B6(129S4)-Et(cre/ERT2)296Rdav/J

Stock No: 009577
Protocol 29081: Standard PCR Assay - Generic Cre Melt Curve Analysis
Version 2.3

Notes

Melting curve analysis is done using a Roche Light Cycler 480.

This assay will NOT distinguish hemizygous from homozygous transgenic animals.

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 = 83°C +/- 1.0°C
Internal positive control Tm = 86.5°C+/- 1.0°C

Transgene = ~100 bp
Internal positive control = 324 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR1084		GCG GTC TGG CAG TAA AAA CTA TC		Transgene Forward	A	Cre F
oIMR1085		GTG AAA CAG CAT TGC TGT CAC TT		Transgene Reverse	A	Cre R
oIMR7338		CTA GGC CAC AGA ATT GAA AGA TCT		Internal Positive Control Forward	A	
oIMR7339		GTA GGT GGA AAT TCT AGC ATC ATC C		Internal Positive Control Reverse	A	

Reaction A

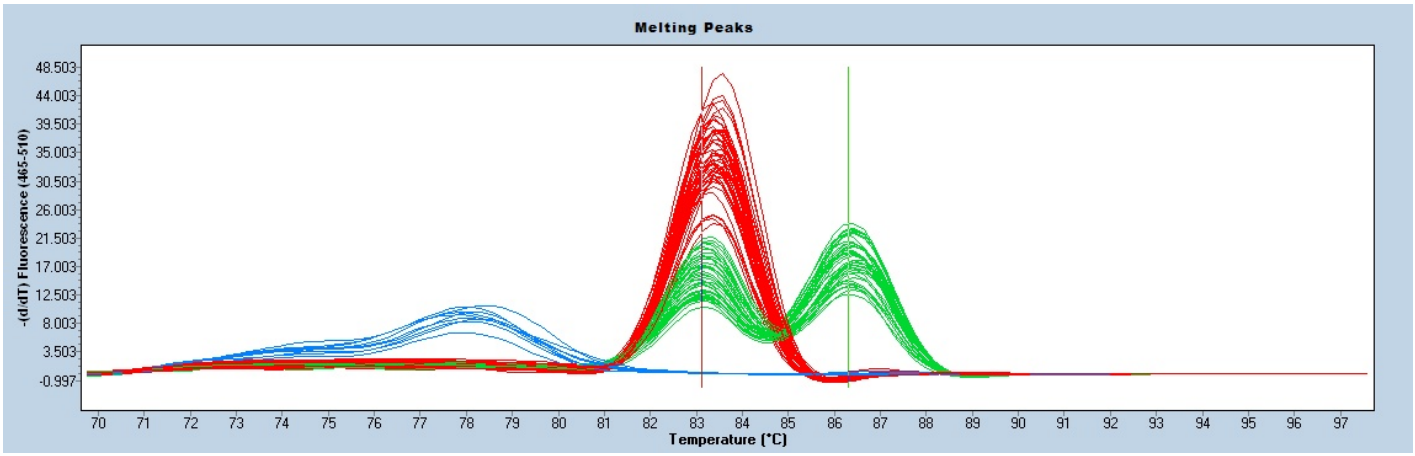
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR1084	0.50 uM
oIMR1085	0.50 uM
oIMR7338	0.50 uM
oIMR7339	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

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)
48.503
44.003
39.503
35.003
30.503
26.003
21.503
17.003
12.503
8.003
3.503
-0.997
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)