C57BL/6-*Dyrk1a*^{tm1Jdc}/J

Stock No: 027801
Protocol 29408: Standard PCR Assay - Dyrk1a<tm1Jdc>-Alternate 1
Version 1.2

Notes

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

Mutant = 232 bp
Heterozygote = 132 bp and 232 bp
Wild type = 132 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
25066		TAC CTG GAG AAG AGG GCA AG		Forward	A	
25067		GGC ATA ACT TGC ATA CAG TGG		Reverse	A	

Reaction A

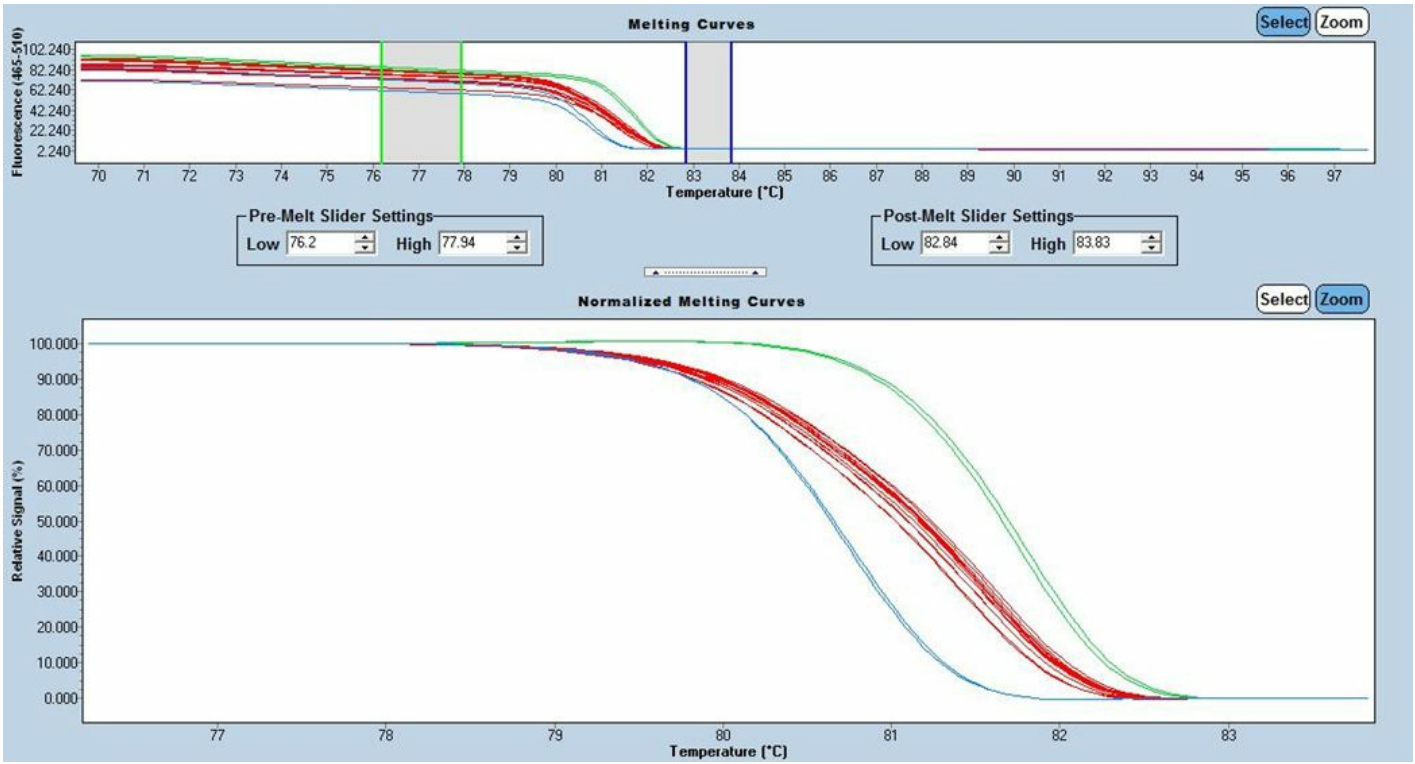
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTPS-kapa	0.26 mM
25066	0.50 uM
25067	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

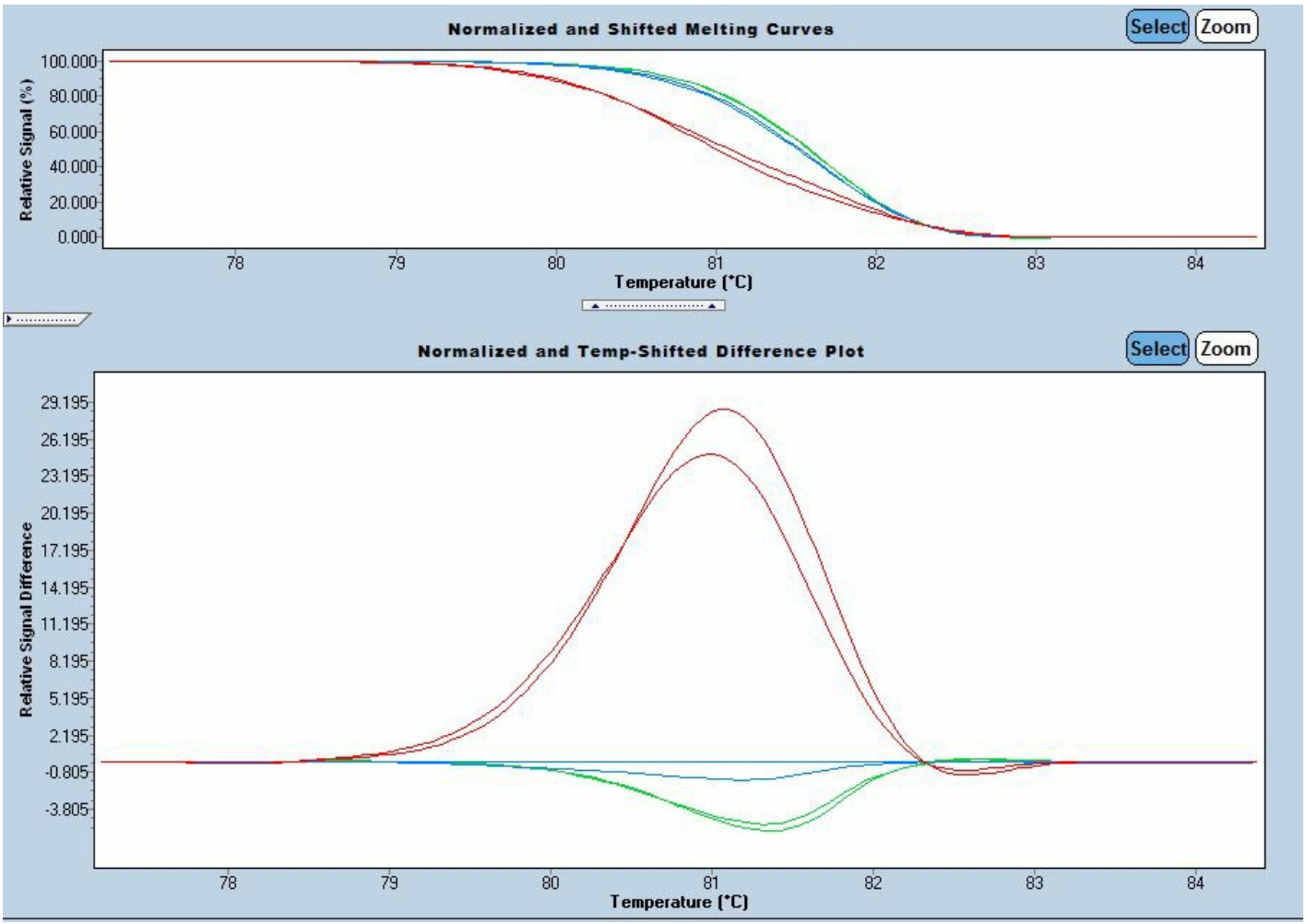
Cycling

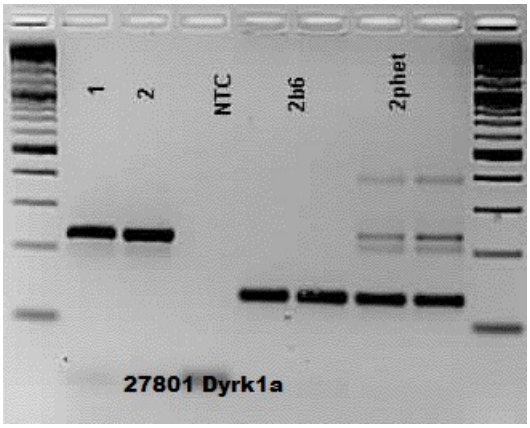
STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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 Curves
Select
Zoom
Fluorescence (465-510)
102.240
82.240
62.240
42.240
22.240
2.240
70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97
Temperature (°C)
Pre-Melt Slider Settings
Low 76.2
High 77.94
Post-Melt Slider Settings
Low 82.84
High 83.83
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
77 78 79 80 81 82 83
Temperature (°C)


Normalized and Shifted Melting Curves
Select
Zoom
Relative Signal (%)
100.000
80.000
60.000
40.000
20.000
0.000
78 79 80 81 82 83 84
Temperature (°C)
Normalized and Temp-Shifted Difference Plot
Select
Zoom
Relative Signal Difference
29.195
26.195
23.195
20.195
17.195
14.195
11.195
8.195
5.195
2.195
-0.805
-3.805
78 79 80 81 82 83 84
Temperature (°C)


1
2
NTC
2b6
2phet
27801 Dyrk1a