

Genotyping Protocol: MMRRC line 219

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

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy kit. 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.

Primer Information:

- 1) Name: m219 F Sequence: AAA CTT CTG GGT CAC CTC TGG ATG
2) Name: m219 Rwt Sequence: CTT AGG ATG GCA GGT TCA AGA CCA
2) Name: m219 Rneo Sequence: TTG TGC CCA GTC ATA GCC GAA TAG

Primer location: m219 F & Rwt: Mus musculus DNA repair protein (XRCC1) gene

Primer location: m219 Rneo: Synthetic construct neomycin resistance gene

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	3.2
m219 F	IDT	25µM	0.3
m219 Rwt	IDT	25µM	0.3
m219 Rneo	IDT	25µM	0.3
FastStart taq	Roche	5 U/µl	0.2
sterile water			12.7

PCR Setup:

Final Reaction: 19µl master mix & 1µl DNA template

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) 69°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

Wildtype = 484 bp

Positive = 1600 bp

Heterozygous = 484 bp, 1600 bp, and an intermediate band that is a heteroduplex of the other two