01.22.16 MLS

Genotyping Protocol: **MMRRC 36725**

Assay Type: Precision Melt SNP analysis – can distinguish between T and G in intron 4 of *Nfkbid*.

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 T to G transversion in the beginning of intron 4 of the nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, delta gene (*Nfkbid*). Details can be found on the Mutagenetix database under "bumble" (http://mutagenetix.utsouthwestern.edu).

Wild type sequence – the location of the T to G transversion is colored in red.
AGGATGAGGA AGGAGACACG **T**GAGTATAGG GGGAAAGGGT GATGTGGACC

Primer Information:
1) Name: M36725 bumble F   Sequence: 5'- CCA GGA TGA GGA AGG AGA CA -3'
2) Name: M36725 bumble R   Sequence: 5'- GTG GAG CAG CAA TGA GAA CA -3'

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

Assay Name: M36725 Bumble PCR

Master Mix Components:

component	manufacturer	concentration	µl/rxn
Precision Melt Supermix	BioRad (Cat# 172-5110)	2X	10
M36725 bumble F	Sigma	2µM	1
M36725 bumble 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 and BioRad Precision Melt Analysis software.

Cycle Parameters:
1)   95°C   2 minutes
2)   95°C   10 seconds
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:
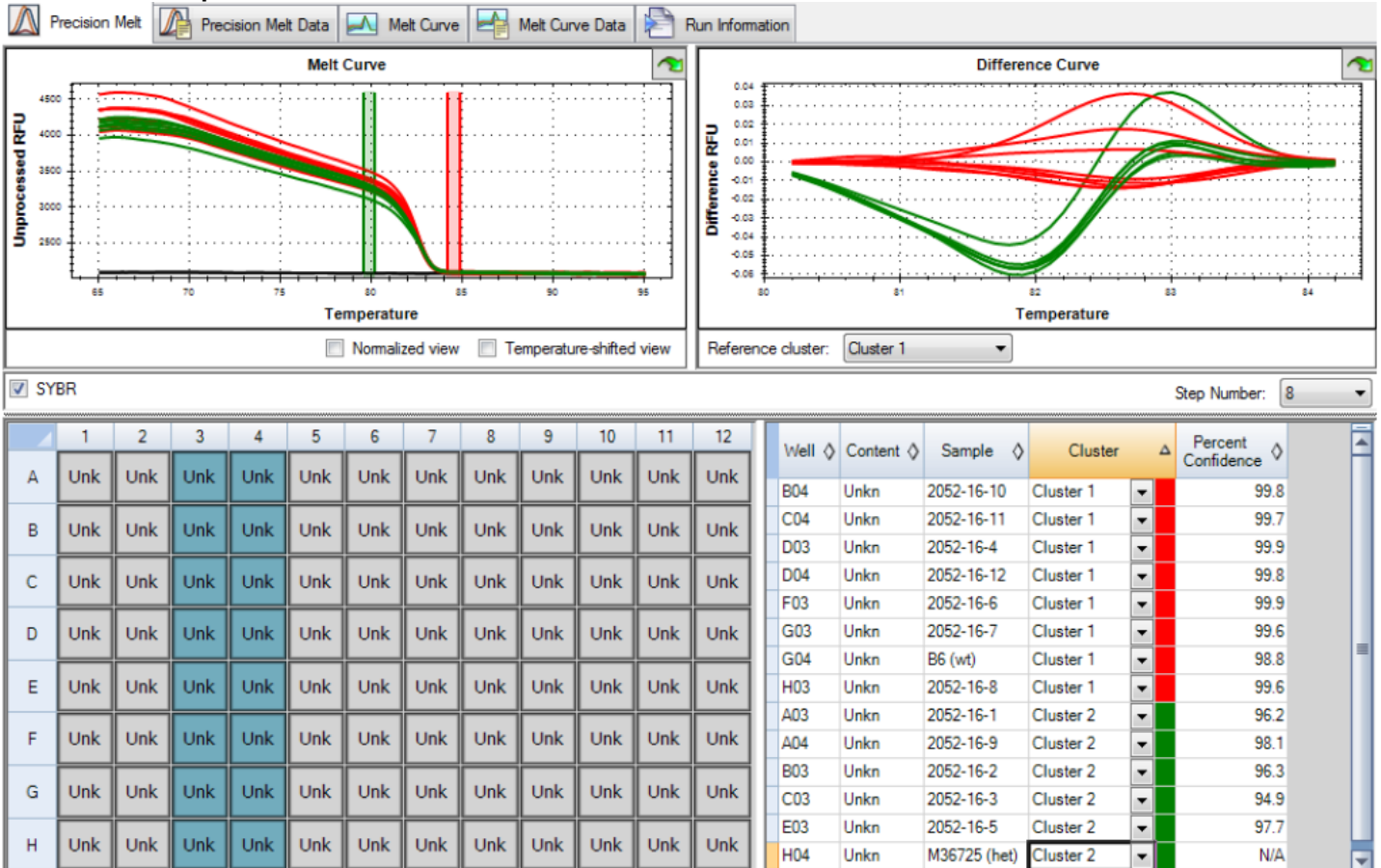Results were analyzed with BioRad Precision Melt Analysis Software

BioRad Data Output:

In this example, Cluster 1(red) are wild-type samples, Cluster 2(green) are heterozygous samples.