B6.Cg-Tg(Gfap-TK)7.1Mvs/J

Stock No: 005698
Protocol 20363: Standard PCR Assay - Tg(Gfap-TK)7.1Mvs Alternate2
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 = 290 bp
Internal positive control = 190 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
25783		GTC AAC GGG GGA CAT AAA AG		Internal Positive Control Forward	A	
25784		CTT CTA AGA GTT AAA CAA TAC CAG CTT		Internal Positive Control Reverse	A	
30290		GGG GCC TCG GTC CTA GT		Transgene Forward	A	
30291		GAT GGC AGG GGT ACG AAG		Transgene Reverse	A	

Reaction A

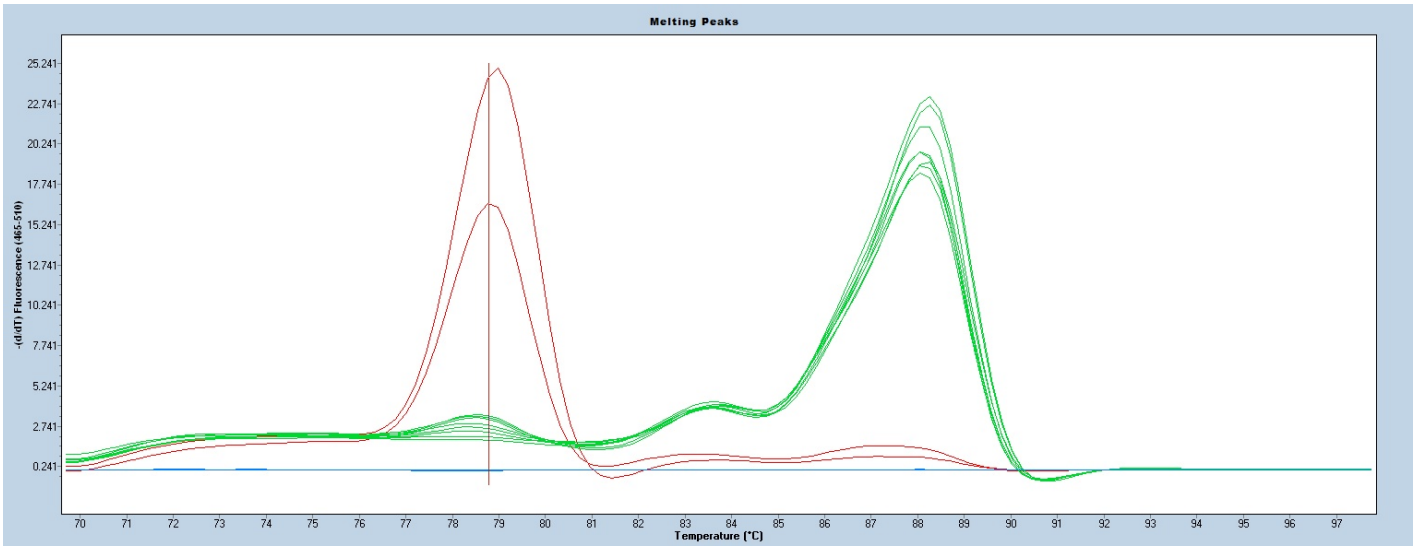
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
25783	0.50 uM
25784	0.50 uM
30290	0.50 uM
30291	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

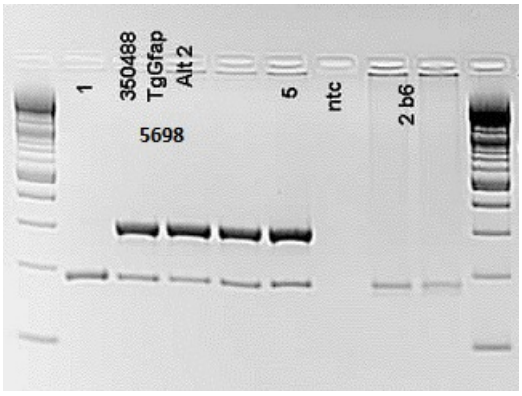
Cycling

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)
25.241
22.741
20.241
17.741
15.241
12.741
10.241
7.741
5.241
2.741
0.241
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)


1
350488 TgGfap Alt 2
5698
5
ntc
2 b6