B6(Cg)-*Sgta*$^{tm1.1Drmcr}$/J

Stock No: 029290
Protocol 29086: Standard PCR Assay - Generic Neo
Version 2.3

Notes

Melting curve analysis is performed using a Roche Light Cycler 480.
PLEASE NOTE: This assay will distinguish heterozygous from homozygous Tcrd<tm1Mom> mutant mice.

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 Tm = 90°C +/- 1.0°C
Wild type Tm = 82°C+/- 1.0°C
Mut = 280 bp
IPC = 206 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR6916		CTT GGG TGG AGA GGC TAT TC		Mutant Forward	A	Neo
oIMR6917		AGG TGA GAT GAC AGG AGA TC		Mutant Reverse	A	Neo
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

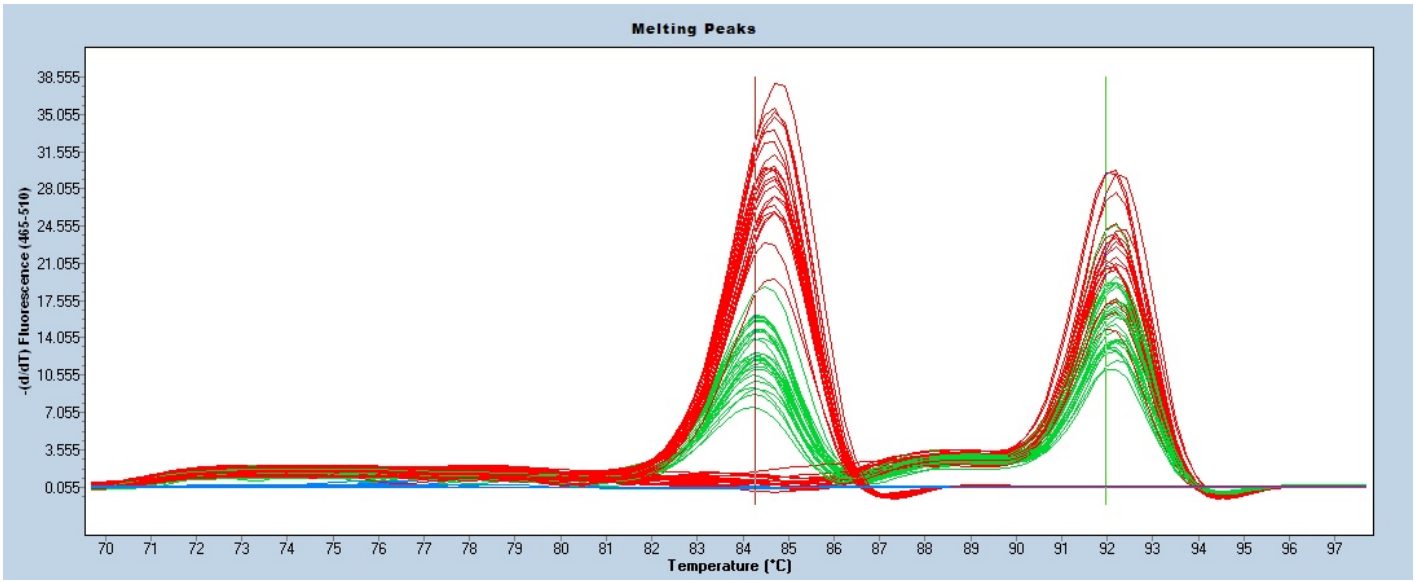
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR6916	0.50 uM
oIMR6917	0.50 uM
oIMR8744	0.50 uM
oIMR8745	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

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)
38.555
35.055
31.555
28.055
24.555
21.055
17.555
14.055
10.555
7.055
3.555
0.055
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)