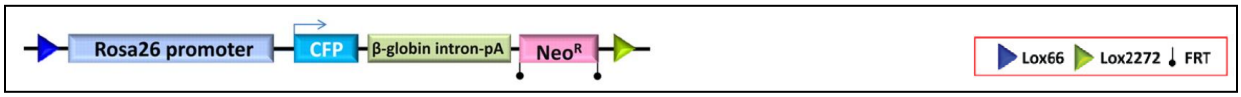
06.12.16 MS

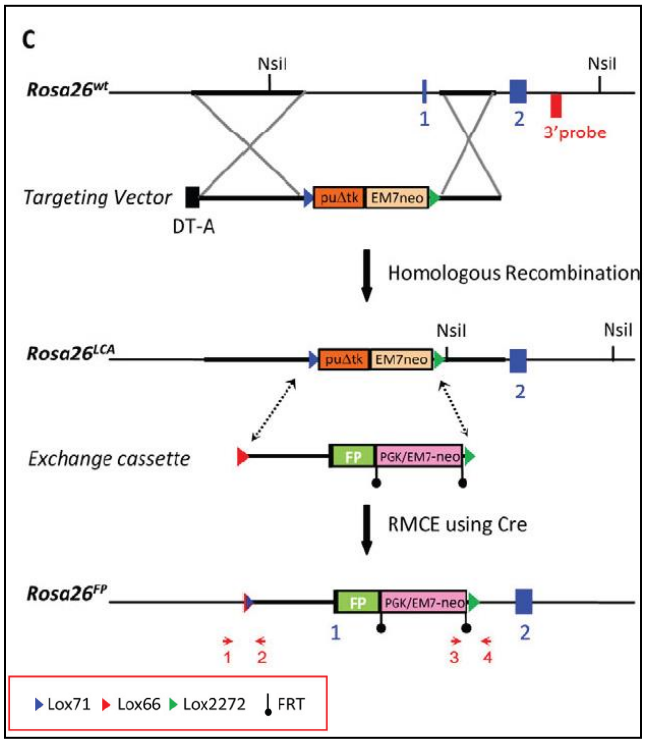
Genotyping Protocol: **MMRRC 36288**

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 contains CFP controlled by the Rosa26 promoter. The CFP was inserted into the Rosa26 locus on Chromosome 6 via recombinase-mediated cassette exchange (RMCE). Details can be found in Chen et al (2011) Disease Models and Mechanisms 4:537-547.





Primer Information:
1) Name: Rosa26.S1 Sequence: 5'-AGA CTT ATC TAC CTC ATA GGT G-3'
2) Name: pRosaR1 Sequence: 5'-GGT CTT ACA GTA ATC TCT G-3'
Primer location: Primers are located on either side of the inserted Lox66 site, within mouse Chromosome 6.

Assay name: Rosa26 CFP 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
Rosa26.S1	Sigma	25µM	0.3
pRosaR1	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

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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:

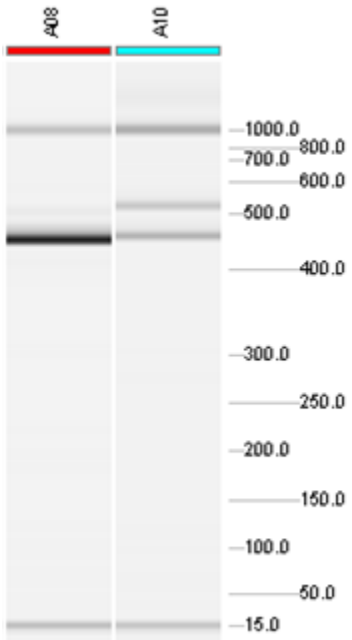
1)	95°C	3 minutes
2)	94°C	30 seconds
3)	58°C	30 seconds
4)	72°C	30 seconds
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	10 minutes
7)	4°C	hold until refrigerate product

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:
WT: 464bp
MUT: 500bp

Example gel:



Lane A08 displays a WT sample (464bp product).
Lane A10 displays a heterozygous product (464bp and ~510bp product)

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