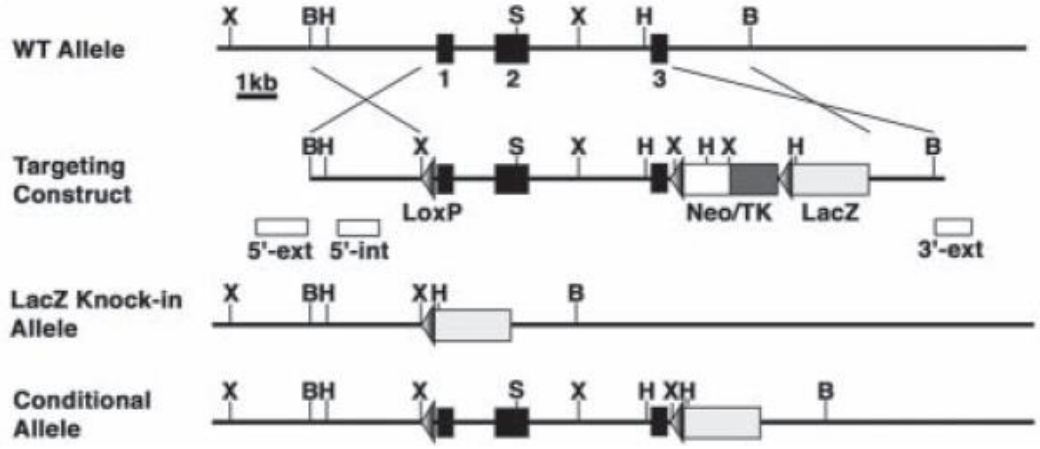
07.31.14 MLS

Genotyping Protocol: **MMRRC 32805**

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: A LacZ cassette is inserted directly at the end of exon 3 of *Nr2f2*. There is a *loxP* site before exon 1 and another directly after exon 3.Details can be found in Takamoto et al (2005) Development 132:2179-2189.



Primer Information:

1) Name: Nr2f2.wtF	Sequence: CAT CCG GGA TAT GTT ACT GTC CGG
2) Name: Nr2f2.wtR	Sequence: TGG GGA AGC TAA GTG TTG ATC TGA TTC C
3) Name: LacZ R 2/6/07	Sequence: GCC GTG GGT TTC AAT ATT GGC TTC

Primer location: Nr2f2.wtF and Nr2f2.wtR are located on either side of the stop codon of Nr2f2. LacZ R 2/6/07 is located in the LacZ gene.

Assay name: Nr2f2 Flox PCR

Mut 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
Nr2f2.wtF	Sigma	25µM	0.3
LacZ R 2/6/07	Sigma	25µM	0.3
FastStart *Taq*	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.

Cycle Parameters:

1)	95°C	3 minutes
2)	94°C	1 minute
3)	66°C	1 minute
4)	72°C	1 minute 45 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

WT 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
Nr2f2.wtF	Sigma	25µM	0.3
Nr2f2.wtR	Sigma	25µM	0.3
FastStart *Taq*	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.

Cycle Parameters:

1)	95°C	3 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	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).

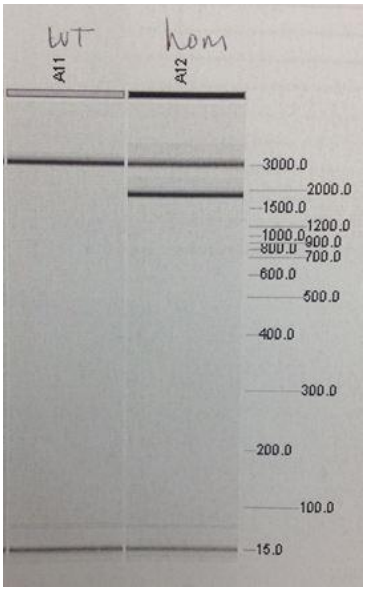
Alignment Marker: QX Alignment Marker 15bp/1kb (Cat# 929521)
Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected products:
WT: 786bp
Mut: ~1600bp

Genotypes:
Heterozygous: 786bp product on WT gel, ~1600bp product on Mut gel
Homozygous: no product on WT gel, ~1600bp product on Mut gel
Wild-type: 786bp product on WT gel, no product on Mut gel

Example gels:

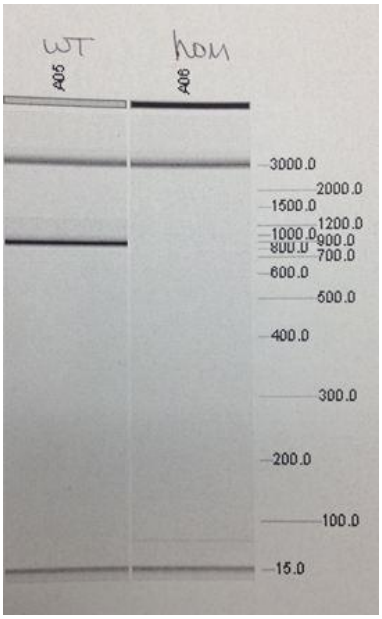
Mut Gel:



Lane A11 displays a sample negative for the Mutant allele. (no product)
Lane A12 displays a sample positive for the Mutant allele (1600bp product)

Please note: the 15bp and 3kb bands are reference markers for the QIAxcel method and do not represent expected products.

WT Gel:



Lane A05 displays a sample positive for the WT alle (786bp product)
Lane A06 displays a sample negative the the WT allele (no product)

Please note: the 15bp and 3kb bands are reference markers for the QIAxcel method and do not represent expected products.