05.11.15 MLS

Genotyping Protocol: **MMRRC 34580**

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 the protocol provided with EMD Millipore's KOD Xtreme Hot Start DNA Polymerase Kit (Cat#71975-3). Kit directions were followed for amplification from mouse tails.

Strain Description: This strain carries a Calb1-iCre transgene. The transgene consists of a mouse calbindin (*Calb1*) minimal promoter, an iCre gene, and an SV40 late polyA signal.

Primer Information:
1) Name: Calb1iCre.F Sequence: 5'-CGG CGG GAT AAA TAC AGA GA-3'
2) Name: Calb1iCre.R Sequence: 5'-CAC AGT CAG CAG GTT GGA GA-3'

Primer location: Calb1iCre.F is located in the *Calb1* promoter. Calb1icre.R is located in the iCre gene.

Assay name: Calb-iCre PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
KOD Xtreme Buffer	Millipore	2X	10
KOD Xtreme dNTPs	Millipore	2mM	4
Calb1iCre.F	Sigma	25µM	0.3
Calb1iCre.R	Sigma	25µM	0.3
KOD Xtreme *Taq*	Millipore (Cat# 71975-3)	1 U/µl	0.4
sterile water			3

PCR Setup:
Final Reaction: 18µl master mix & 2µl DNA template (10-20ng/µl)

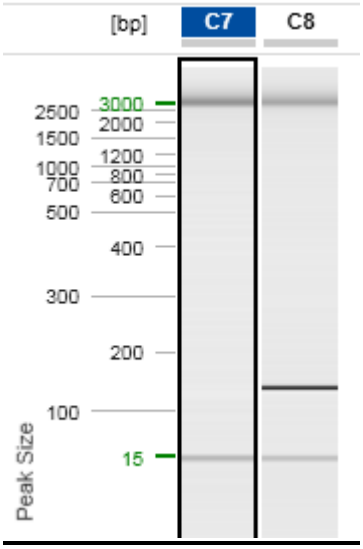
All reactions were performed in 200µl thin walled PCR tubes and were run in Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 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:
Transgene positive: 150bp
Transgene negative: no product

Example Gel:



Lane C7 displays a transgene negative sample (no product).
Lane C8 displays a transgene positive sample (150bp product).

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