

STOCK Tg(TIE2GFP)287Sato/J

Stock No: 003658

Protocol 30028: QPCR Assay - humanized GFP/ER fusion qPCR

Version 5.0

Notes

Taqman qPCR protocols are run on an ABI 7500, 7700, 7900 or the Roche LightCycler 480. Use an appropriate instrument-specific Fluorophore/Quencher combination. The transgene genotype is determined by comparing δ Ct values of each unknown sample against known homozygous and hemizygous controls, using appropriate endogenous references.

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 = 67 bp

IPC = 74 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR1544		CAC GTG GGC TCC AGC ATT		Internal Positive Control Forward	A	
oIMR1858		TGG TCC CAA TTC TCG TGG AA		Transgene	A	
oIMR1859		CCT CTC CGC TGA CAG AAA ATT T		Transgene	A	
oIMR3580		TCA CCA GTC ATT TCT GCC TTT G		Internal Positive Control Reverse	A	
TmoIMR0031	Fluorophore-1	TGC CCA TTC ACA TCG CCA TCC A	Quencher-1	Tg Probe		
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	
oIMR1544	0.40 uM
oIMR1858	0.40 uM
oIMR1859	0.40 uM
oIMR3580	0.40 uM
Tg Probe	0.15 uM
IC Probe	0.15 uM
DNA	

Cycling

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

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