

STOCK *Gt(ROSA)26Sor^{tm1(CAG-Brainbow2.1)Cle}/J*

Stock No: 013731

Protocol 29479: Standard PCR Assay - *Gt(ROSA)26Sor^{tm1(CAG-Brainbow2.1)Cle}*

Version 3.2

Notes

This assay does not work well without the use of a Hotstart Taq polymerase.

8916 is the common primer

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 = 300 bp

Heterozygote = 300 bp and 386 bp

Wild type = 386 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
11341		GAA TTA ATT CCG GTA TAA CTT CG		Mutant Forward	A	directly after mCerulean
oIMR8545		AAA GTC GCT CTG AGT TGT TAT		Wild type Forward	A	
oIMR8916		CCA GAT GAC TAC CTA TCC TC		Common	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
11341	0.50 uM
oIMR8545	0.50 uM
oIMR8916	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.

