

09.22.10 MS
10.09.19 MLS

Genotyping Protocol: **MMRRC 14112**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals. This strain is a knock-in at the *Rosa26* location on Chromosome 6.

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 μ l of Extraction Solution, 12.5 μ l Tissue Preparation Solution and 50 μ l of Neutralization Solution B.

Primer Information:

WT:

- 1) Name: M14112 A Sequence: 5'-AAA GTC GCT CTG AGT TGT TAT-3'
2) Name: M14112 C Sequence: 5'-GGA GCG GGA GAA ATG GAT ATG-3'

Mut:

- 3) Name: M14112 A Sequence: 5'-AAA GTC GCT CTG AGT TGT TAT-3'
4) Name: M14112 B Sequence: 5'-GCG AAG AGT TTG TCC TCA ACC-3'

Primers A and C bind to mouse Chromosome 6 while primer B binds to the insert.

Assay Name: R26M2rtTA PCR

PCR Master Mix Components: Run separate reaction for Mutant assay and WT assay:

Master Mix for WT assay:

component	manufacturer	concentration	μ /rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
M14112 A	Sigma or IDT	25 μ M	0.3
M14112 C	Sigma or IDT	25 μ M	0.3
sterile water			5.4

Master Mix for Mut assay:

Component	manufacturer	concentration	μ /rxn
Extract-N-Amp PCR Reaction Mix	Sigma	2X	10
M14112 A	Sigma or IDT	25 μ M	0.3
M14112 B	Sigma or IDT	25 μ M	0.3
sterile water			5.4

PCR Setup:

Final Reaction: 16 μ l master mix & 4 μ l DNA template (10-20ng/ μ l)

All reactions were performed in 200 μ l thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler

Cycle Parameters:

WT and KO assays use the same 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

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Product Analysis:

3 Controls should be run, one each of a WT, Het, and Hom animal.

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

Expected Products:

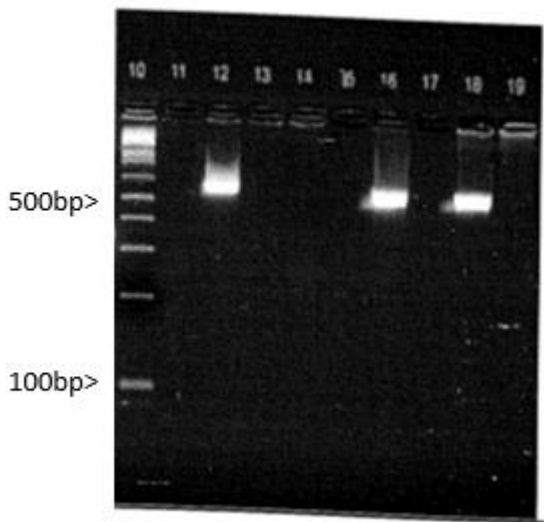
Wild type product: 500 bp on WT assay

Mutant gene product: 300 bp on Mut assay

Heterozygous animals will have both the 300 and 500 bp products.

Example gels:

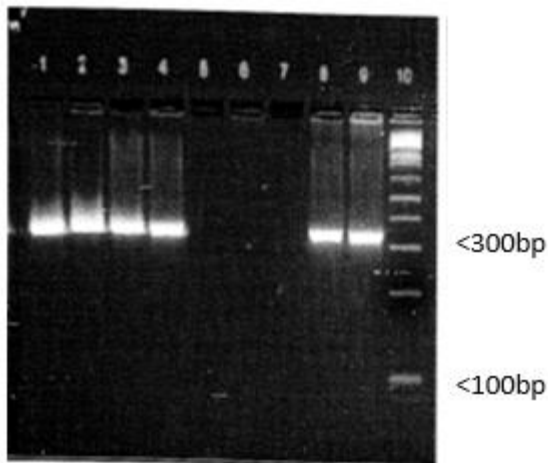
WT Gel:



Lanes 12, 16, 18 display samples positive for the wild-type allele (500bp product)
Lanes 11, 13, 14, 15, 17, 19 display samples negative for the wild-type allele (no product)

Lane 10 displays a 1kb+ ladder
(Invitrogen Cat#10787018)

Mutant Gel:



Lanes 1, 2, 3, 4, 8, 9 display samples positive for the mutant allele (300bp product)
Lanes 5, 6, 7 display samples negative for the mutant allele (no product)

Lane 10 displays a 1kb+ ladder
(Invitrogen Cat#10787018)