FVB.Cg-*Pde6b*$^{+}$ *Lrrk1*$^{tm1.1Mjff}$ *Tyr*$^{c\text{-}ch}$/J

Stock No: 022880
Protocol 31378: Standard PCR Assay - Generic Pde6b Alternate1
Version 1.0

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 = ~550 bp
Heterozygote = 318 bp and ~550 bp
Wild type = 318 bp

>chr5:108819374+108819691 318bp CATGTCCTACAGCCCCTCTC ACCATTTGCAAGGAAAGCAC

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
36758		CAT GTC CTA CAG CCC CTC TC		Common	A	
36759		ACC ATT TGC AAG GAA AGC AC		Wild type Reverse	A	
oIMR2093		AAG CTA GCT GCA GTA ACG CCA TTT		Mutant Reverse	A	

Reaction A

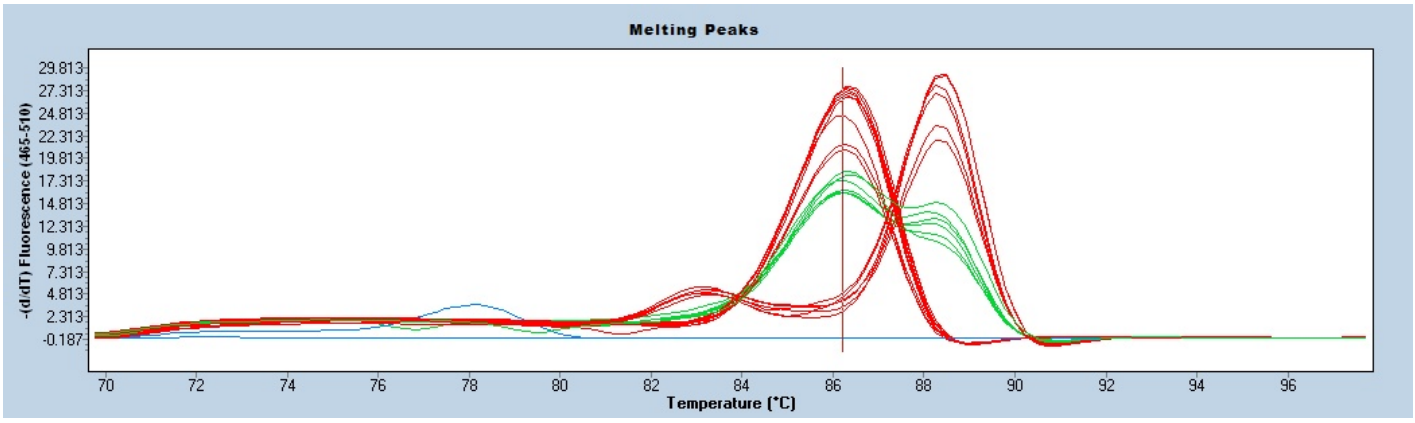
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
36758	0.50 uM
36759	0.50 uM
oIMR2093	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

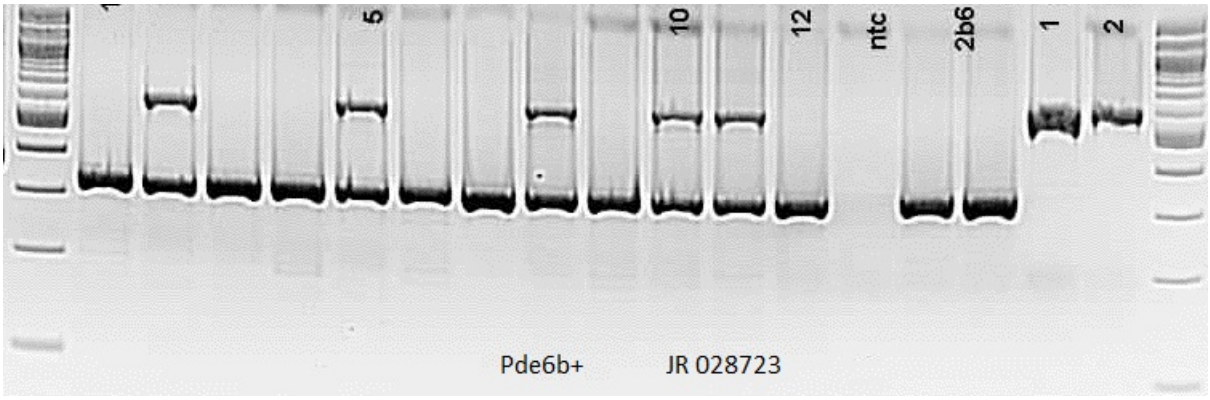
Cycling

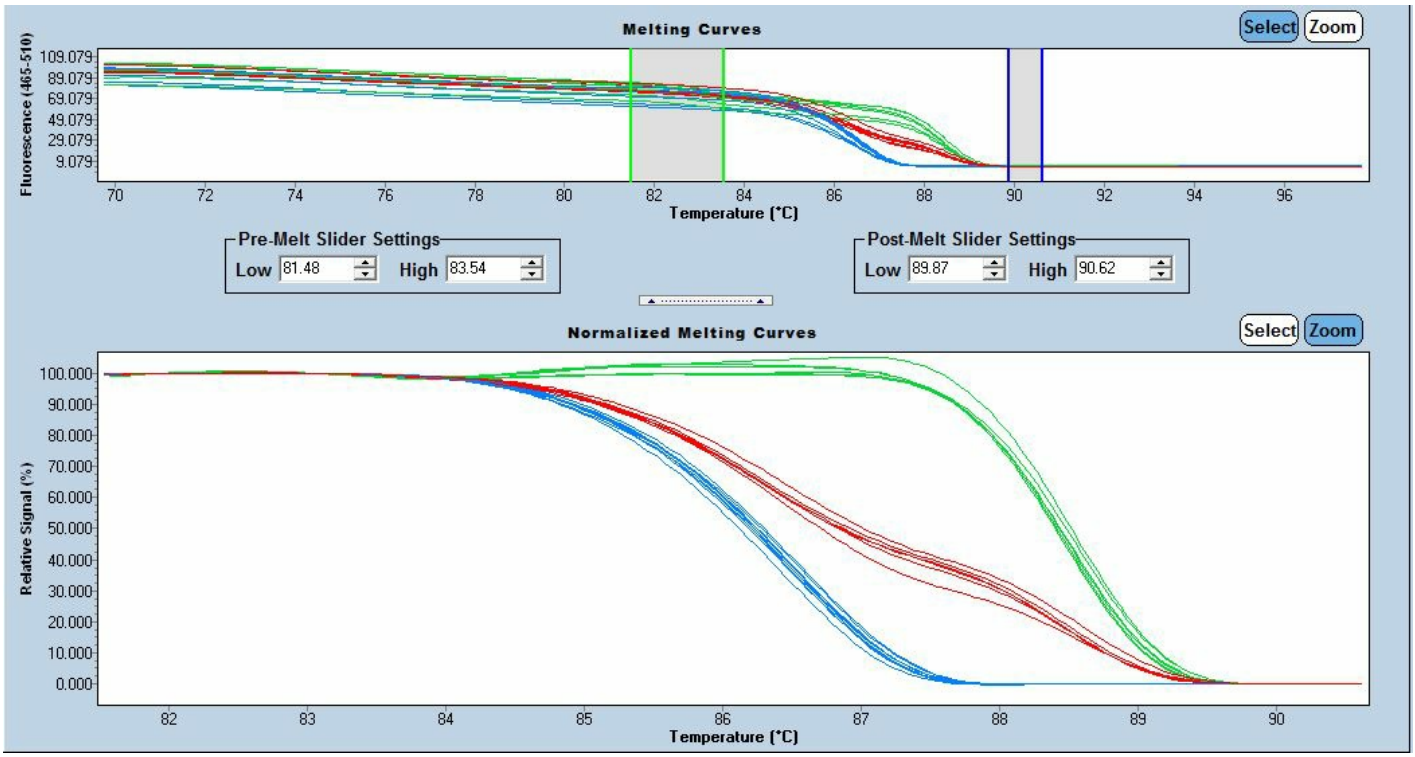
STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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)
29.813
27.313
24.813
22.313
19.813
17.313
14.813
12.313
9.813
7.313
4.813
2.313
-0.187
70
72
74
76
78
80
82
84
86
88
90
92
94
96
Temperature (°C)


5
10
12
ntc
2b6
1
2
Pde6b+
JR 028723


Melting Curves
Select
Zoom
Fluorescence (465-510)
109.079
89.079
69.079
49.079
29.079
9.079
70
72
74
76
78
80
82
84
86
88
90
92
94
96
Temperature (°C)
Pre-Melt Slider Settings
Low 81.48
High 83.54
Post-Melt Slider Settings
Low 89.87
High 90.62
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
82
83
84
85
86
87
88
89
90
Temperature (°C)