06.20.16 MLS

Genotyping Protocol: **MMRRC 33007**

Assay Type: PCR- cannot distinguish heterozygous animals from homozygous animals. Can distinguish hemizygous and wild-type 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 three transgenes: tet-Rag1, tet-Rag2 and tet-tTA. They are cointegrated. Details can be found in Shockett et al (2004) Molecular Immunology 40:813-829.

Primer Information:
1) Name: RAG1R Sequence: 5'-CAG GCA GTC CAA GTG CTA TGA GAT G-3'
2) Name: TX3'UTRF Sequence: 5'-CGA ATT TCT GCC ATT CAT CCG C -3'
3) Name: RAG2R Sequence: 5'-GCA CAG TCT TGC CAG GAG GAA TC-3'
4) Name: TTA-Rev Sequence: 5'-ATC TCA ATG GCT AAG GCG TC-3'
5) Name: CMV-F1 Sequence: 5'-TGA CCT CCA TAG AAG ACA CC-3'

Primer location: RAG1R is located in Exon 1 of mouse *Rag1*. RAG2R is located in Exon 2 of mouse *Rag2*. TX3'UTRF is located in the transgene construct. TTA-Rev and CMV-F1 are located in the tet-tTA transgene.

Assay name: M33007 PCR

This assay consists of three separate PCRs, one to detect each transgene. Since the three transgenes are cointegrated, all test results should agree.

Rag1 Tg PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
RAG1R	Sigma	25µM	0.3
TX3'UTRF	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

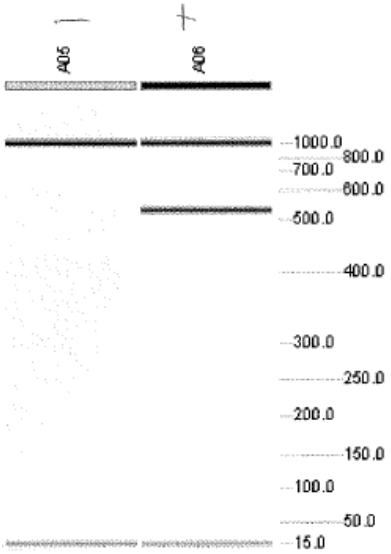
Cycle Parameters:
1) 95°C 3 minutes
2) 94°C 30 seconds
3) 70°C 30 seconds
4) 72°C 30 seconds
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 minutes
7) 4°C hold until refrigerate product

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/1kb (Cat# 929521)
Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected products:
Rag1 transgene (Tg) positive: ~506bp
Rag1 transgene (Tg) negative: no product

Example gel:



Lane A05 displays a sample negative for the Rag1 Tg (no product)
Lane A06 displays a sample positive for the Rag1 Tg (~506bp product)

Please note: the 15bp and 1kb bands are reference markers specific to the QIAxcel method and do not represent expected products.

Rag2 Tg PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
RAG2R	Sigma	25µM	0.3
TX3'UTRF	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 95°C 3 minutes
2) 94°C 1 minute
3) 70°C 1 minute

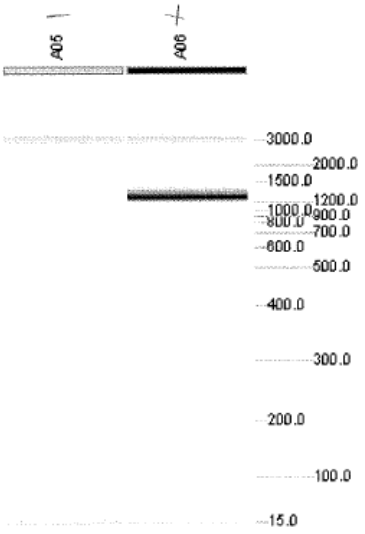
4) 72°C 1 minute 10 seconds
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 minutes
7) 4°C hold until refrigerate product

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 products:
Rag2 Tg positive: ~1258bp
Rag2 Tg negative: no product

Example gel:



Lane A05 displays a sample negative for the Rag2 Tg (no product)
Lane A06 displays a sample positive for the Rag2 Tg (~1258bp product)

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

Tet-tTA Tg PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
TTA-Rev	Sigma	25µM	0.3
CMV-F1	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

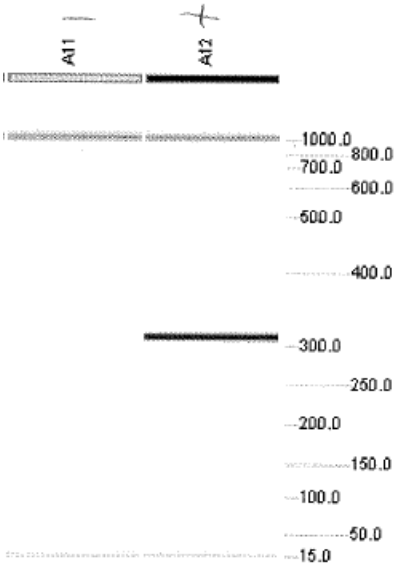
1)	95°C	3 minutes
2)	94°C	30 seconds
3)	63°C	30 seconds
4)	72°C	30 seconds
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	10 minutes
7)	4°C	hold until refrigerate product

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/1kb (Cat# 929521)
Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected products:
Tet-tTA Tg positive: ~290bp
Tet-tTA Tg negative: no product

Example gel:



Lane A11 displays a sample negative for the tet-tTA Tg (no product)
Lane A06 displays a sample positive for the tet-tTA Tg (~290bp product)

Please note: the 15bp and 1kb bands are reference markers specific to the QIAxcel method and do not represent expected products.