

## Genotyping Protocol: **MMRRC 16992**

**Assay Type:** PCR to detect transgene positive animals- cannot distinguish hemizygous animals from homozygous animals.

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

**Primer Information:**

- 1) Name: cre1                      Sequence: 5'-GGT CGA TGC AAC GAG TGA TGA GG-3'
- 2) Name: cre2                      Sequence: 5'-GCT AAG TGC CTT CTC TAC ACC TGC G-3'

**Primer location:**              Cre1 and Cre2 both bind to the Cre Recombinase sequence in the transgene

**Assay Name: GFAP-Cre/Esr1**

**PCR Master Mix Components:**

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
cre1	Sigma-Genosys	25µM	0.3
cre2	Sigma-Genosys	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

**Cycle Parameters:**

- 1) 94°C                      3 minutes
- 2) 94°C                      1 minute
- 3) 66°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/1000bp (Cat# 929521)

Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)

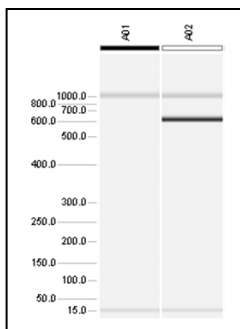
Method: AH320

Injection: 20s at 2kV

Separation: 320s at 6kV

Positive = 600 bp  
Negative = no band

**Example gel:**



Lane A01 displays a negative sample (no product). Lane A02 displays a positive sample (600bp product).

\*Please note: the 15bp and 1000bp bands seen in Lanes A01 and A02 are reference markers specific to the Qiaxcel method and do not represent amplification products\*