C57BL/6-Tg(tetO-MAPT*A152T)L1Lms/J

Stock No: 028979
Protocol 19651: Standard PCR Assay - Tg(tetO-MAPT)
Version 1.0

Notes

This assay will NOT distinguish hemizygous from homozygous transgenic animals.

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

Transgene = 375 bp
Internal positive control = 200 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
17350		AGC TCG TTT AGT GAA CCG TCA		Transgene Forward	A	
28986		TCT TCC ATC ACT TCG AAC TCC		Transgene Reverse	A	
oIMR8744		CAA ATG TTG CTT GTC TGG TG		Internal Positive Control Forward	A	
oIMR8745		GTC AGT CGA GTG CAC AGT TT		Internal Positive Control Reverse	A	

Reaction A

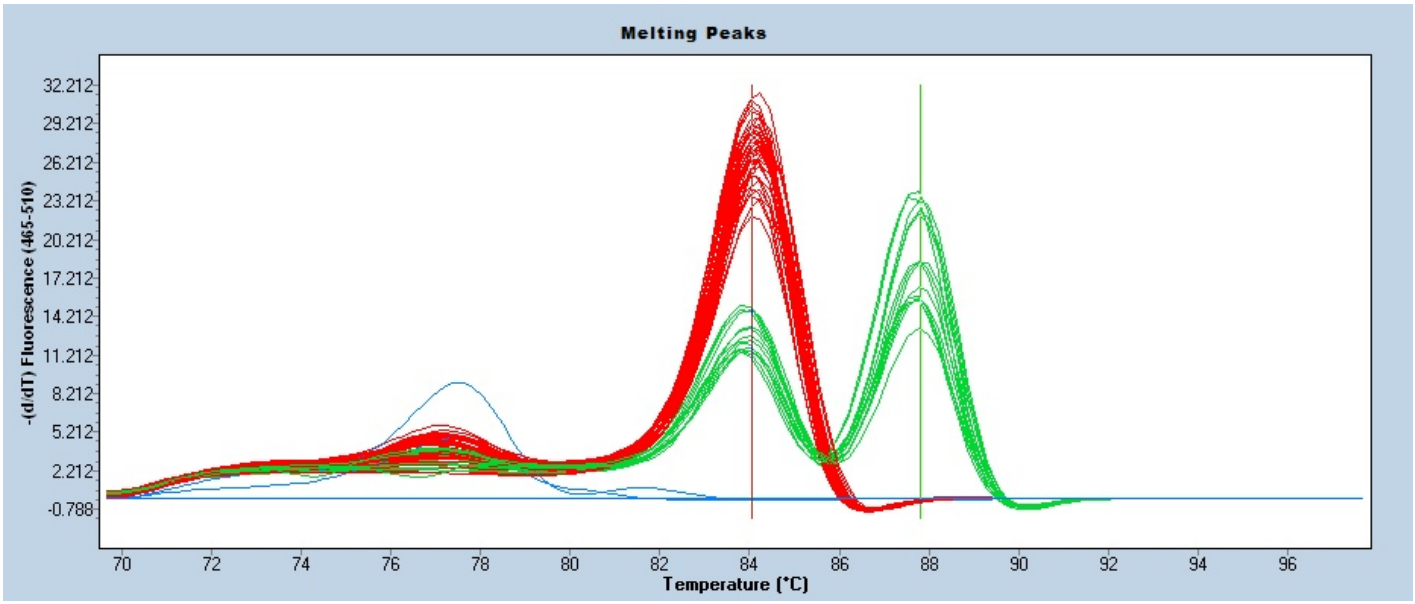
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
17350	0.50 uM
28986	0.50 uM
oIMR8744	0.50 uM
oIMR8745	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

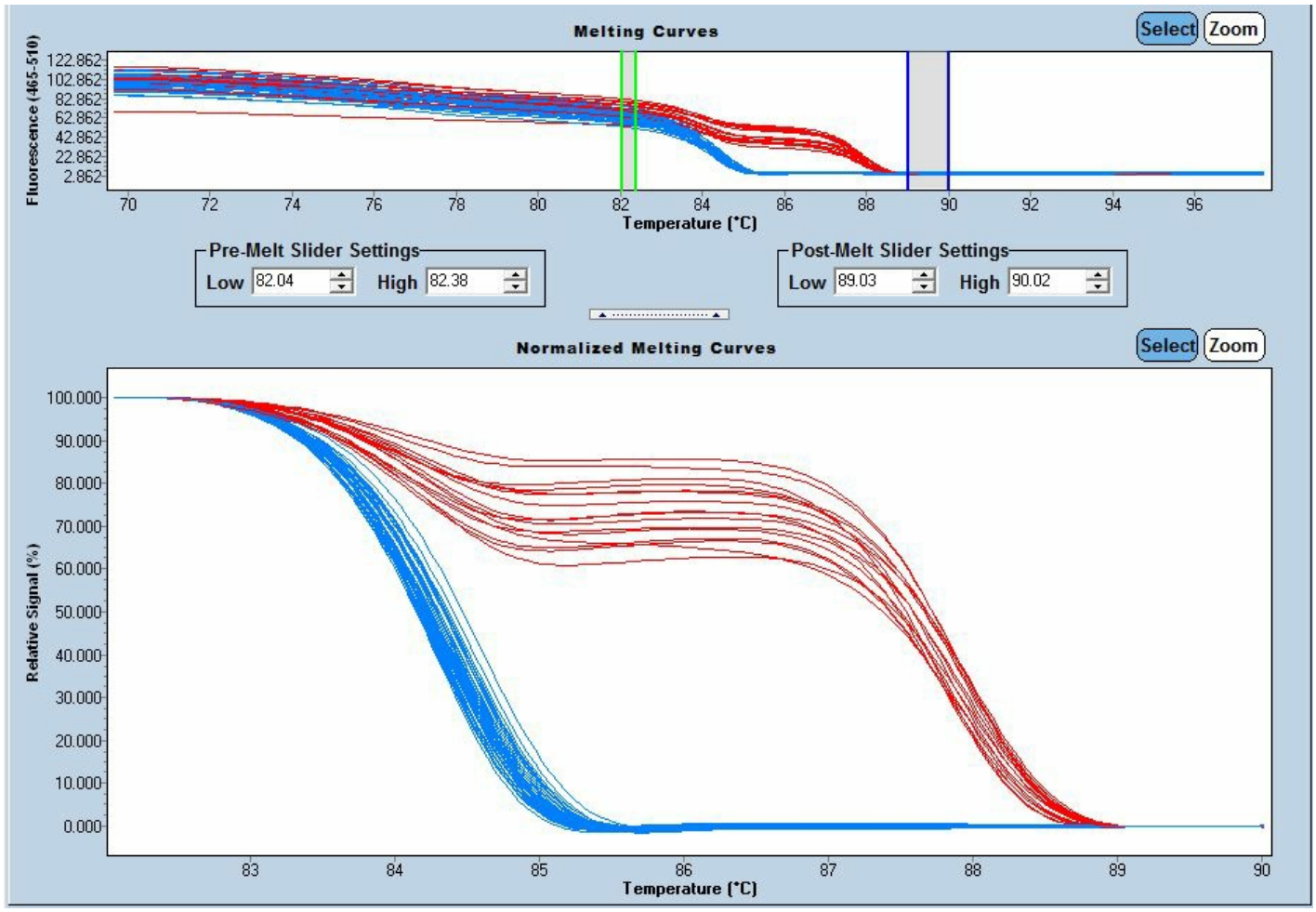
Cycling

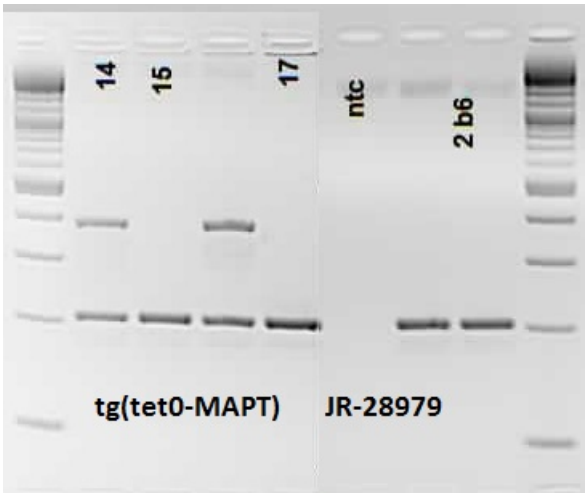
STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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.


Melting Peaks
-(d/dT) Fluorescence (465-510)
32.212
29.212
26.212
23.212
20.212
17.212
14.212
11.212
8.212
5.212
2.212
-0.788
70
72
74
76
78
80
82
84
86
88
90
92
94
96
Temperature (°C)


Melting Curves
Select
Zoom
Fluorescence (465-510)
122.862
102.862
82.862
62.862
42.862
22.862
2.862
70
72
74
76
78
80
82
84
86
88
90
92
94
96
Temperature (°C)
Pre-Melt Slider Settings
Low 82.04
High 82.38
Post-Melt Slider Settings
Low 89.03
High 90.02
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
83
84
85
86
87
88
89
90
Temperature (°C)


14
15
17
ntc
2 b6
tg(tet0-MAPT)
JR-28979