B6.FVB-Tg(Acta2-cre)1Rkl/J

Stock No: 029925
Protocol 31980: Standard PCR Assay - Tg(Acta2-cre)1Rkl-Alternate 2
Version 1.0

Notes

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 = ~300 bp
Internal positive control = 521 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
15495		ACA TGT CCA TCA GGT TCT TGC		Transgene Reverse	A	
31704		AGT GGC CTC TTC CAG AAA TG		Internal Positive Control Forward	A	
31705		TGC GAC TGT GTC TGA TTT CC		Internal Positive Control Reverse	A	
38112		GGT GTT AGT TGA GAA CTG TGG AG		Transgene Forward	A	

Reaction A

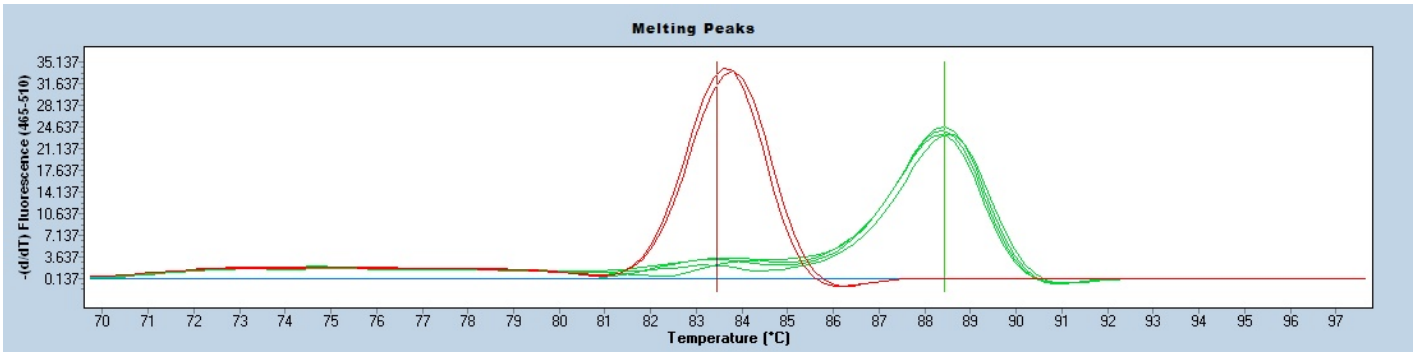
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
15495	0.50 uM
31704	0.50 uM
31705	0.50 uM
38112	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

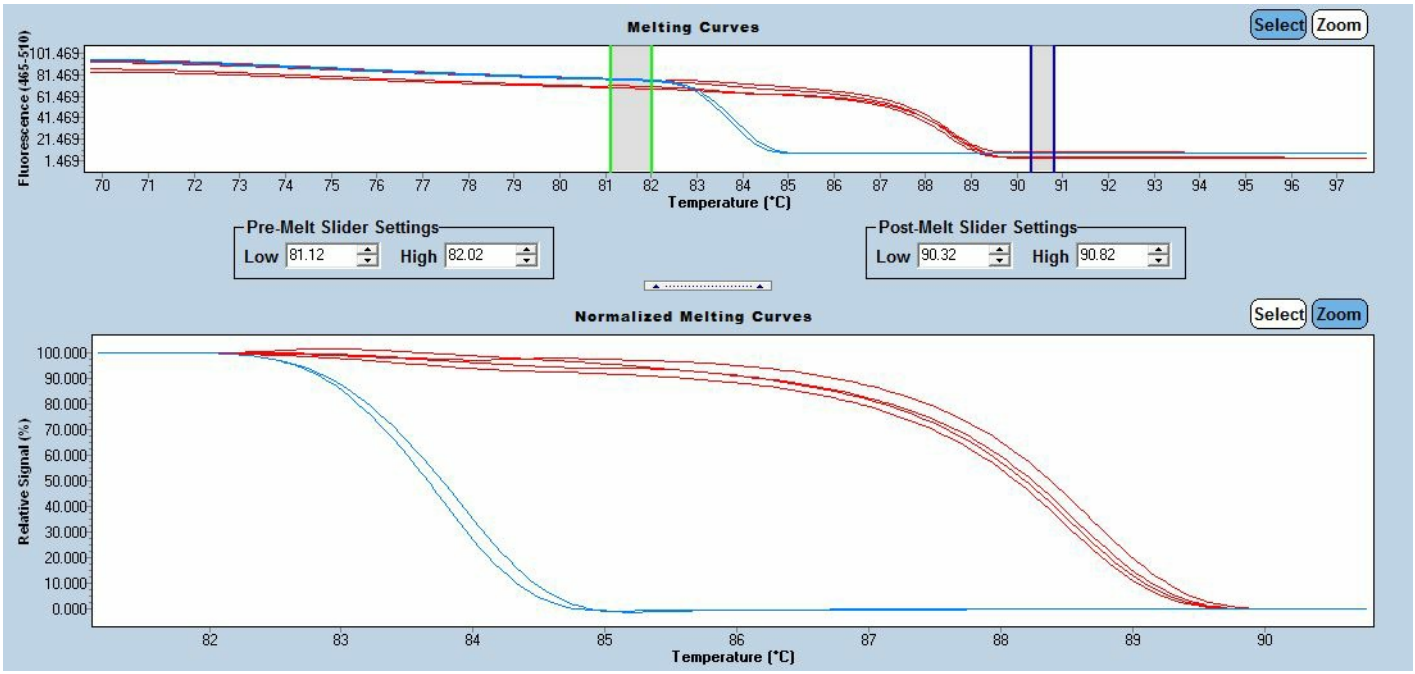
Cycling

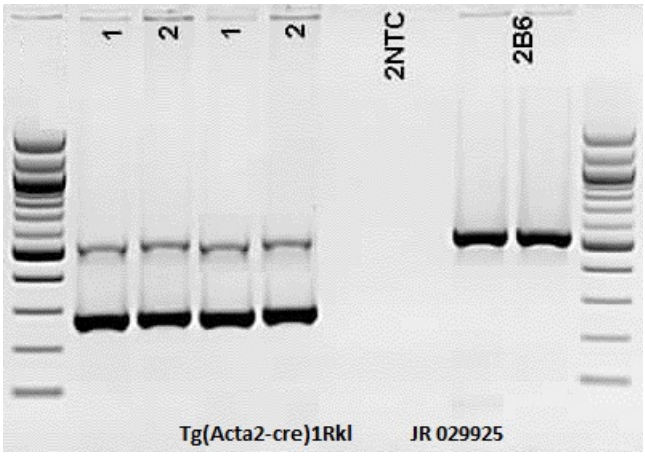
STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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)
35.137
31.637
28.137
24.637
21.137
17.637
14.137
10.637
7.137
3.637
0.137
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)


Melting Curves
Select
Zoom
Fluorescence (465-510)
101.469
81.469
61.469
41.469
21.469
1.469
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 81.12
High 82.02
Post-Melt Slider Settings
Low 90.32
High 90.82
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
82 83 84 85 86 87 88 89 90
Temperature (°C)


1
2
1
2
2NTC
2B6
Tg(Acta2-cre)1Rkl
JR 029925