B6;129-*Vmn1r51*^{tm1Dlc}/J

Stock No: 025197
Protocol 29681: Standard PCR Assay - Generic LacZ Melt Curve Analysis
Version 4.2

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

Transgene Tm = 87°C +/- 1.0°C
Internal positive control Tm = 82.1°C+/- 1.0°C

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR0040		CGT GGC CTG ATT CAT TCC		Mutant Reverse	A	
oIMR3054		ATC CTC TGC ATG GTC AGG TC		Mutant Forward	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

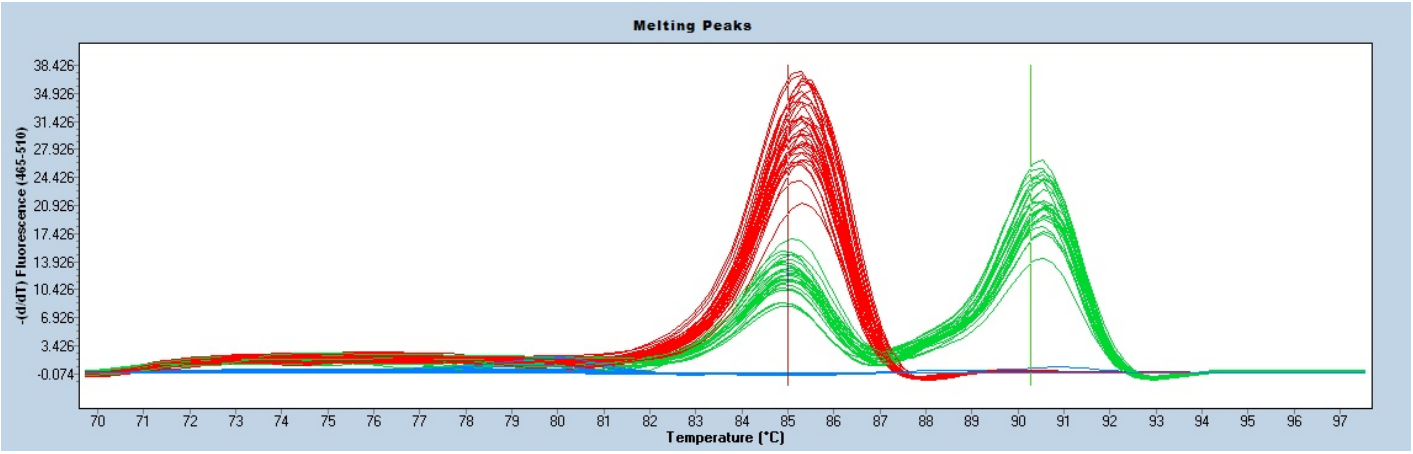
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR0040	0.50 uM
oIMR3054	0.50 uM
oIMR8744	0.50 uM
oIMR8745	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

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)
38.426
34.926
31.426
27.926
24.426
20.926
17.426
13.926
10.426
6.926
3.426
-0.074
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)