

Genotyping Protocol: **MMRRC 37407**

Assay Type: PCR- can distinguish heterozygous animals from homozygous 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: Exon 1 of mouse tumor susceptibility gene 101 (*Tsg101*) on Chromosome 7 was flanked by a floxed Neo cassette, which was inserted 3kb upstream. A loxp site was also inserted 230bp into intron 1 of *Tsg101*. Details can be found in Krempler et al (2002) J Biol Chem 277(45):43216-23.

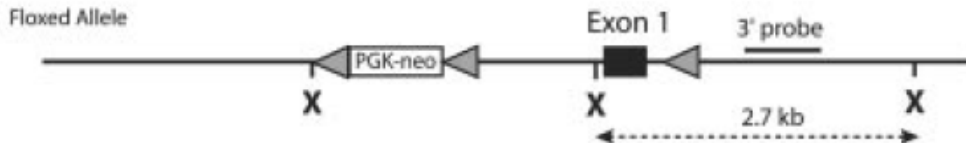


Image from Krempler et al (2002) J Biol Chem 277(45):43216-23.

Primer Information:

1) Name: M37407 flox F	Sequence: 5'- AGA GGC TAT TCG GCT ATG ACT G-3'
2) Name: M37407 flox R	Sequence: 5'-TTC GTC CAG ATC ATC CTG ATC-3'
2) Name: M37407 wt F	Sequence: 5'-GTT CGC TGA AGT AGA GCA GCC AG-3'
2) Name: M37407 wt R	Sequence: 5'-CAT TTC TGG AGT CCG ATG CGC AG-3'

Primer location: M37407 wt F and M37407 wt R are located in intron 1 of *Tsg101*. M37407 flox F and M37407 flox R are located in the neo cassette.

Assay name: Tsg101 flox PCR**FLOX PCR Master Mix Components:**

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M37407 flox F	Sigma	25µM	0.3
M37407 flox 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 Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

1)	95°C	4 minutes
2)	94°C	30 seconds
3)	62°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

WT PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
M37407 wt F	Sigma	25µM	0.3
M37407 wt R	Sigma	25µM	0.3
FastStart Taq	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 Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 95°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 100-3kb (Cat# 929553)

Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected products:

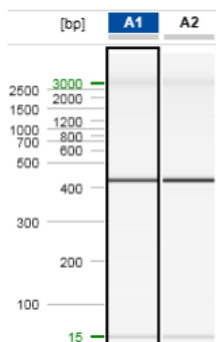
Flox allele: 421bp product
WT allele: 261bp product

Genotyping results:

Heterozygous: 421bp product from Flox PCR, 261bp product from WT PCR
Homozygous mutant (floxed allele): 421bp product from Flox PCR, no product from WT PCR
Wild-type: no product from Flox PCR, 261bp product from WT PCR

Example Gels:

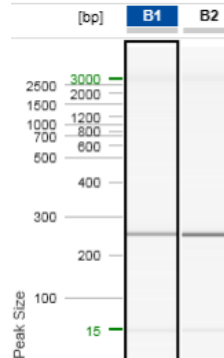
Flox gel:



Lanes A1 and A2 display samples positive for the flox allele (421bp product on Flox gel).

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

WT gel:



Lanes B1 and B2 display samples positive for the WT allele (261bp product on WT gel).

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