B6.129P2-*H2-K1*^{tm1Bpe} *H2-D1*^{tm1Bpe}/DcrJ

Stock No: 019995
Protocol 19658: Standard PCR Assay - H2-D1<tm1Bpe> Alternate3
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 = 257 bp
Heterozygote = 160 bp and 257 bp
Wild type = 160 bp
Two protocols are provided because of different band sizes.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
28995		CTG GTT GCC CCA GCT TC		Wild type Forward	A	
28996		CGA TTT ATA TTC CTC CAG GGT A		Common	A	
28997		GAG GAC ATT CCA ATC ATA GGC		Mutant Forward	A	

Reaction A

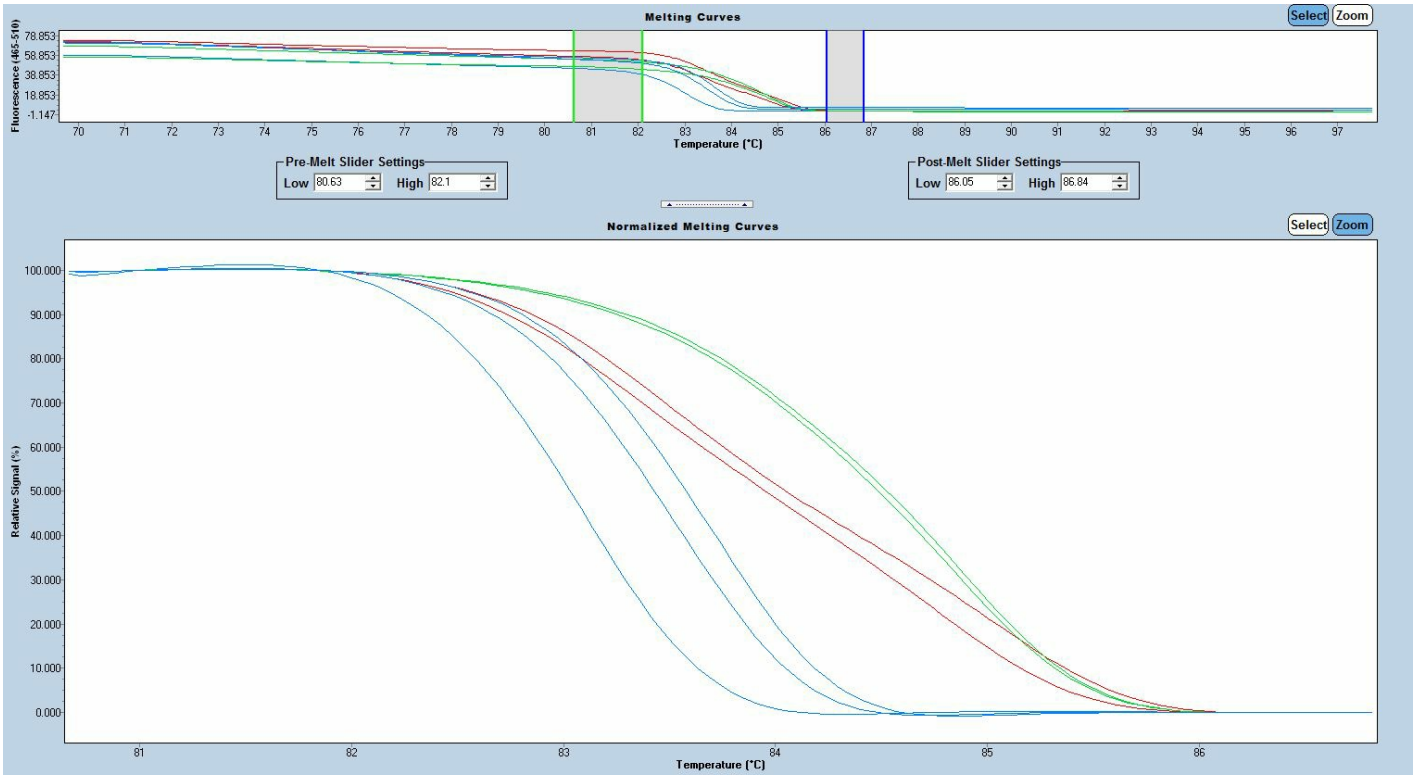
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
28995	0.50 uM
28996	0.50 uM
28997	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

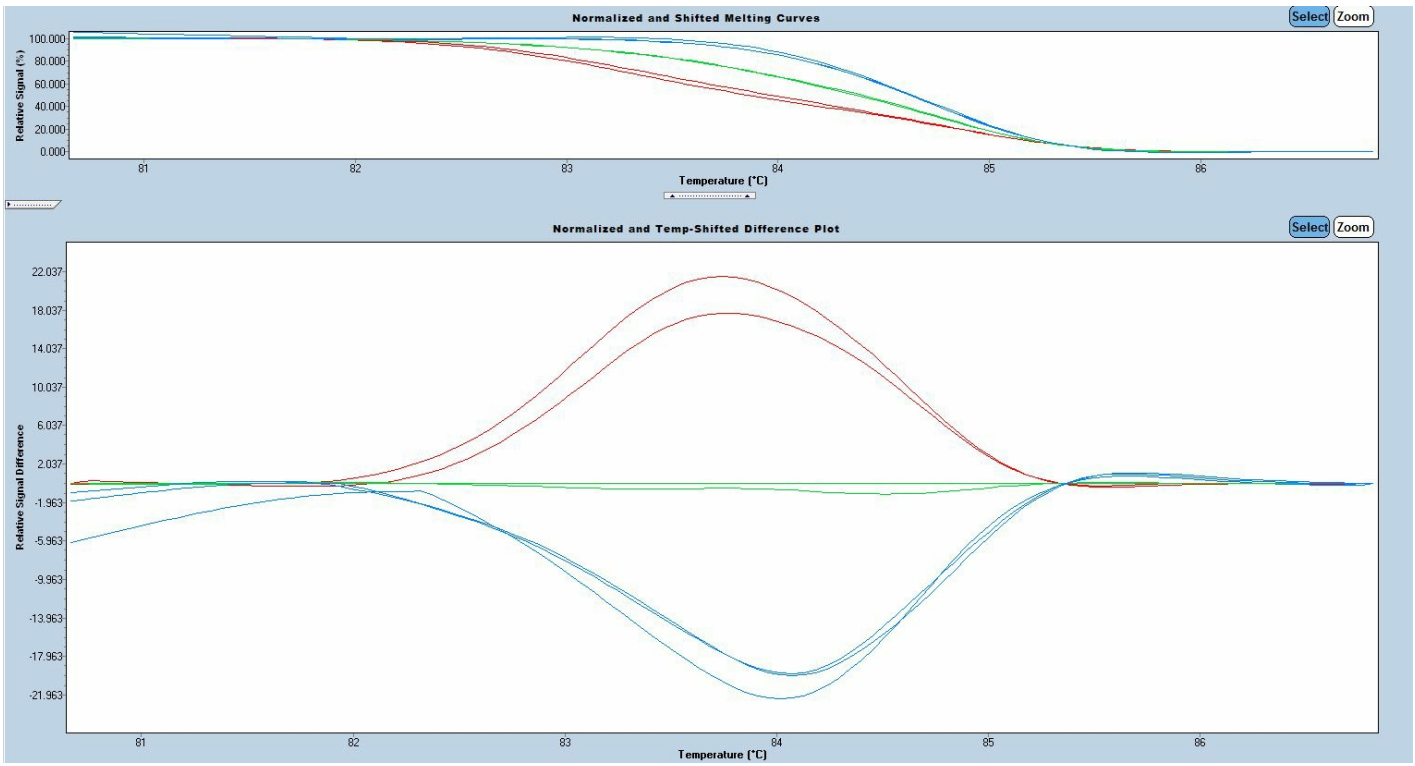
Cycling

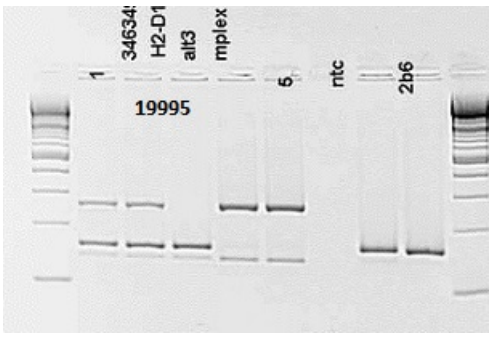
STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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)
78.853
58.853
38.853
18.853
-1.147
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 80.63
High 82.1
Post-Melt Slider Settings
Low 86.05
High 86.84
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
81 82 83 84 85 86
Temperature (°C)


Normalized and Shifted Melting Curves
Select
Zoom
Relative Signal (%)
100.000
80.000
60.000
40.000
20.000
0.000
81 82 83 84 85 86
Temperature (°C)
Normalized and Temp-Shifted Difference Plot
Select
Zoom
Relative Signal Difference
22.037
18.037
14.037
10.037
6.037
2.037
-1.963
-5.963
-9.963
-13.963
-17.963
-21.963
81 82 83 84 85 86
Temperature (°C)


1
34634
H2-D1
alt3
mplex
6
ntc
2b6
19995