

B6.Cg-Gt(ROSA)26Sor^{tm9}(CAG-tdTomato)Hze/J

Stock No: 007909

Protocol 29436: Standard PCR Assay - Gt(ROSA)26Sor(tdTomato-WPRE)

Version 2.2

Notes

Melt curve analysis is done using a Roche Light Cycler 480.

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 T_m = 81°C +/- 1.0°C

 Wild type T_m = 87°C +/- 1.0°C

Mutant = 196 bp

Heterozygote = 297 bp and 196 bp

Wild type = 297 bp

For Stock# 012567

Mutant = ~315 bp

Wild type = 297 bp

For Stock #7914

Mutant = ~200 bp

Wild type = 297 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR9020		AAG GGA GCT GCA GTG GAG TA		Wild type Forward	A	
oIMR9021		CCG AAA ATC TGT GGG AAG TC		Wild type Reverse	A	
oIMR9103		GGC ATT AAA GCA GCG TAT CC		Mutant Reverse	A	WPRE
oIMR9105		CTG TTC CTG TAC GGC ATG G		Mutant Forward	A	tdTomato

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
oIMR9020	0.50 uM
oIMR9021	0.50 uM
oIMR9103	0.50 uM
oIMR9105	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.



