

STOCK *Vsx1^{tm2Mci}/ChowJ*

Stock No: 026703

Protocol 27476: Standard PCR Assay - Generic IRES

Version 1.2

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 = 187 bp

Internal positive control = 415 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
21238		CTG TCC CTG TAT GCC TCT GG		Internal Positive Control Forward	A	
21239		AGA TGG AGA AAG GAC TAG GCT ACA		Internal Positive Control Reverse	A	
24712		CTT GGA ATA AGG CCG GTG T		Transgene Forward	A	
24713		AGC TTC CAG AGG AAC TGC TTC		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
21238	0.50 uM
21239	0.50 uM
24712	0.50 uM
24713	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.

