

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

Stock No: 031158

Protocol 31572: Standard PCR Assay - Tg(Cspg4-ZsYellow1)1Rkl

Version 1.0

Notes

This assay will NOT distinguish hemizygous from homozygous transgenic animals.

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 = ~450 bp

Internal positive control = 521 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
31704		AGT GGC CTC TTC CAG AAA TG		Internal Positive Control Forward	A	
31705		TGC GAC TGT GTC TGA TTT CC		Internal Positive Control Reverse	A	
37191		GTT AAG TAC TGG ACA GCC CTT GC		Transgene Forward	A	
37192		GTA CTC GGT GAA GAT CCG GTC GCC G		Transgene Reverse	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
31704	0.50 uM
31705	0.50 uM
37191	0.50 uM
37192	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.

