

STOCK *Igs2^{tm2}(ACTB-tdTomato,-EGFP)Luo/J*

Stock No: 013751

Protocol 29011: Standard PCR Assay - *Igs2*<tm#ACTB-tdTomato,-EGFP>

Version 2.2

Notes

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

Mutant = 230 bp

Heterozygote = 230 bp and 323 bp

Wild type = 323 bp

This assay cannot distinguish the *Igs*<tm1ACTB-tdTomato,-EGFP from *Igs*<tm2ACTB-tdTomato,-EGFP. To distinguish run in conjunction with Generic EGFP^{C-terminus}-MADM TG

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
11362		TGG AGG AGG ACA AAC TGG TCA C		Wild type Reverse	A	
11363		TTC CCT TTC TGC TTC ATC TTG C		Wild type Forward	A	
oIMR7320		TCA ATG GGC GGG GGT CGT T		Mutant Reverse	A	CMV Promoter

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
11362	0.50 uM
11363	0.50 uM
oIMR7320	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles (Touchdown)
6	94.0	--	
7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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

JAX uses a 'touchdown' cycling protocol and therefore has not calculated the optimal annealing temperature for each set of primers.

