

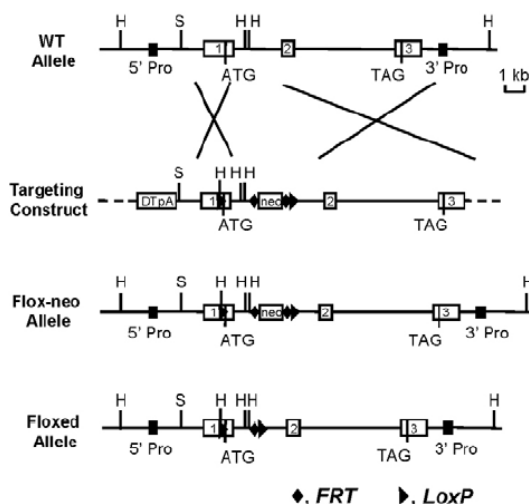
01.19.11 MS
02.17.14 MLS

Genotyping Protocol: **MMRRC 32804**

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 200µl of AE buffer once.

Strain Description: This strain has a *loxP* site inserted into exon 1 upstream of the *Nr2f1* start codon and a neo cassette and second *loxP* site inserted into intron 1 of the *Nr2f1* gene. The neo cassette was removed by flp mediated recombination. Nuclear receptor subfamily 2, group F, member 1 (*Nr2f1*) is found on mouse Chromosome 13. Details can be found in Tang et al (2010) Development 137:725-734.



Primer Information:

- 1) Name: Nr2f1.F Sequence: 5'-AGT AGA GCG AGG TCC ATG TC-3'
2) Name: Nr2f1.R Sequence: 5'-GTG AAC CAG TCA ATT TCG AAG-3'

Primer location: Nr2f1.R is located at the beginning of exon 2 of the *Nr2f1* gene. Nr2f1.F is located further downstream within exon 2.

Assay name: **Nr2f1 PCR**

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
10X Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
Nr2f1.F	Sigma	25µM	0.3
Nr2f1.R	Sigma	25µM	0.3
FastStart Tag	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

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.

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Cycle Parameters:

- | | | |
|----|--|--------------------------------|
| 1) | 95°C | 3 minutes |
| 2) | 94°C | 30 seconds |
| 3) | 61°C | 30 seconds |
| 4) | 72°C | 30 seconds |
| 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 the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/1kb (Cat# 929521)

Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)

Method: AM320

Injection: 10s at 5KV

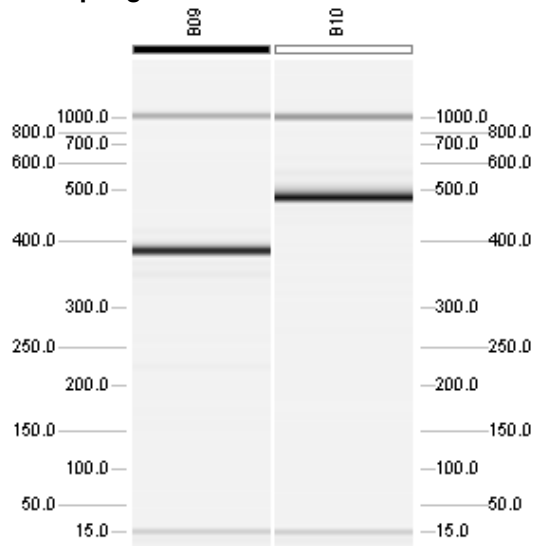
Separation: 320s at 6KV

Expected products:

Wld type (WT): 390bp

Mutant: 503bp

Example gel:



Lane B09 displays a WT sample (390bp product).
Lane B10 displays a homozygous sample (503bp product).

Please note: the 15bp and 1kb bands are reference markers specific to the QIAxcel method and do not represent expected products.