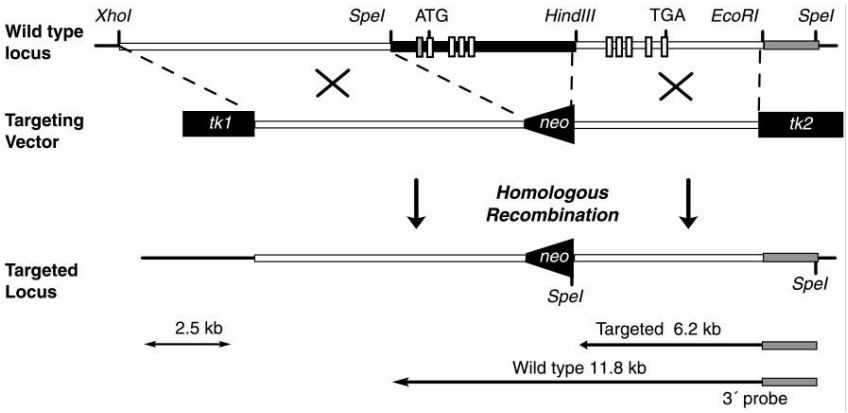
03.19.14 MLS

Genotyping Protocol: **MMRRC 36768**

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.

Mutation Information: This strain contains a neomycin resistance cassette, which replaces the first noncoding exon and the first three coding exons of mouse *Zyxin* on Chromosome 6. This removes amino acids 1-333 of the *Zyxin* gene. Details can be found in Hoffman et al (2003) Mol Cell Biol. 23(1):70-79.



Primer Information:

1) Name: M36768 Zyx WTF Sequence: 5'-TGG ACG AAG TTT CCG TGT GTT G-3'
2) Name: M36768 Zyx WTR Sequence: 5'-TAC AAG GGC GAA GTC AGG GCG AGT G-3'
2) Name: M36768 Neo Sequence: 5'-GAC CGC TTC CTC GTG CTT TAC-3'

Primer Location: M36768 Zyx WTR and WTF are located in the promoter region of mouse *Zyxin* on either side of the *Spe*I site. M36768 Zyx WTF pairs with M36768 Neo, which is located in the inserted Neomycin cassette.

Assay Name: Zyxin KO PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M36768 Zyx WTR	Sigma	25µM	0.3
M36768 Zyx WTF	Sigma	25µM	0.3
M36768 Neo	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

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 an Applied Biosystems 2700 thermocycler.

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Cycle Parameters:

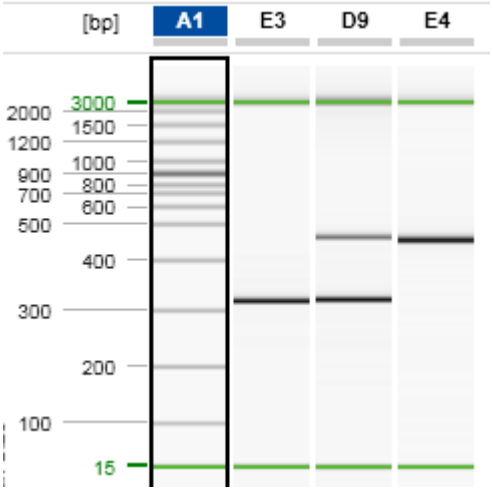
1)	95°C	3 minutes
2)	94°C	30 seconds
3)	66°C	30 seconds
4)	72°C	30 seconds
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	10 minutes
7)	4°C	hold until refrigerate product

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/3Kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected products:
Mutant allele: 473bp
WT allele: 327bp

Example gel:



Lane A1 displays a 15bp-3kb size marker
Lane E3 displays a Wild-Type sample (327bp product)
Lane D9 displays a heterozygous sample (327bp and 473bp products)
Lane E4 displays a homozygous mutant sample (473bp product)

*Please note: the 15bp and 3kb bands are reference markers specific to the QIAxcel method and do not represent expected