sequence: 5'- ATG GAA AAC ACA ATC TCA GAG GA -3'
3) Name: SI-75 Sequence: 5'- GAT TGG GAA GAC AAT AGC AGG CAT GC -3'

Primer location: Primers UB761 and UB764 bind to the *Lrp2* gene. Primer SI-75 binds to the *neo* cassette used to knock out the gene. For details see Willnow, TE et al (1996) PNAS 93:8460-8464.

Assay name: gp330 (LRP2) KO PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
REDExtract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
UB761	Sigma	10µM	0.3
UB764	Sigma	10µM	0.3
SI-75	Sigma	10µM	0.3
sterile water			5.1

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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 94^{o}C 3 minutes
2) 94^{o}C 1 minute
3) 62^{o}C 1 minute
4) 72^{o}C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72^{o}C 10 minutes
7) 4^{o}C hold until refrigerate product

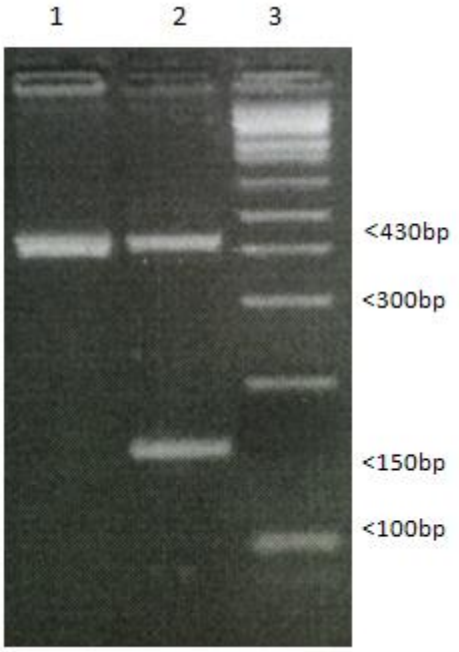
Product Analysis:

Products were analyzed by gel electrophoresis on 3% agarose gels.

Wild-type allele: 430 bp band
Mutant allele: 150 bp band
Heterozygote: Both 430 bp and 150 bp bands

Control DNA: C57BL/6 (WT) and Heterozygous animal

Example of Gel:



Lane 1 displays a WT sample (430bp product)
Lane 2 displays a heterozygous sample (430bp and 150bp products)
Lane 3 displays 1Kb+ Ladder (Invitrogen Cat#10787-018)