

B6.Cg-Tg(Rgs4-EGFP)4Lvt/J

Stock No: 007894

Protocol 22102: Probe Assay - Fluorescent Proteins (Generic GFP)

Version 6.0

Notes

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

This assay will NOT distinguish hemizygous from homozygous transgenic animals.

This protocol can also be used for YFP.

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

Transgene = 92

IPC = 74 bp

The Jackson Laboratory designed this protocol using GFP sequence, however, we have found that it can be used to genotype for other fluorescence proteins. It is possible that mismatches in the primer sequences may make data interpretation challenging for other fluorescence proteins.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
10201		AGT GCT TCA GCC GCT ACC		Transgene Forward	A	
10202		GAA GAT GGT GCG CTC CTG		Transgene Reverse	A	
13731	Fluorophore-1	TTC AAG TCC GCC ATG CCC GAA	Quencher-1	MUT 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	
10201	0.40 uM
10202	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

