

Genotyping Protocol: **MMRRC 31756**

Assay Type: PCR: cannot distinguish homozygous animals from hemizygous animals; can distinguish positive animals from negative animals

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Strain Description: This strain carries the *c-fos*-tTA transgene designed by Dr Mark Mayford. The transgene consists of the *c-fos* promoter region and the tTA gene, which is inserted into the *AccI* site of exon 1 of mouse *c-fos*. The transgene contains an artificial intron and splice sites at the 5' end and a SV40 polyadenylation signal at the 3' end.

Primer Information:

- | | |
|--------------------|--|
| 1) Name: htTA Tg F | Sequence: 5'-ACC TGG ACA TGC TGT GAT AA-3' |
| 2) Name: htTA Tg R | Sequence: 5'-TGC TCC CAT TCA TCA GTT CC-3' |

Primer location: Both primers are located in the htTA transgene.

Assay name: htTA PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer (with MgCl ₂)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
htTA Tg F	Sigma	25µM	0.3
htTA Tg R	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:

Final Reaction: 19µl master mix & 1µl 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:

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|----|--|--------------------------------|
| 1) | 95°C | 3 minutes |
| 2) | 94°C | 30 seconds |
| 3) | 61°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

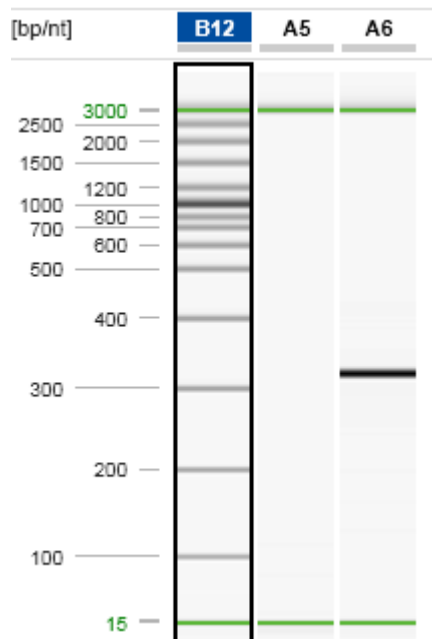
Separation: 320s at 6KV

Expected product:

Positive: 310bp

Negative: no product

Example gel:



Lane B12 displays a 15bp-3kb size marker

Lane A5 displays a negative sample (no product)

Lane A6 displays a positive sample (310bp product)

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