129S/Sv-*Kras*^{tm4Tyj}/J

Stock No: 008180
Protocol 29388: Standard PCR Assay - Kras<tm4Tyj> Alt1
Version 1.2

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 (embryonic lethal) = 100 bp
Heterozygote = 100 bp and 250 bp
Wild type = 250 bp

After Cre-recombination a bandsize of ~300 is expected.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
22907		TGT CTT TCC CCA GCA CAG T		Wild type Forward	A	
22908		CTG CAT AGT ACG CTA TAC CCT GT		Common	A	
oIMR9592		GCA GGT CGA GGG ACC TAA TA		Mutant Forward	A	

Reaction A

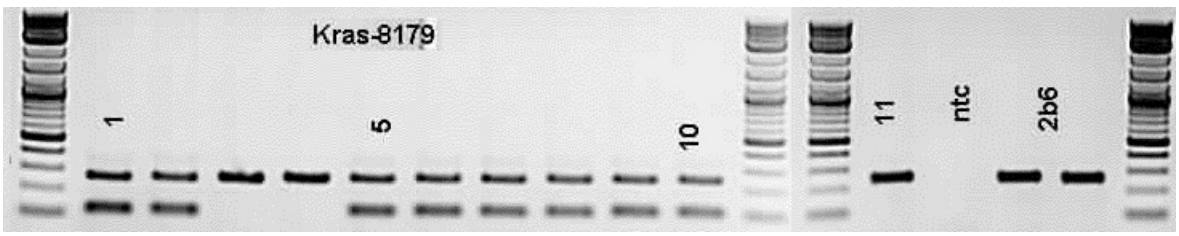
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
22907	0.50 uM
22908	0.50 uM
oIMR9592	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

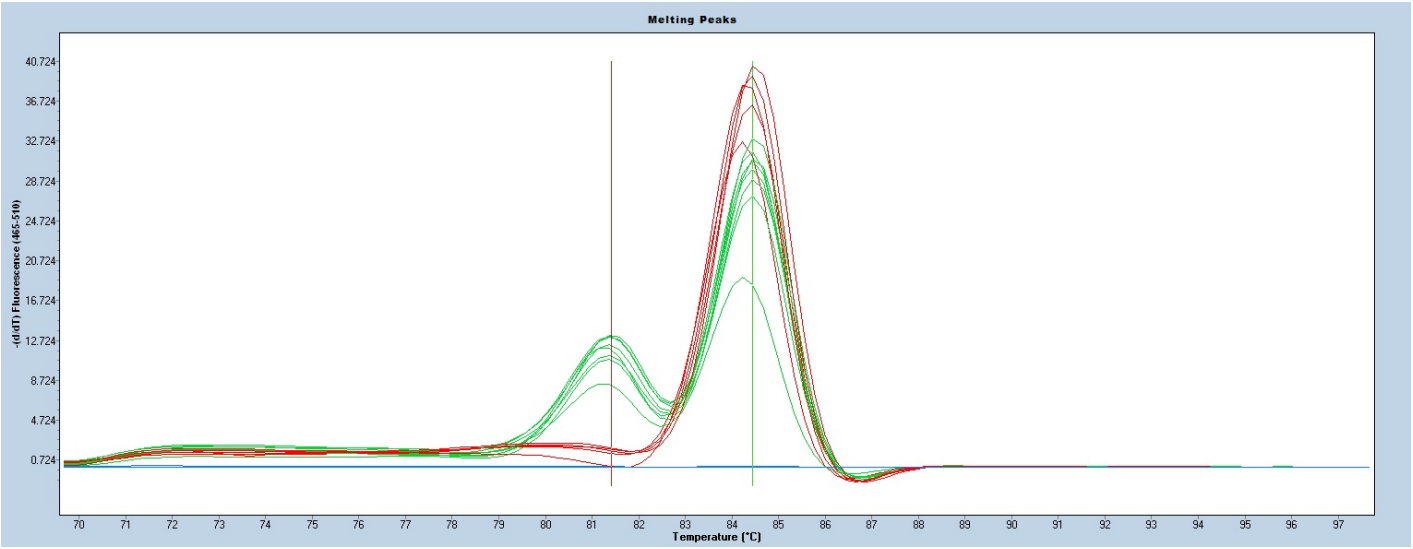
Cycling

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)
40.724
36.724
32.724
28.724
24.724
20.724
16.724
12.724
8.724
4.724
0.724
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)