12.15.11 MS
04.10.17 MLS

# Genotyping Protocol: **MMRRC 34570**

**Assay Type**: PCR- detects Cre recombinase transgene positive animals; cannot distinguish between heterozygous and homozygous 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 has a lentiviral vector transgene construct containing an EGFP/cre fusion gene under control of the mouse Thy1.2 minimal promoter.

**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'

**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 or IDT | 25µM | 0.3 |
| Cre 3 | Sigma or IDT | 25µM | 0.3 |
| sterile water | | | 5.4 |

**PCR Setup**:
Final Reaction: 16µl master mix & 4µl DNA template

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 10minutes
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: 232bp product
Transgene negative: no product

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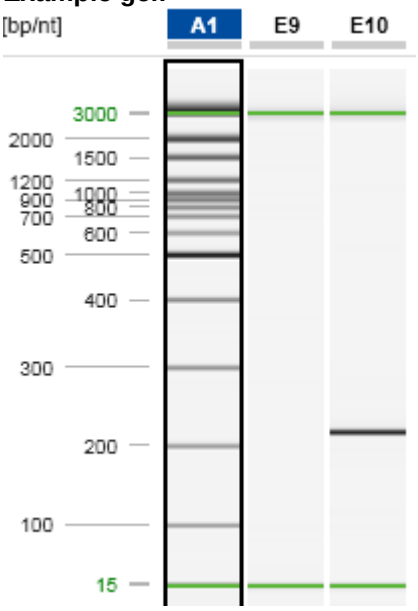
**Example gel:**




Lane A1 displays a 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.*

Lanes 9, 10, 11, 13 displays Cre recombinase positive samples (232bp product).
Lanes 12 and 14 display negative samples (no product).
Lanes 15 and 16 are extraction and PCR blanks, respectively.
Lane 17 is a negative control, and Lane 18 is a positive control.
Lane 19 displays 1Kb+ Ladder (Invitrogen Cat# 10787-018).