

## STOCK *Gt(ROSA)26Sor<sup>tm1.1(CAG-EGFP)Fsh</sup>/Mmjax*

Stock No: 010701

Protocol 29811: Standard PCR Assay - *Gt(ROSA)26Sor<tm(CAG)>*

Version 2.2

### 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 = 320bp

Heterozygote = 320bp and 603bp

Wild type = 603bp

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
9655		CCA GGC GGG CCA TTT ACC GTA AG		Mutant	A	CAG
oIMR8545		AAA GTC GCT CTG AGT TGT TAT		Common	A	
oIMR8546		GGA GCG GGA GAA ATG GAT ATG		Wild type	A	

#### Reaction A

COMPONENT	FINAL CONCENTRATION
ddH <sub>2</sub> O	
Kapa 2G HS buffer	1.30 X
MgCl <sub>2</sub>	2.60 mM
dNTP KAPA	0.26 mM
9655	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.

