NOD.Cg-*Tnfrsf1b*^{tm1Imx} *Tnfrsf1a*^{tm1Imx}/J

Stock No: 024314
Protocol 29676: Standard PCR Assay - Tnfrsf1b<tm1 Imx> MCA
Version 2.2

Notes

Melting curve analysis is done using a Roche Light Cycler 480.

The genotyping protocol(s) presented here have been optimized for reagents and conditions used by The Jackson Laboratory (JAX). To genotype animals, JAX recommends researchers validate the assay independently upon receipt of animals into their facility. Reaction cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Expected Results

HET 88C +/-1.0 area 3
HET2 91C +/-1.0 area 5
HOM 88C +/-1.0 area 8
WT 91C +/- 1.0 area 8

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR0837		CCG GTG GAT GTG GAA TGT GTG		Mutant	A	PGK R
oIMR0838		AGA GCT CCA GGC ACA AGG GC		Common	A	
oIMR0839		AAC GGG CCA GAC CTC GGG T		Wild type	A	

Reaction A

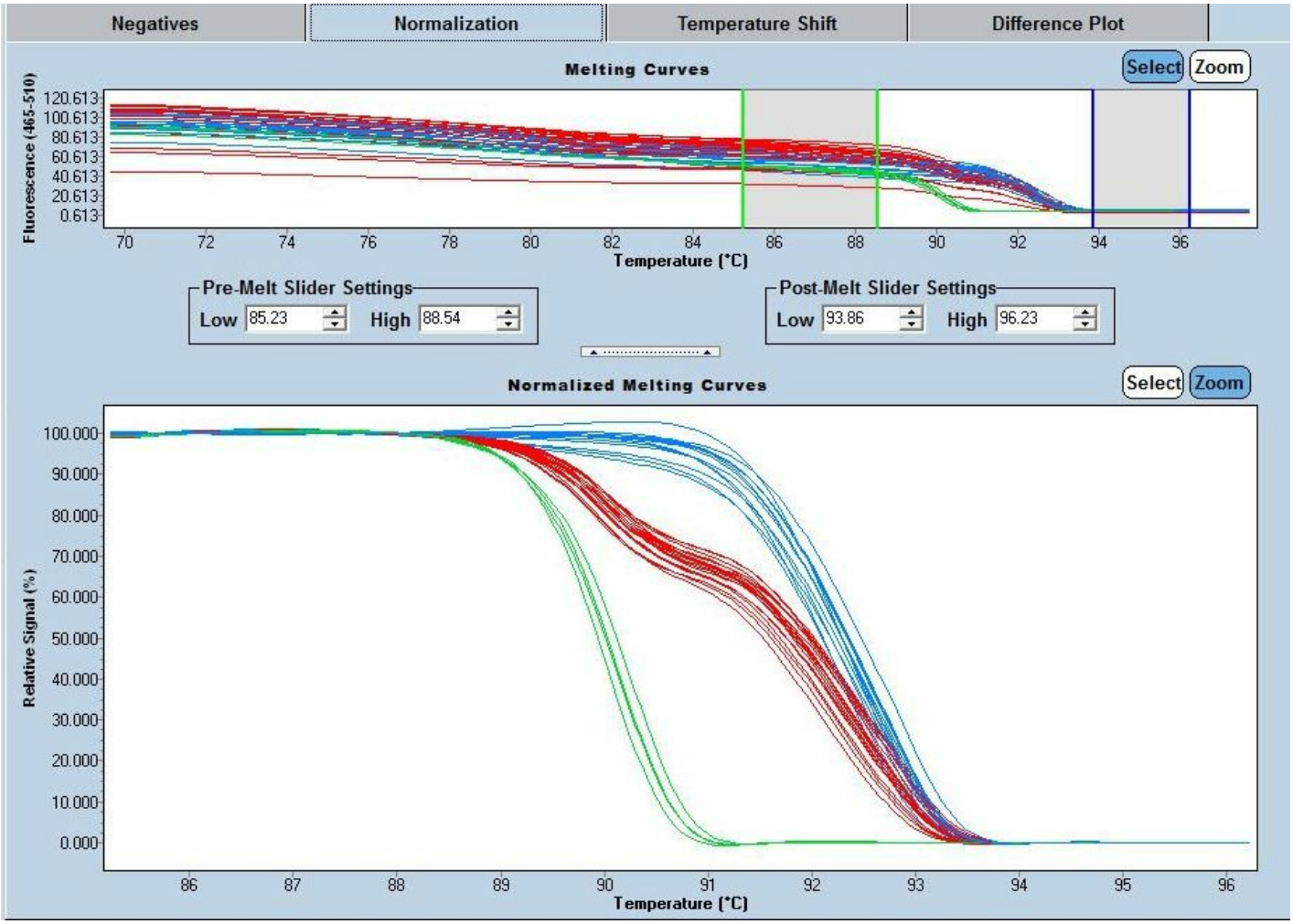
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR0837	0.50 uM
oIMR0838	0.50 uM
oIMR0839	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

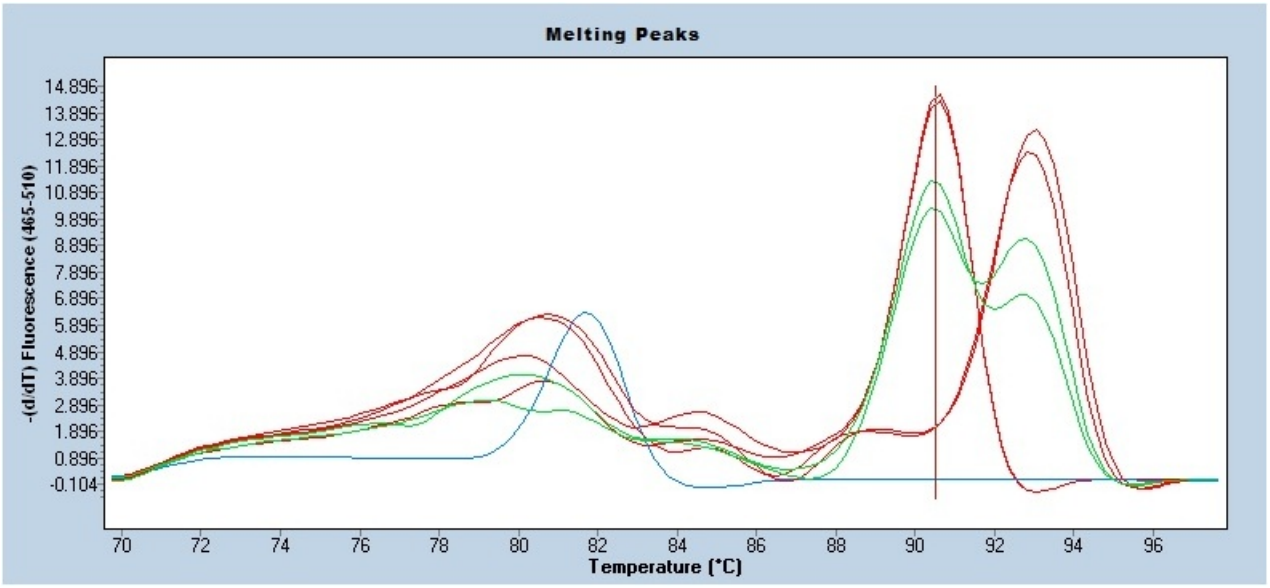
Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.


Negatives
Normalization
Temperature Shift
Difference Plot
Melting Curves
Select
Zoom
Fluorescence (465-510)
120.613
100.613
80.613
60.613
40.613
20.613
0.613
70 72 74 76 78 80 82 84 86 88 90 92 94 96
Temperature (°C)
Pre-Melt Slider Settings
Low 85.23
High 88.54
Post-Melt Slider Settings
Low 93.86
High 96.23
Normalized Melting Curves
Select
Zoom
Relative Signal (%)
100.000
90.000
80.000
70.000
60.000
50.000
40.000
30.000
20.000
10.000
0.000
86 87 88 89 90 91 92 93 94 95 96
Temperature (°C)


Melting Peaks
-(d/dT) Fluorescence (465-510)
14.896
13.896
12.896
11.896
10.896
9.896
8.896
7.896
6.896
5.896
4.896
3.896
2.896
1.896
0.896
-0.104
70 72 74 76 78 80 82 84 86 88 90 92 94 96
Temperature (°C)