10.03.14 MLS

Genotyping Protocol: **MMRRC 36021**

Assay Type: PCR- cannot distinguish hemizygous animals from homozygous animals. Can distinguish transgene positive from transgene negative animals.

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat# XNAT2R). Kit directions for fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Strain Description: This strain carries a Thy1-Cre-ERT2-bGHpA transgene. The transgene consists of a mouse *Thy1* minimal promoter, a rabbit beta globin intron, a Cre-ERT2 fusion gene and a bovine growth hormone polyA signal.

Primer Information:
1) Name: Cre 5 Sequence: 5'-GCG GCA TGG TGC AAG TTG AAT-3'
2) Name: Cre 3 Sequence: 5'-CGT TCA CCG GCA TCA ACG TTT-3'

Primer location: Cre 5 and Cre 3 are located in Cre Recombinase gene.

Assay name: Cre Recombinase PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat# XNAT2R)	2X	10
Cre 5	Sigma	25µM	0.3
Cre 3	Sigma	25µM	0.3
sterile water			5.4

PCR Setup:
Final Reaction: 16µl master mix & 4µl DNA template (10-20ng/µl)

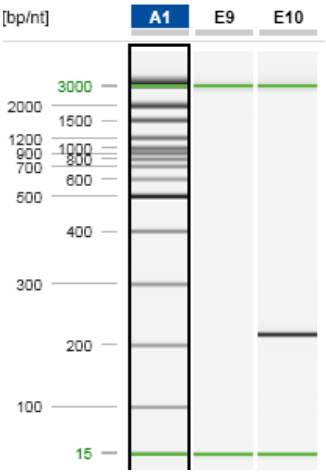
All reactions were performed in 200µl thin walled PCR tubes and were run in Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 94°C 3 minutes
2) 94°C 1 minute
3) 60.8°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

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:
Transgene positive: 232 bp
Transgene negative: no product

Example Gel:



Lane A1 displays 15bp-3kb size marker.
Lane E9 displays a transgene negative sample (no product).
Lane E10 displays a transgene positive sample (232bp product).

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