

## C.FVB-Tg(Cspg4-ZsYellow1)1Rkl/J

Stock No: 031158

Protocol 32038: Probe Assay - Generic YFP 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= 97 bp

IPC = 74 bp

### Sequence

Tg Sequence:

CTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCC  
TGGCCACCCCTCGTGACCACCTcggCTACGGCcTGCAGT  
GCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGAC

### JAX Protocol

#### Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
38214		ATC TGC ACC ACC GGC AAG		Transgene Forward	A	
38215		TCA TGT GGT CGG GGT AGC		Transgene Reverse	A	
38216	Fluorophore-1	CTT CGG CTA CGG CCT GCA G	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	
38214	0.40 uM
38215	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

