

B6.129S4-Gt(ROSA)26Sor^{tm3(phiC31*)Sor}/J

Stock No: 007743

Protocol 19469: Standard PCR Assay - PhiC31 Alternate3

Version 4.0

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

Tg Tm = 90

IPC Tm = 87

Transgene = 237 bp

Internal Positive control = 320 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
21157		CTC CCA ACC CCA GAG GTA GT		Internal Positive Control Forward	A	
21225		AGA CCC CAG ATC CAG AAA GG		Internal Positive Control Reverse	A	
28721		CCA CCG TGA TGA GAA TCC TG		Mutant Forward	A	
28722		CTC TGC TCA GGC CCT TGC		Mutant Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
21157	0.50 uM
21225	0.50 uM
28721	0.50 uM
28722	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.

