STOCK *Gt(ROSA)26Sor$^{tm5(ACTB-tTA)Luo}$ Igs7$^{tm94.1(tetO-GCaMP6s)Hze}$*/J

Stock No: 024112
Protocol 28732: Standard PCR Assay - Igs7<tm*(tetO-GCaMP6f)Hze>-Alternate 1
Version 2.2

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

Mutant = 330 bp
Heterozygote = 330 bp and 468 bp
Wild type = 468 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
19693		TCC CCT GGC ACA ACG TAA G		Mutant Reverse	A	chicken HS4 chromatin insulator
22159		GTG TAG CCC TGG CTT TTC TG		Common	A	
22330		GAA CTC ACA GTG GCC AGT CA		Wild type Reverse	A	

Reaction A

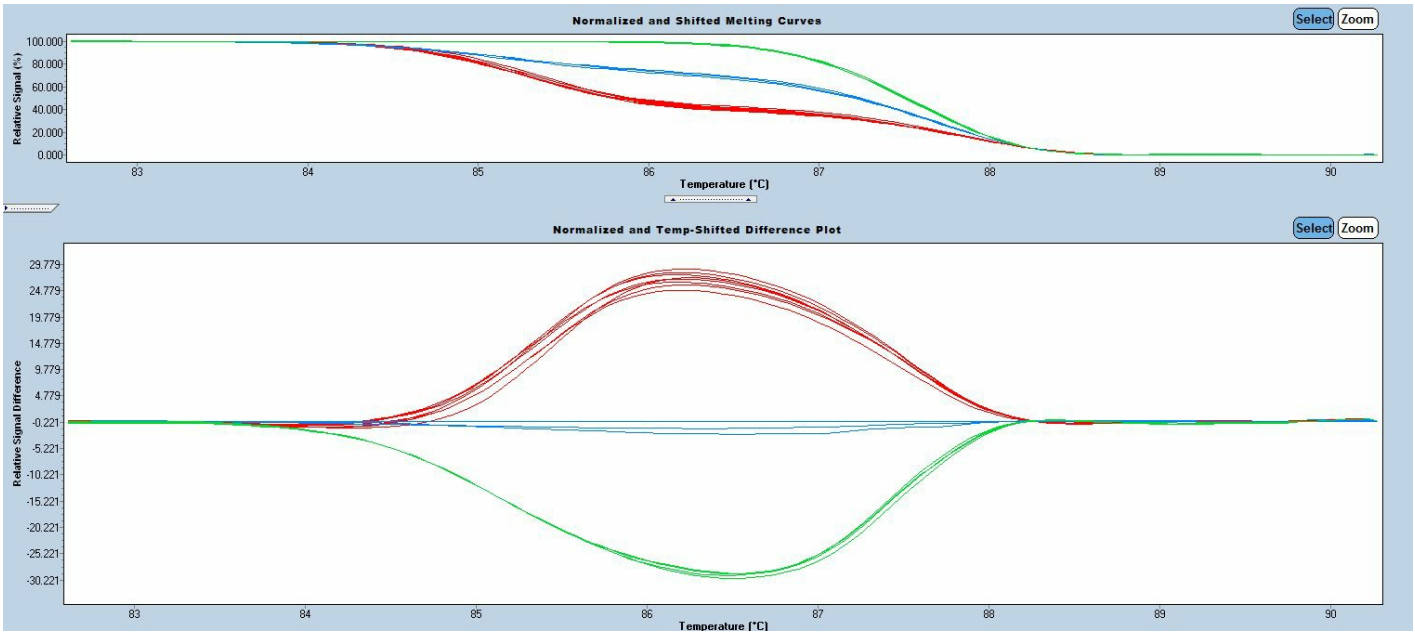
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
19693	0.50 uM
22159	0.50 uM
22330	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

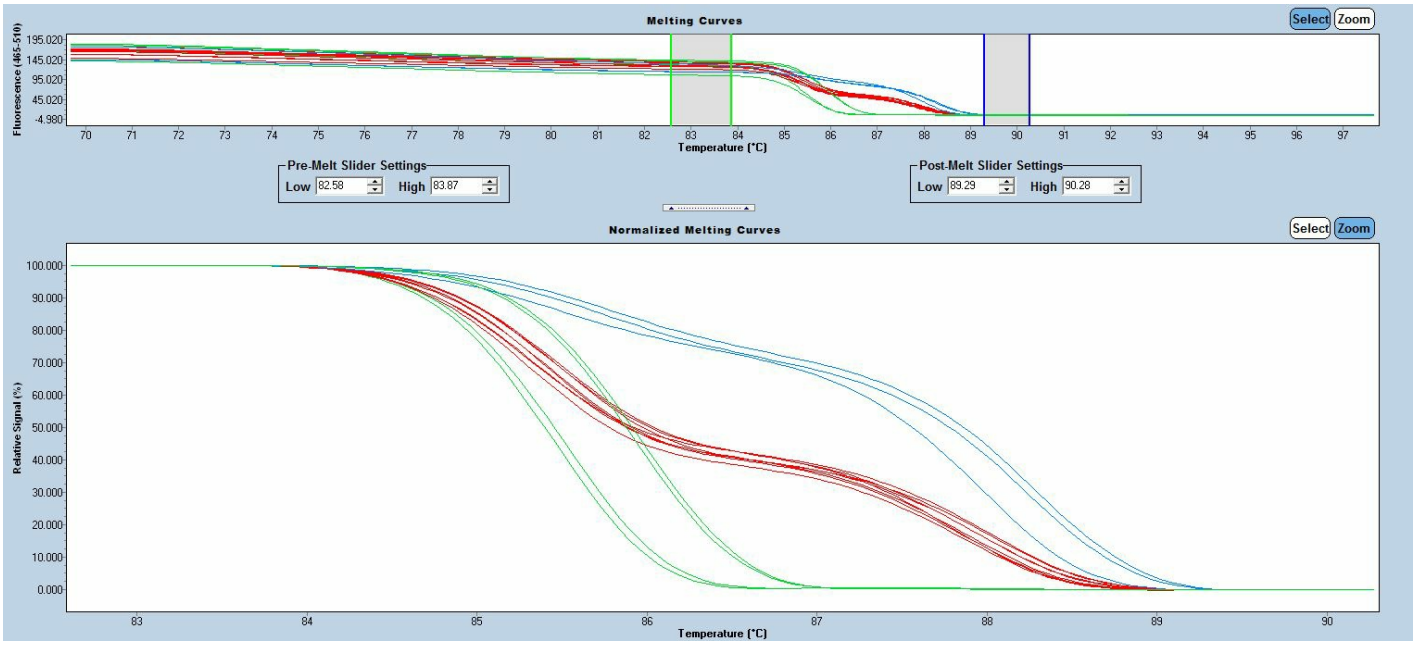
Cycling

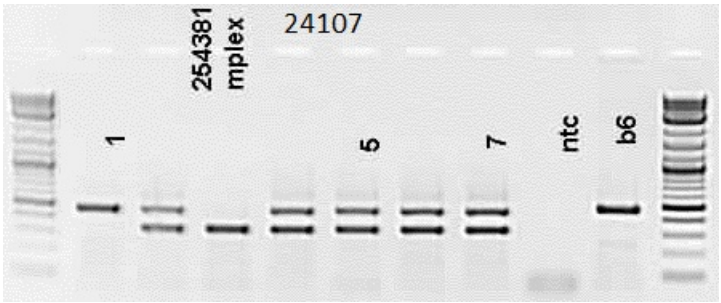
STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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.


Normalized and Shifted Melting Curves
Select
Zoom
Relative Signal (%)
100.000
80.000
60.000
40.000
20.000
0.000
83
84
85
86
87
88
89
90
Temperature (°C)
Normalized and Temp-Shifted Difference Plot
Select
Zoom
Relative Signal Difference
29.779
24.779
19.779
14.779
9.779
4.779
-0.221
-5.221
-10.221
-15.221
-20.221
-25.221
-30.221
83
84
85
86
87
88
89
90
Temperature (°C)


Melting Curves
Select
Zoom
Fluorescence (465-510)
195.020
145.020
95.020
45.020
-4.980
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 82.58
High 83.87
Post-Melt Slider Settings
Low 89.29
High 90.28
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
83
84
85
86
87
88
89
90
Temperature (°C)


24107
254381
mplex
1
5
7
ntc
b6