

## Genotyping Protocol: **MMRRC 422**

**Assay Type:** PCR - cannot distinguish hemizygous animals from homozygous animals, can only 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 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: 422 F                      Sequence: 5'-CATCTGCGGACTGGAAAAACAAC-3'  
 2) Name: 422 Ra                    Sequence: 5'-GCATCGGTAAACATCTGCTCAAAC-3'

**Primer location:**     422 F and 422 Ra: Reverse tet transactivator gene (rtTA)

**Assay Name: MCKrtTA**

**PCR Master Mix Components:**

Component	Manufacturer	Concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat# XNAT2R)	2X	10
422 F	IDT	25µM	0.3
422 Ra	IDT	25µM	0.3
Sterile Water			5.4

**PCR Setup:**

Final Reaction: 16 µl master mix & 4 µl DNA template (10-20 ng/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) 94°C                      3 minutes
- 2) 94°C                      30 seconds
- 3) 61°C                      30 seconds
- 4) 72°C                      1minute
- 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

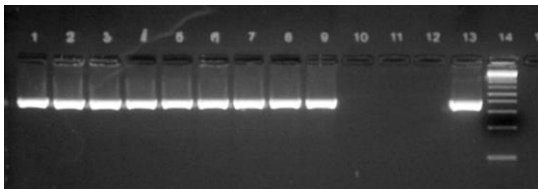
**Product Analysis:**

All products were analyzed on a 3% agarose gel with ethidium bromide staining

Transgene Positive = 402 bp

Transgene Negative = no band

**Example of Gel:**



Lanes 1-9: samples positive for the transgene (402bp band).  
 Lanes 10 and 11 are extraction and PCR blanks, respectively.  
 Lane 12 is a WT control (no product).  
 Lane 13 is a positive control (402bp band).  
 Lane 14 is 1Kb+ Ladder (Invitrogen Cat# 10787-018)