

Genotyping Protocol: **MMRRC 37586**

Assay Type: PCR- cannot distinguish heterozygous animals from homozygous 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 200ul of AE buffer once.

Strain Description: This strain carries a transgene in which the human *TSHR* gene is driven by the bovine *Tg* promoter. This causes the human *TSHR A-subunit* to be expressed in the thyroid. Details can be found in Pichurin *et al.* (2006) J Immunol 176(1):668-676.

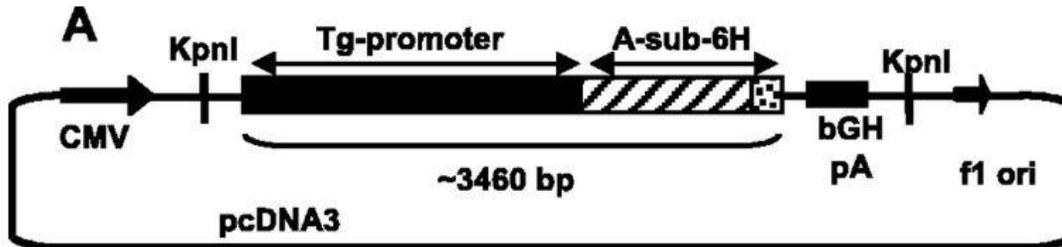


Image from Pichurin *et al.* (2006) J Immunol 176(1):668-676.

Primer Information:

- 1) Name: M37586 F Sequence: 5'- GCC TGG AGA ATC CCA TGG ACA GAG GAG CCT GG -3'
 2) Name: M37586 R Sequence: 5'- CTC CTG ATG GCA CTC GCA GGG TGG AGA CGA AC -3'

Primer location: M37586 F is located in the promoter region of bovine *Tg*. M37586 R is located in Exon 1 of human *TSHR*.

Assay names: hTSHR Tg PCR**PCR Master Mix Components:**

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche (Cat#12032953001)	10X	2
dNTPs	Promega (Cat# U1515)	1.25 mM	3.2
M37586 F	Sigma or IDT	25 µM	0.3
M37586 R	Sigma or IDT	25 µM	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 (10-20 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 4 minutes
- 2) 94°C 30 seconds
- 3) 68°C 30 seconds
- 4) 72°C 30 seconds
- 5) Repeat steps 2-4 34 times for a total of 35 cycles
- 6) 72°C 7 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 100-2.5Kb (Cat# 929559)

Method: AM320

Injection: 10s at 5KV

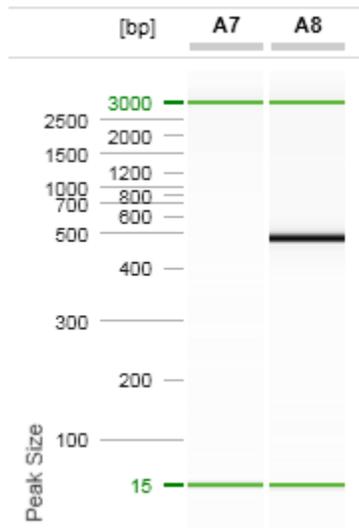
Separation: 320s at 6KV

Expected product:

Transgene positive: 460bp

Transgene negative: no product

Example gel:



Lane A7 displays a wild-type sample (no product)
Lane A8 displays a transgene positive sample (460bp product)

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