

## C57BL/6N-Gt(ROSA)26Sor<sup>tm1(CAG-111)</sup>Cook/J

Stock No: 031928

Protocol 33142: Probe Assay - Gt(Rosa)26Sor(CAG) alternate 2

Version 3.0

### Notes

The size of the mutant band can vary between different Gt(Rosa)26sor(CAG) strains.

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

>[chr6:113025964-113026059](#) 96bp CTTCCCTCGTGATCTGCAAC CAGGACAACGCCACACA

Mutant= 140 bp

Wild Type = 96 bp

Fam=Mut

Hex=Wt

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
17062		GTT ATG TAA CGC GGA ACT CCA		Mutant Reverse	A	
24500		CAG GAC AAC GCC CAC ACA		Wild type Reverse	A	
36158		CTT CCC TCG TGA TCT GCA AC		Common	A	
39816	Fluorophore-1	ACG GGG TCA TTA GTT CAT AGC CCA	Quencher-1			
40263	Fluorophore-2	CCA GTC TTT CTA GAA GAT GGG CGG	Quencher-2			

#### Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
17062	0.40 uM
24500	0.40 uM
36158	0.40 uM
Wt Probe	0.15 uM
Mutant Probe	0.15 uM
DNA	

#### Cycling

STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	
4		--	repeat steps 2-3 for 40 cycles
5	4.0	--	Forever

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.

Endpoint Fluorescence Scatter Plot

