# 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 TGAGTATAGG 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

## 01.22.16 MLS

### **BioRad Data Output:** Precision Melt Precision Melt Data Melt Curve Melt Curve Data Run Information Melt Curve Difference Curve 0.03 0.02 Unprocessed RFU Difference RFU 0.01 0.00 -0.01 -0.02 -0.03 -0.04 -0.05 -0.06 Temperature Temperature Reference cluster: Normalized view Temperature-shifted view Cluster 1 SYBR Step Number: 8 2 3 4 5 7 9 11 12 6 8 10 Percent Confidence Well ♦ Content ♦ Sample ♦ Cluster Unk B04 Unkn 99.8 2052-16-10 Cluster 1 C04 2052-16-11 Cluster 1 99.7 Unkn В Unk D03 Unkn 2052-16-4 Cluster 1 99.9 D04 Unkn 2052-16-12 Cluster 1 99.8 С Unk F03 Unkn 2052-16-6 Cluster 1 99.9 Unk G03 2052-16-7 Cluster 1 99.6 D Unk Unkn • G04 98.8 Unkn B6 (wt) Cluster 1 • Unk Unk 99.6 Unk Unk Unk Unk Unk Unk Unk H03 2052-16-8 Cluster 1 Ε Unk Unk Unk Unkn A03 Unkn 2052-16-1 Cluster 2 96.2 Unk A04 Unkn 2052-16-9 Cluster 2 98.1 B03 Unkn 2052-16-2 Cluster 2 96.3 G Unk C03 Unkn 2052-16-3 Cluster 2 94.9 E03 Unkn 2052-16-5 Cluster 2 97.7 Unk H04 Unkn M36725 (het) Cluster 2 N/A