C57BL/6-Tg(THY1-SNCA)1Sud/J

Stock No: 008389
Protocol 24362: Standard PCR Assay - Smn1<tm1Msd>
Version 5.2

Notes

validation/LC Conversion data attached to rec 169358

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

Wt=410 bp
Mut=110 bp
Two public protocols are supplied- different band sizes.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR3439		TTT TCT CCC TCT TCA GAG TGA T		Common	A	
oIMR3440		CTG TTT CAA GGG AGT TGT GGC		Wild type Reverse	A	
oIMR7210		GGT AAC GCC AGG GTT TTC C		Mutant Reverse	A	LacZ

Reaction A

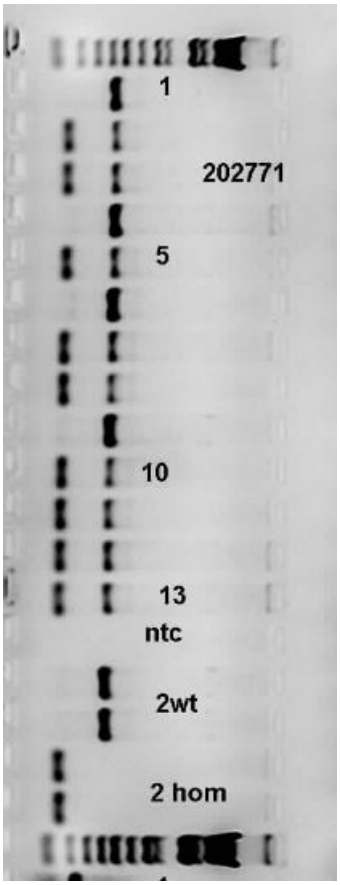
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR3439	0.50 uM
oIMR3440	0.50 uM
oIMR7210	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

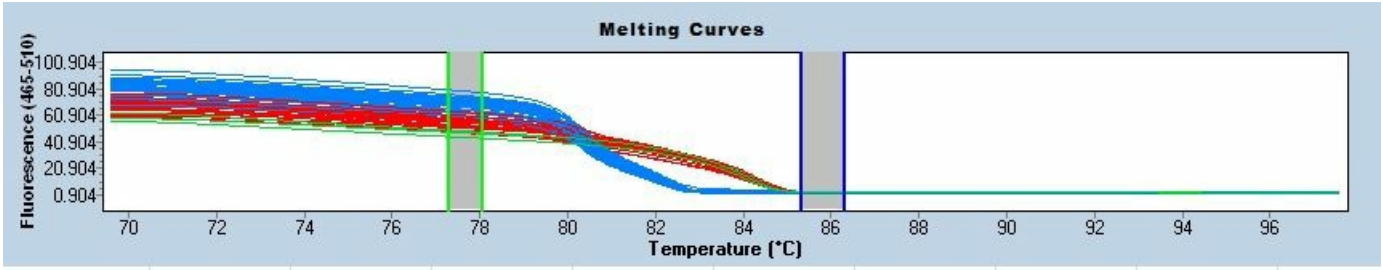
Cycling

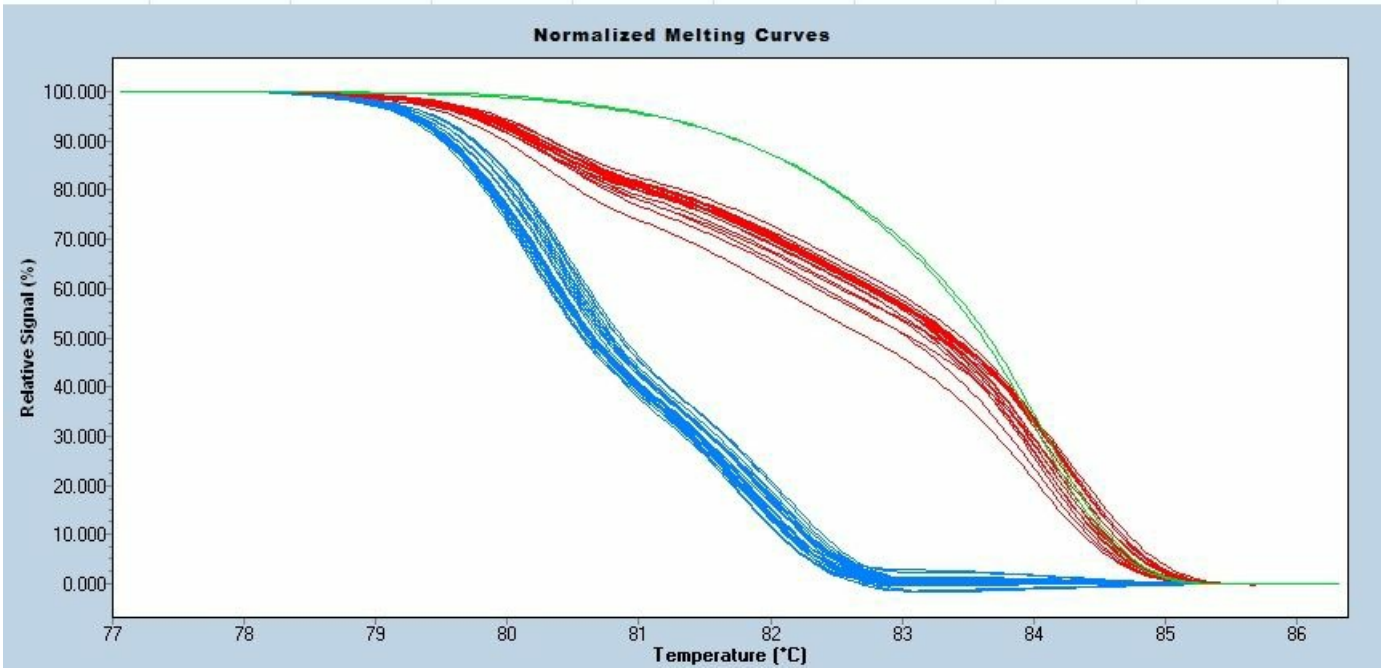
STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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.


1
202771
5
10
13
ntc
2wt
2 hom


Melting Curves
Fluorescence (465-510)
100.904
80.904
60.904
40.904
20.904
0.904
70
72
74
76
78
80
82
84
86
88
90
92
94
96
Temperature (°C)


Normalized Melting Curves
Relative Signal (%)
100.000
90.000
80.000
70.000
60.000
50.000
40.000
30.000
20.000
10.000
0.000
77
78
79
80
81
82
83
84
85
86
Temperature (°C)