

C57BL/6-Tg(Nr4a1-EGFP/cre)820Khog/J

Stock No: 016617

Protocol 32458: Standard PCR Assay - Tg(Nr4a1-EGFP/cre)820Khog Alternate1

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 = ~200 bp

Internal positive control = 521 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
15020		CTG AAC TTG TGG CCG TTT AC		Mutant Reverse	A	EGFP
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	
39157		GAC ACC CGC TTC CTT GTC T		Transgene Forward	A	Nr4a1

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
15020	0.50 uM
31704	0.50 uM
31705	0.50 uM
39157	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.

