01.20.09 MS
08.02.10 HB updated
04.05.12 MS
08.22.16 MLS

Genotyping Protocol: **MMRRC 30532**

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 200ul of AE buffer once.

Strain Characteristics: This strain carries a phosphoglycerol kinase-neomycin resistance cassette inserted into the tumor necrosis factor receptor superfamily, member 10b (*Dr5)* on Chromosome 14, resulting in the replacement of exons 3, 4 and part of 5. Details can be found in Finnberg et al (2005) Mol Cell Biol 25:2000-2013.

Primer Information:
1) Name: M30532 Dr5 1 Sequence: 5'-GAC AAC TCA CAT GTA CGT GAC TC-3'
2) Name: M30532 Dr5 2 Sequence: 5'-ATG GCT ATC ACA ACT GAG GGA CTG CC-3'
3) Name: M30532 Dr5 3 Sequence: 5'-GGG TGG GAT TAG ATA AAT GCC TGC TC-3'

Primer location: M30532 Dr5 1 and 2 are located within the *Dr5* gene on Chromosome 14. M30532 Dr5 3 is located within the inserted cassette.

Assay name: Dr5 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
M30532 Dr5 1	Sigma or IDT	25µM	0.3
M30532 Dr5 2	Sigma or IDT	25µM	0.3
M30532 Dr5 3	Sigma or 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) 95°C 5 minutes
2) 94°C 1 minute
3) 63.5°C 1 minute
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 minutes
7) 4°C hold until refrigerate product

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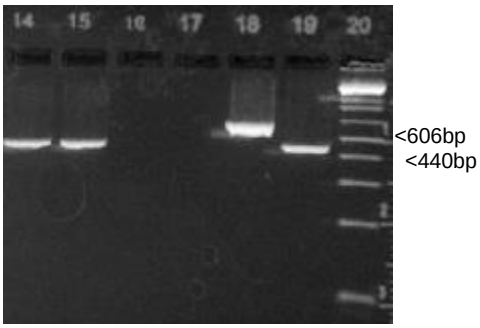
Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining.

Heterozygous: 440bp, 606bp
Homozygous mutant: 440bp
Wild Type: 606bp

Example gel:



Wells 14 and 15 are homozygous for the mutant allele. Wells 16 and 17 are blanks. Well 18 is a WT control and Well 19 is a homozygous control. Well 20 is 1Kb+ Ladder (Invitrogen Cat# 10787-018).