08.16.16 MLS

# Genotyping Protocol: **MMRRC 36698**

**Assay Type:** Precision Melt SNP analysis – can distinguish between heterozygous and homozygous samples.

**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 a G to A single nucleotide change in Exon 39 of the mouse *Myh9* gene. This results in a glutamic acid to lysine amino acid change at codon 1841 (E1841K). Details can be found in Zhang et al (2012) Blood 119(1): 238–250.

**Primer Information:**
1) Name: M36698 melt F Sequence: 5'- CCC CTT TCA GTC AGC ATT TC-3'
2) Name: M36698 melt R Sequence: 5'- GCA CGT CCT TCA GCT TCT TC-3'

**Primer Location**: M36698 melt F and melt R are located on either side of the SNP: M36698 melt F is in Intron 38 and M36698 melt R is in Exon 39 of mouse *Myh9.*

WT Mouse *Myh9* sequence (red letters with tan highlights are Exon 39):

CAGCAGCTACTGAGTGTCCAGGAAAGACTAGCACCTTAGCTTAGCCTCCAGGGGCCCCAGGAAGCGGAAGCTCCGGTTGCCATCTTGCTGGCTGAGCTA
TCTGGTGGAGGGTGCCCCCTTTCAGTCAGCATTTCTCTCCTACCCAG**GGAGCGCCAGGCAGCCTCCAAGCAGGTGCGCCGGACG**G**AGAAGAAGCTGAAG**
**GACGTGCTGCTGCAGGTGGAGGACGAGCGGAGGAACGCGGAACAGTTCAAGGACCAG**GTCTGTGCTCTGCAGGGCCTGGGTTCTGTCTGTGCTCAGTAG
AGCCTTGGGCTGCACTCAGTGTCTCACCAGCGTCCCTGGCCACCTGTTTCTGCAG

M36698 melt F
M36698 melt R
Nucleotide which changes from G to A in mutant allele
Codon 1841

**Assay Name: M36698 SNP PCR**

**Master Mix Components:**

| component | manufacturer | concentration | µl/rxn |
|---|---|---|---|
| Precision Melt Supermix | BioRad (Cat# 172-5110) | 2X | 10 |
| M36698 melt F | Sigma or IDT | 2µM | 1 |
| M36698 melt R | Sigma or IDT | 2µM | 1 |
| sterile water | | | 6 |

**PCR Setup**:
Final Reaction: 18µl master mix & 2µl DNA template (DNA is diluted to 5ng/µl, for 10ng total for each reaction)

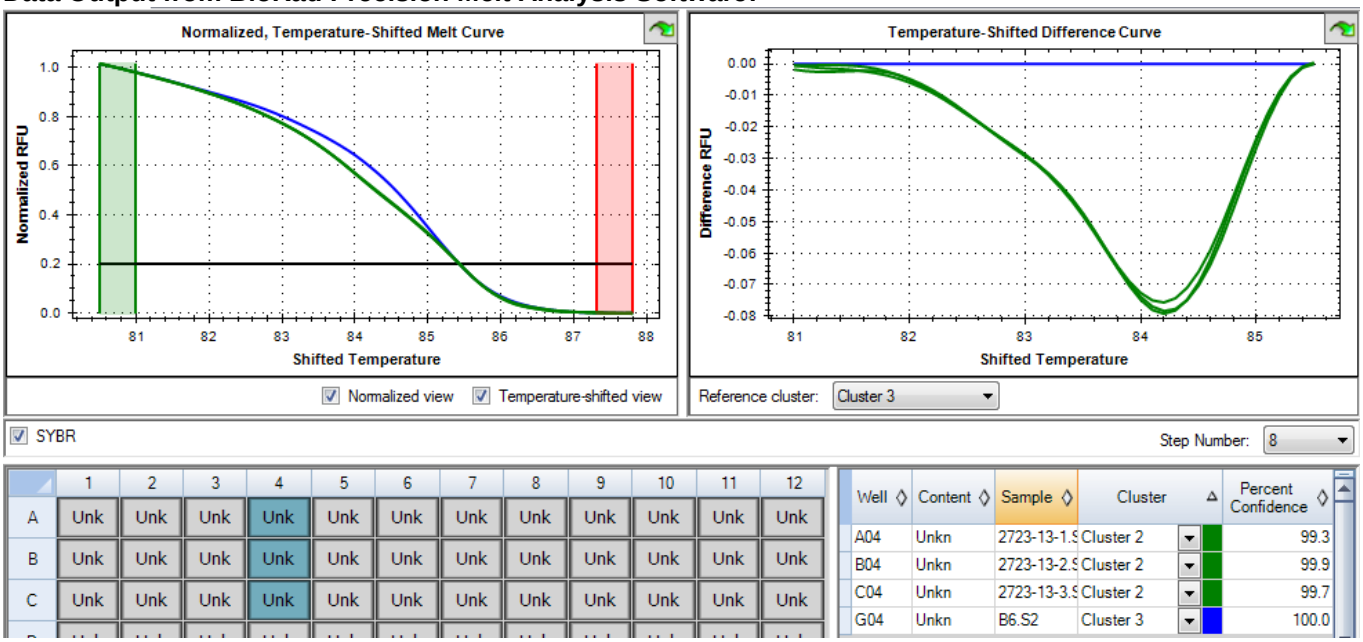
All reactions were performed in either BioRad Hard-Shell PCR Plates(Cat#HSP9601) with Optical Tape(Cat#2239444) covers or LifeTechnologies' Optical 8-tube strips (Cat#4316567) with Optical Caps (Cat#4323032). All were run in a BioRad CFX96 RealTime System utilizing BioRad CFX Manager software.

**Cycle Parameters**:
1) 95ºC 2 minutes
2) 95ºC 10 seconds
3) 60ºC 30 seconds (+plate read)
4) 72ºC 30 seconds
5) Repeat steps 2-4 39 times for a total of 40 cycles
6) 95ºC 30 seconds
7) 60ºC 1 minute
8) 65-95ºC (in 0.2ºC increments) 10 seconds/step (+plate read)

**Product Analysis**:
Results were analyzed with BioRad Precision Melt Analysis Software.
*If products are run on a gel, a 91bp product can be expected.*

**Expected Results:**

**Data Output from BioRad Precision Melt Analysis Software:**




In this example, Cluster 2 (green) are heterozygous samples and Cluster 3 (blue) is a WT control.

## For Genotype Confirmation via Sequencing (Optional):

**Primer Information**:
1) Name: M36698 551 F Sequence: 5'-TTG CCA TCT TGC TGC TGA GCT ATC TG-3'
2) Name: M36698 552 R Sequence: 5'-ACC TGC AGC AGC ACT TCA GCT TCT TC-3'

**Primer location**: Primers are located on either side of the G to A single nucleotide change in Exon 39 of *Myh9*.

**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 |
| M36698 551 F | Sigma or IDT | 25µM | 0.3 |
| M36698 552 R | Sigma or IDT | 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**:

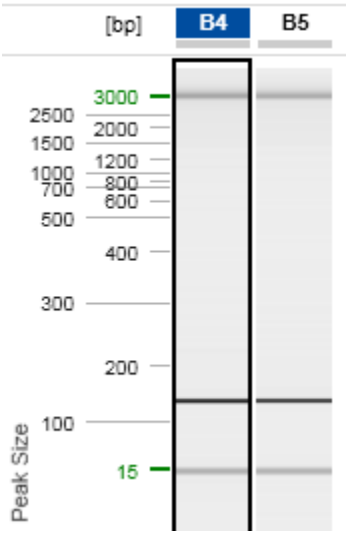
| | | |
|---|---|---|
| 1) | 95°C | 3 minutes |
| 2) | 94°C | 20 seconds |
| 3) | 66°C | 25 seconds |
| 4) | 72°C | 30 seconds |
| 5) | Repeat steps 2-4 34 times for a total of 35 cycles | |
| 6) | 72°C | 10 minutes |
| 7) | 4°C | hold until refrigerate product |

**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 product**: 138bp

**Example gel**:




Lanes B4 and B5 display the 138bp product from the sequencing PCR (M36698 551 F and M36698 552 R). All samples will produce this 138bp product.

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

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

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 M36698 551 F primer (25µM) in a 1.5ml tube. The tube is submitted to the sequencing core.

**Analysis:** In mutant allele, G changes to A

**Example Chromatograms**:
Green: A
Red: T
Blue: C
Black: G

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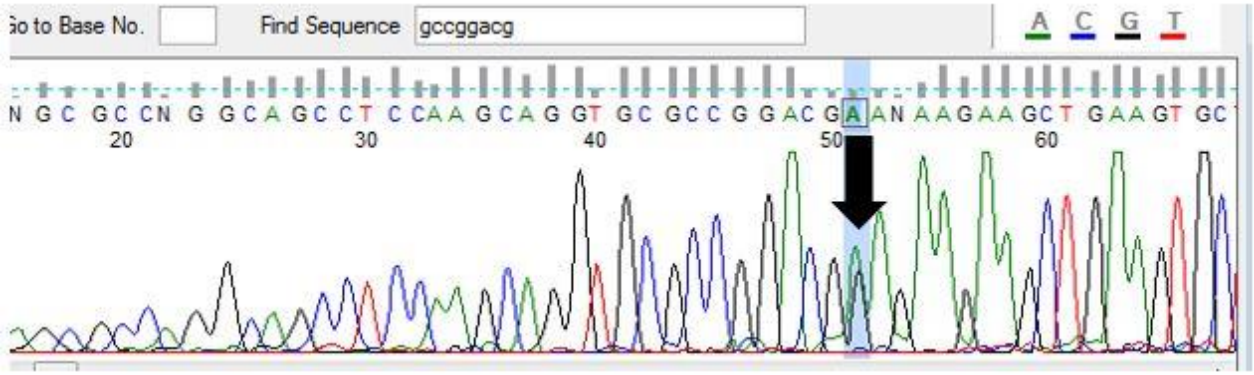
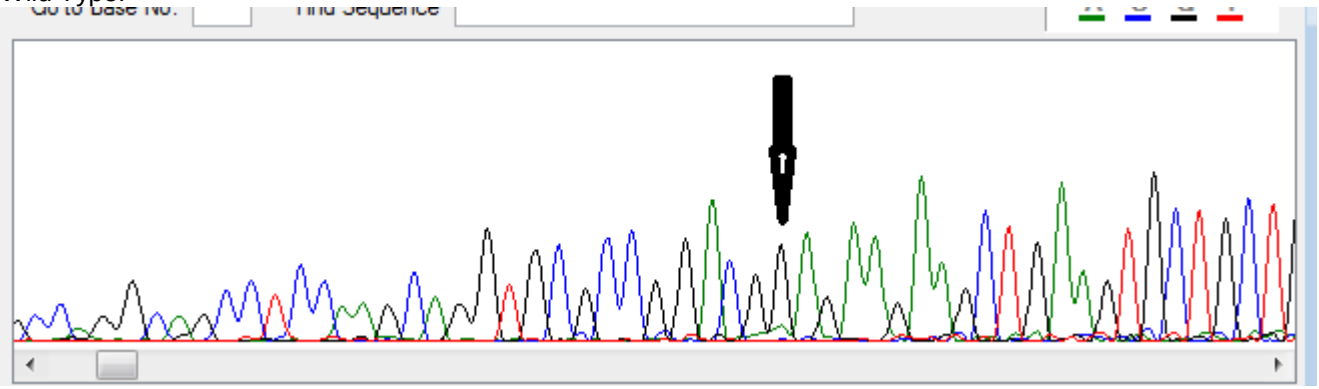
Heterozygous Mutant:




GCCGGACG A/G

Wild Type:



GCCGGACG G