

Genotyping Protocol: **MMRRC 31779**

Assay Type: PCR: cannot distinguish homozygous animals from hemizygous animals; can distinguish transgene positive animals from transgene 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 *Klk8*-tTa transgene designed by Dr Mark Mayford. The transgene consists of a ~120kb BAC containing the whole mouse kallikrein related-peptidase 8 (*Klk8*) gene. A human codon-optimized tetracycline-regulated transactivator gene (htTA) was inserted into the first exon upstream of the *Klk8* start codon.

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:

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

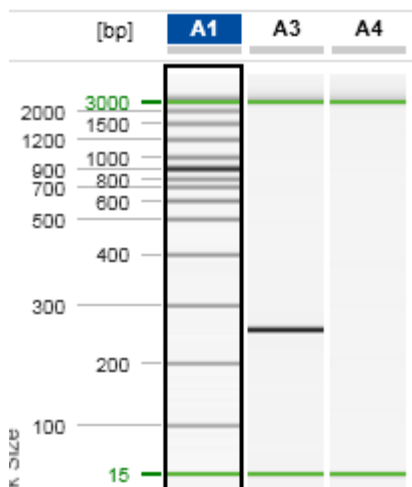
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Expected products:

Positive: 281bp

Negative: no product

Example gel:



Lane A1 displays a 15bp-3kb size marker

Lane A3 displays a transgene positive sample (281bp product)

Lane A4 displays a transgene negative sample (no product)

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