STOCK *Gli1*$^{tm3(cre/ERT2)Alj}$/J

Stock No: 007913
Protocol 26533: Standard PCR Assay - Gli1<tm3(cre/ERT2)Alj>alternate3
Version 3.2

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 = ~100 bp
Heterozygote = ~100 bp and 136 bp
Wild type = 136 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	
oIMR1085		GTG AAA CAG CAT TGC TGT CAC TT		Transgene Reverse	A	
oIMR7888		GGG ATC TGT GCC TGA AAC TG		Wild type Forward	A	
oIMR7889		CTT GTG GTG GAG TCA TTG GA		Wild type Reverse	A	

Reaction A

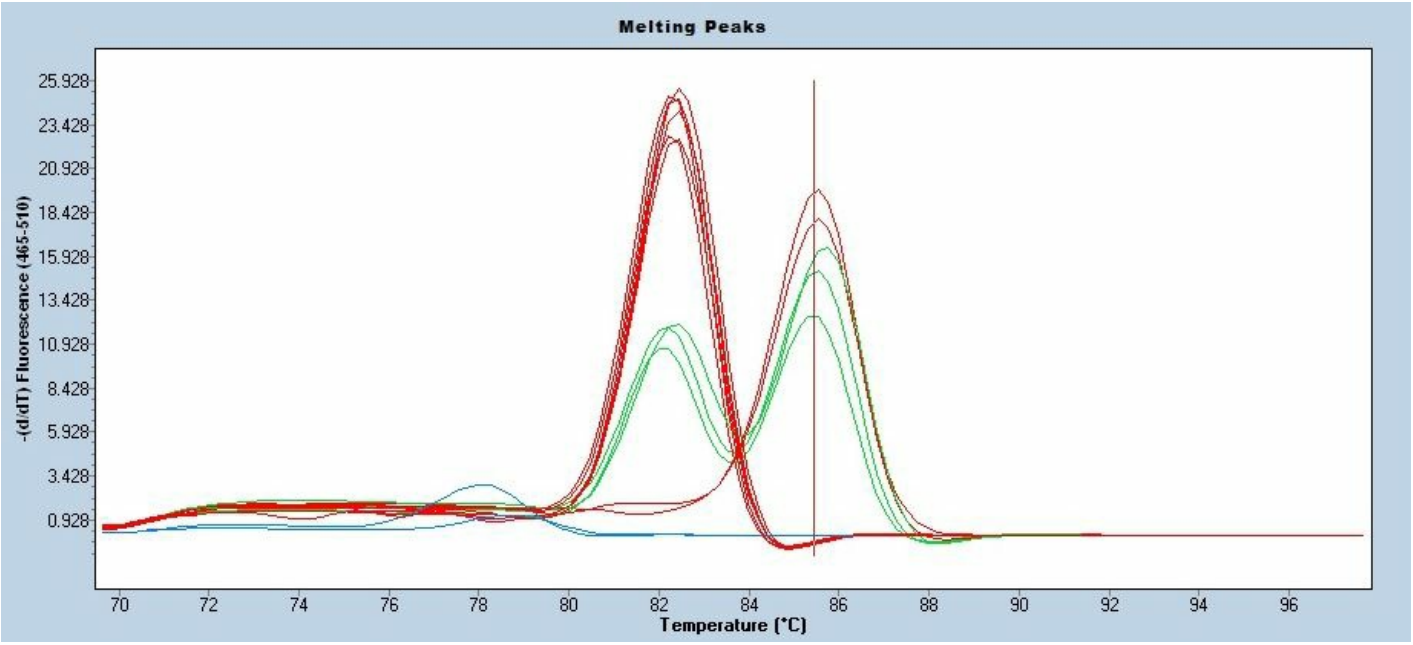
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR1084	0.50 uM
oIMR1085	0.50 uM
oIMR7888	0.50 uM
oIMR7889	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

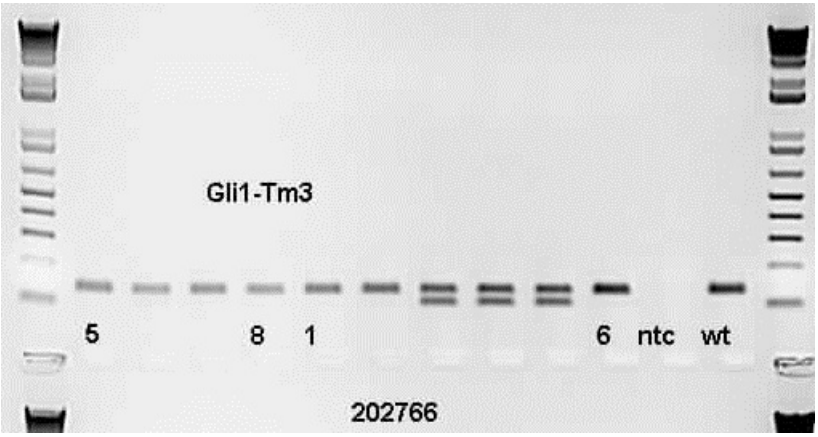
Cycling

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)
25.928
23.428
20.928
18.428
15.928
13.428
10.928
8.428
5.928
3.428
0.928
70
72
74
76
78
80
82
84
86
88
90
92
94
96
Temperature (°C)


Gli1-Tm3
5
8
1
6
ntc
wt
202766