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

Stock No: 007743
Protocol 29915: Standard PCR Assay - Gt(ROSA)26Sor<tm1sor> STD
Version 4.2

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 = 340 bp
Heterozygote = 340 bp and ~650 bp
Wild type = ~650 bp
Separated by gel electrophoresis on a 1.5% agarose gel.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR8052		GCG AAG AGT TTG TCC TCA ACC		Mutant Reverse	A	SA site
oIMR8545		AAA GTC GCT CTG AGT TGT TAT		Common	A	ROSA26
oIMR8546		GGA GCG GGA GAA ATG GAT ATG		Reverse	A	ROSA26

Reaction A

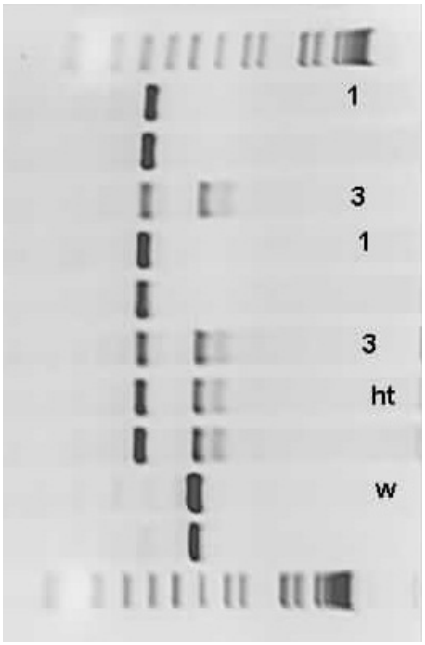
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR8052	0.50 uM
oIMR8545	0.50 uM
oIMR8546	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

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.


1
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