Genotyping Protocol: MMRRC 36192

Assay Type: Precision Melt SNP analysis – can distinguish between G and A at nucleotide 12212 of mouse *Tgm3*.

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 an ENU-induced G to A transversion at nucleotide 12212 – the first nucleotide of exon 3 - in the mouse transglutaminase 3 gene (*Tgm3*). Details can be found on the Mutagenetix database under "tortellini" (http://mutagenetix.utsouthwestern.edu).

Wild type sequence – the location of the G to A transversion is colored in red.
TTTATAATGC TCTTCAACCC GTGGTTGCAA GGTAGGTCTT TAAGCACGGC ATTCCCCACA CATCCCTGAT

Primer Information:

1) Name: M36192 tortellini F Sequence: 5'-GGG CCT CCT CTC TGA AAC TT-3' 2) Name: M36192 tortellini R Sequence: 5'-GAA TGC CGT GCT TAA AGA CC-3'

Primer Location: Primers are located on either side the G to A transversion in *Tgm3*.

Assay Name: M36192 Tortellini PCR

Master Mix Components:

component	manufacturer	concentration	μl/rxn
Precision Melt Supermix	BioRad (Cat# 172-5110)	2X	10
M36192 tortellini F	Sigma	2µM	1
M36192 tortellini R	Sigma	2µM	1
sterile water			3

PCR Setup:

Final Reaction: 15µl master mix & 5µl DNA template (5ng/µl)

All reactions were performed in either BioRad Hard-Shell PCR Plates(Cat#HSP9601) with Optical Tape(Cat#2239444) covers or LifeTechnologies Optical 8-tube strips (Cat#4316567) with Optical Caps (Cat#4323032). Analysis was performed in a BioRad CFX96 RealTime System utilizing BioRad CFX Manager software.

Cycle Parameters:

1)	95°C	2 minutes
2)	95°C	10 seconds
a)	CO ⁰ C	20

3) 60°C 30 seconds (+plate read)

4) 72°C 30 seconds 5) Repeat steps 2-4 39 times for a total of 40 cycles 6) 95°C 30 seconds 7) 60°C 1 minute

8) 65-95°C (in 0.2°C increments) 10 seconds/step (+plate read)

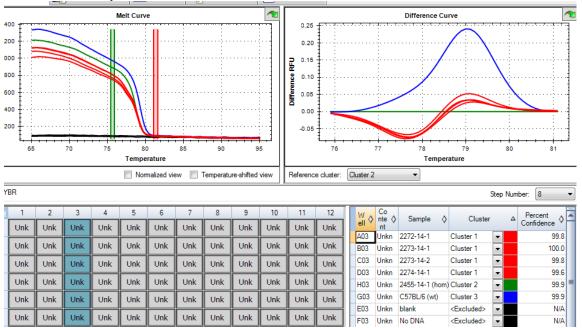
Product Analysis:

Results were analyzed with BioRad Precision Melt Analysis Software

Expected Results:

M36192 Homozygous	M36192 Heterozygous	C57BL/6 Wild type
Cluster 2	Cluster 1	Cluster 3

BioRad Data Output:



In this example, Cluster 1 are heterozygous samples, Cluster 2 are homozygous samples, and Cluster 3 are WT samples.

For Genotype Confirmation via Sequencing (Optional):

Primer Information:

1) Name: M36192 seq F Sequence: 5'-CCA TTG CTA TTG CCA GTC CT-3' 2) Name: M36192 seq R Sequence: 5'-GGC ACA GGT TAG CCA TCT CT-3'

Primer location: Primers are located on either side of the G to A transversion at nucleotide 12212 of mouse *Tam3*.

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
M36192 seq F	Sigma	25µM	0.3
M36192 seq 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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

 1)
 95°C
 3 minutes

 2)
 94°C
 20 seconds

 3)
 63°C
 25 seconds

 4)
 72°C
 30 seconds

5) Repeat steps 2-4 34 times for a total of 35 cycles

6) 72°C 10 minutes

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

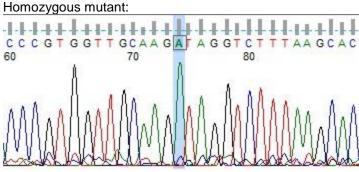
Expected product: 213bp

Sequencing: To confirm that the correct point mutation is present.

PCR product is purified with the QIAquick PCR Purification Kit (Qiagen Cat# 28104). Kit directions are performed, and DNA is eluted with 50µl nuclease-free water. 15µl of the DNA is then added to 1µl M36192 seq F primer (25µM) in a 1.5ml tube. The tube is submitted to the MU DNA Sequencing Core.

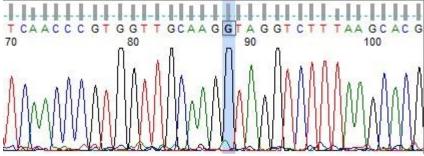
Analysis: In mutant allele, G changes to A

Example Chromatograms:



CCCGTGGTTGCAAGATAGGTCTTTAAGCAC





CCCGTGGTTGCAAGGTAGGTCTTTAAGCAC