

B6;129S-Gt(ROSA)26Sor^{tm65.1(CAG-tdTomato)Hze}/J

Stock No: 021875

Protocol 35288: Standard PCR Assay - Gt(ROSA)26Sor<sup>tm65.1(CAG-tdTomato)Hze</sup>

Version 1.0

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

Mutant = 190 bp

Heterozygote = 190 bp and 402 bp

Wild type = 402 bp

 >[chr6:113025997-113026398](#) 402bp ACGGGCAGTAGGGCTGAG AGCCTGCCAGAAAGACTCC

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
24493		AGC CTG CCC AGA AGA CTC C		Wild type Reverse	A	
44393		ACG GGC AGT AGG GCT GAG		Wild type Forward	A	
45697		TCT AGC TTG GGC TGC AGG T		Mutant Reverse	A	
oIMR5971		GCA ATA GCA TCA CAA ATT TCA C		Mutant Forward	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
24493	0.50 uM
44393	0.50 uM
45697	0.50 uM
oIMR5971	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.

