

C57BL/6J-Tg(Thy1-GCaMP6s)GP4.6Dkim/J

Stock No: 028278

Protocol 20781: Probe Assay - GCaMP6s-Probe

Version 1.0

Notes

This assay cannot distinguish hemi from hom

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

Tg= 85 bp

IPC = 74 bp

This assay distinguishes GCaMP6s from GCaMP6f and GCaMP3

Sequence

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aagacaggtcacgcagtcagagctataggtcggctgagctcactcgagaacgtcta
tatcaaggccgacaagcagaagaacggcatcaaggcgaactc[CaC]atccgc
cacaacatcgaggacggcggcgtgagctcgctaccactaccagcagaacacc
ccatcggcgacggccccgtgctgctgccgacaacc
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JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
27656		TGG TAG TGG TAG GCG AGC TG		Transgene Reverse	A	
31033		ACA AGC AGA AGA ACG GCA TC		Transgene Forward	A	
31034	Fluorophore-1	CGA ACT TCC ACA TCC GCC	Quencher-1	Tg Probe		
oIMR1544		CAC GTG GGC TCC AGC ATT		Internal Positive Control Forward	A	
oIMR3580		TCA CCA GTC ATT TCT GCC TTT G		Internal Positive Control Reverse	A	
TmoIMR0105	Fluorophore-2	CCA ATG GTC GGG CAC TGC TCA A	Quencher-2	IC Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
27656	0.40 uM
31033	0.40 uM
oIMR1544	0.40 uM
oIMR3580	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

