

Genotyping Protocol: **MMRRC Line 16833**

Assay Type: PCR followed by restriction digest. Can distinguish heterozygous and 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.

Mutation Information: This strain carries a complex allele of the mouse p53 gene. The alteration involves the replacement of mouse p53 exon 4 with a human p53 exon 4 that carries a single nucleotide polymorphism at codon 72 coding for arginine (p53.72R).

p53.72R SNP Primer Information:

- | | |
|----------------------|--------------------------------------|
| 1) Name: Trp53-In3-F | Sequence: GCT GGT AGG CTG AGA ACA CA |
| 2) Name: Trp53-In4-R | Sequence: GGC ACA GTC TAC AGG CTG AA |

Assay Name: p53.72R SNP

PCR Master Mix Components:

p53.72R SNP Master Mix

component	manufacturer	concentration	µl/rxn
buffer	Roche	10X	2
dNTP	Roche	1.25mM	3.2
Trp53-In3-F	Sigma	25µM	0.3
Trp53-In4-R	Sigma	25µM	0.3
FastStart Taq	Roche	5 U/µl	0.2
sterile water			13

PCR Setup:

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

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

Cycle Parameters:

p53.72R SNP:

- 1) 95°C 5 minutes
- 2) 94°C 1 minute
- 3) 66°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

Product Analysis:

Products can be analyzed on a 3% agarose gel with ethidium bromide staining (8 µl of product loaded). Usually skip this step and go straight into restriction digest step since failure to amplify is extremely rare.

Predicted Product sizes:

p53.72R SNP: 429 bp

Restriction Digests:

p53.72R SNP (*BtgI*):

10 µl PCR product from above reaction
0.4 µl *BtgI* (NEB – 10U/µl)
2 µl NEB Buffer 3 (NEB)
2 µl 10x BSA (NEB- use 10X working stock)
5.6 µl water
20 µl reaction

Incubate overnight at 37°C

Product Analysis:

All products were analyzed on a 3% agarose gel with ethidium bromide staining (6µl loading dye added to 20µl digest- 20 µl loaded on gel)

Products:

p53.72R SNP:

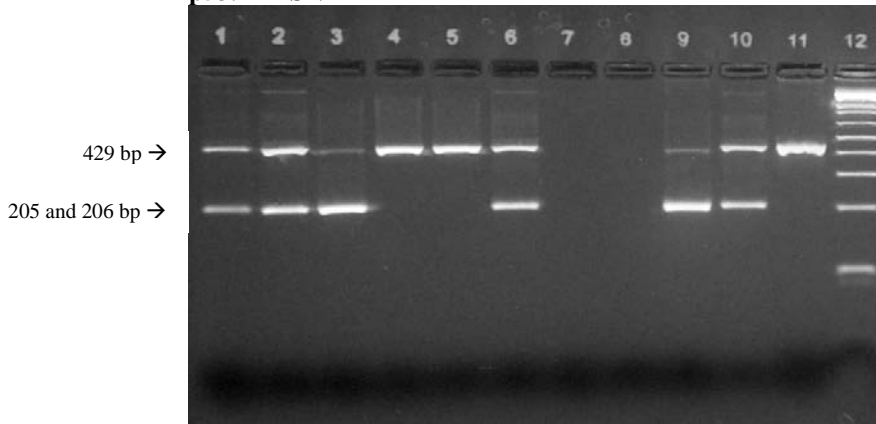
Wt: 205 and 206 bp (this assay is not completely cutting and wt may have faint band at 429bp)

Hom: 429 bp

Het: 429bp & 205/206 bp (higher band and lower band should be equal intensity)

Example of Gel:

p53.72R SNP



Wells 3 and 9 are wild-type for p53.72R SNP. Wells 1, 2, 6, and 10 are heterozygous. Wells 4, 5, and 11 are homozygous. Wells 7 & 8 were no template controls. Well 12 is 1 Kb Plus DNA ladder (Invitrogen Cat. # 10787-018).