

B6.129X1(Cg)-Csf3r^{tm1Link}/J

Stock No: 017838

Protocol 27247: Standard PCR Assay - Csf3r<tm1Link> Alternate1

Version 1.2

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 = 136 bp

Heterozygote = 136 bp and 78 bp

Wild type = 78 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
23594		ACA TAA GCC TGT GGG AAG GA		Common	A	
23595		GCT GGT TCT CCA CTC ATT TG		Wild type Reverse	A	
oIMR8619		CTC CAG ACT GCC TTG GGA AAA		Mutant 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
23594	0.50 uM
23595	0.50 uM
oIMR8619	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.

