B6(SJL)-*Apoe*$^{tm1.1(APOE*4)Adiuj}$/J

Stock No: 027894
Protocol 28934: Standard PCR Assay - Apoe<tm1.1(APOE*4)Adiuj>
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 = 148 bp
Heterozygote = 148 bp and 224 bp
Wild type = 224 bp
Gel on 3% due to nonspecific bands close to wt.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
24463		AAT TTT TCC CTC CGC AGA CT		Common	A	
24464		ACA GCT GCT CAG GGC TAT TG		Wild type Reverse	A	
24465		AGG AGG TTG AGG TGA GGA TG		Mutant Reverse	A	

Reaction A

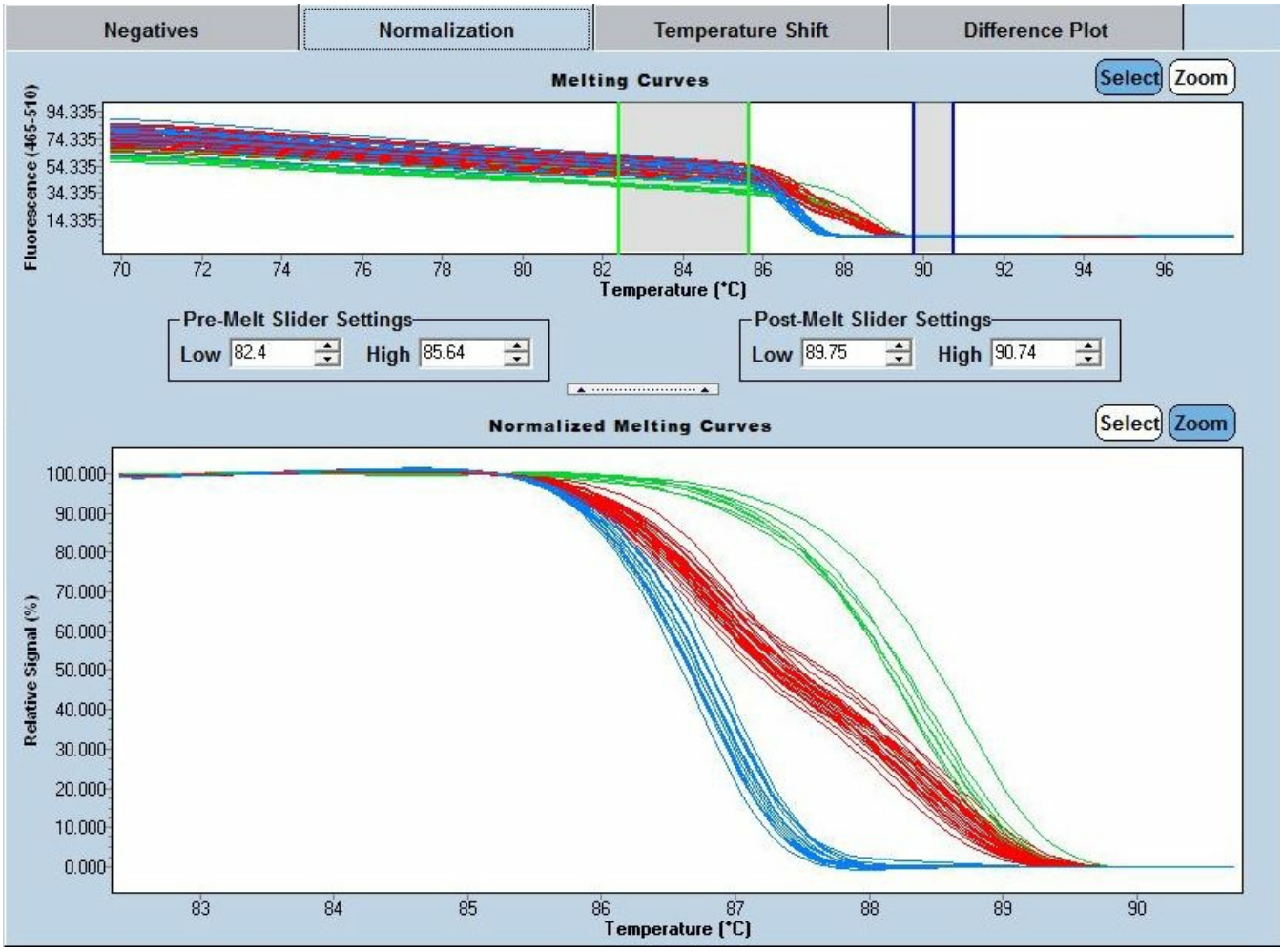
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
24463	0.50 uM
24464	0.50 uM
24465	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

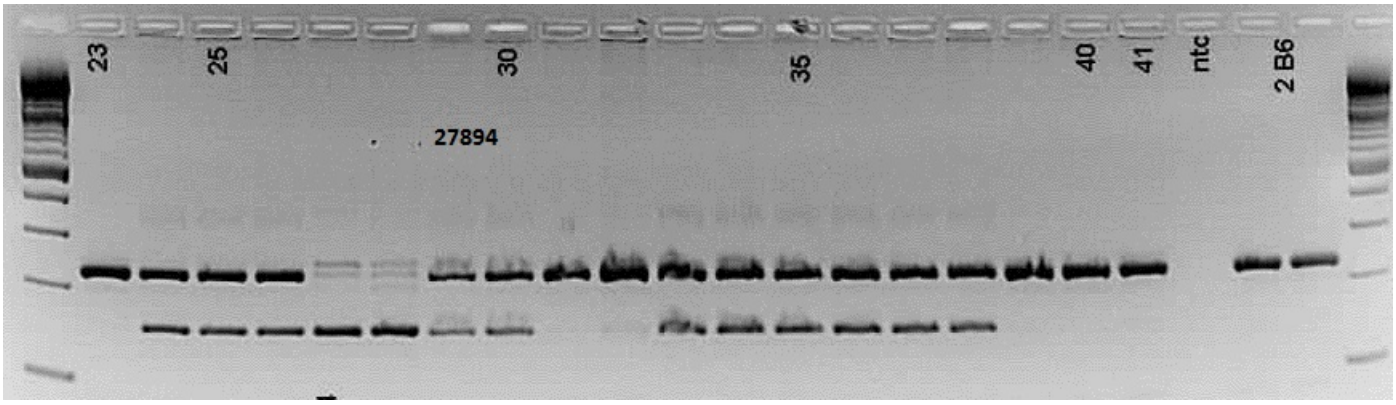
Cycling

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)
94.335
74.335
54.335
34.335
14.335
70
72
74
76
78
80
82
84
86
88
90
92
94
96
Temperature (°C)
Pre-Melt Slider Settings
Low 82.4
High 85.64
Post-Melt Slider Settings
Low 89.75
High 90.74
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)


23
25
30
35
40
41
ntc
2 B6
27894