# Genotyping Protocol: MMRRC 29876

**Assay Type**: PCR- cannot distinguish heterozygous animals from homozygous animals; can distinguish transgene positive animals from transgene negative animals.

**Strain Characteristics:** This strain carries a random insertion of the transgene containing a Cre coding sequence fused to a 2kb fragment of mouse albumin promoter and a 4.6kb fragment of mouse alpha-feto protein enhancer.

**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.

#### **Primer Information:**

1) Name: Cre5 Sequence: 5'-GCG GCA TGG TGC AAG TTG AAT-3'
2) Name: Cre3 Sequence: 5'-CGT TCA CCG GCA TCA ACG TTT-3'

**Primer location**: Both primers are located in the Cre recombinase gene.

Assay Name: AlfpCre 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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

#### **Cycle Parameters:**

1) 95°C 5 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)

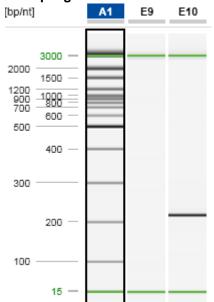
Method: AM320 Injection: 10s at 5KV

Separation: 320s at 6KV

## **Expected products:**

Transgene positive: 232 bp
Transgene negative: no product

Example gel:



Lane A1 displays 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.\*