

Genotyping Protocol: MMRRC line 16832

Assay Type: PCR and restriction enzyme digest - can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Sigma's RedExtract-N-Amp Tissue PCR Kit. 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.

Mutation Information: This strain carries a knock-in mutation. A targeting vector harboring the p53.S389A mutation was created by site-directed mutagenesis and introduced into the mouse genome by homologous recombination in ES cells. The selectable neomycin (NEO) marker, preceded by transcription stops, flanked by loxP sites, was present in intron 11. The targeting vectors were also fitted with a diphtheria toxin A (DTA) expression cassette.

Primer Information:

- | | |
|------------------|---|
| 1) Name: p53in10 | Sequence: GGT GGT GAC AGT TGT GAT AAT AAT |
| 2) Name: p53ex11 | Sequence: TTG CAG AAT GGA AGG AAA GTA |

Both primers are located in the p53 gene. P53in10 is in exon 10 and p53ex11 is in exon 11.

Assay Name: p53.S389A PCR/HinfI Digest

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
REDExtract-N-Amp PCR Reaction Mix	Sigma	2X	10
p53in10	IDT	25µM	0.3
p53ex11	IDT	25µM	0.3
sterile water			5.1

PCR Setup:

Final Reaction: 16µl master mix & 4µl 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 3 minutes
- 2) 94°C 1 minute
- 3) 58 °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:

All products were analyzed on a 3% agarose gel with ethidium bromide staining. Usually skip this step and go straight to digest.

Expected product size: 674 bp

HinfI Digest:

10 µl PCR product from above reaction

0.4 µl HinfI (NEB – 10U/µl)

2 µl NEB Buffer 3 (NEB)

7.6 µl water

20 µl reaction

Incubate for 2 hours at 37°C

Product Analysis:

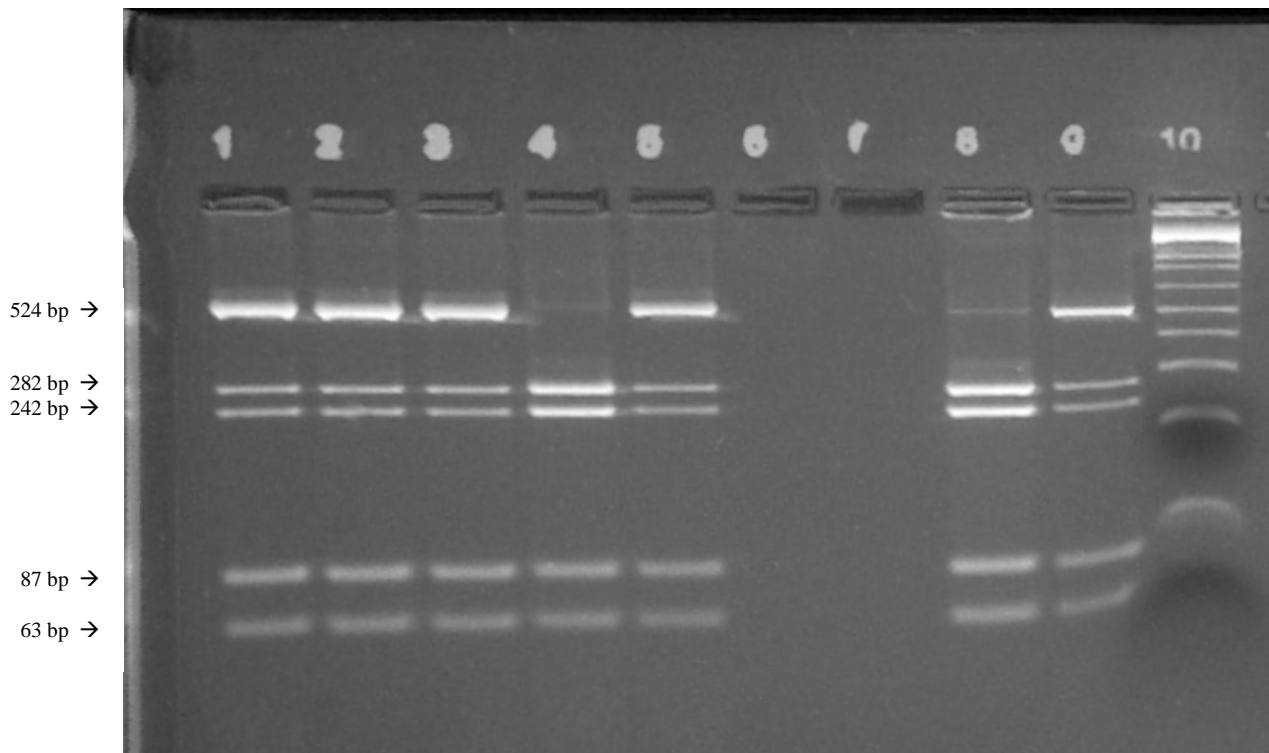
All products were analyzed on a 3% agarose gel with ethidium bromide staining.

Expected product sizes:

WT = 282, 242, 87, and 63 bp

Het = 524, 282, 242, 87, and 63 bp

Hom = 524, 87 and 63 bp

Example of Gel:

Wells 1-3 and 5 are heterozygous. Well 4 is wild-type. Wells 6 and 7 are negative controls. Well 8 is a wild-type control, and well 9 is a heterozygous control. Well 10 is 1 Kb Plus DNA ladder (Invitrogen Cat. # 10787-018).

