B6.129S4-*Ngfr*^{tm1Jae}/J

Stock No: 002213
Protocol 29363: Standard PCR Assay - Ngfr<tm1Jae>
Version 5.2

Notes

This assay does not work well without the use of a Hotstart Taq polymerase.

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 = ~193 bp
Heterozygote = ~193 bp and ~386 bp
Wild type = ~386 bp
Separated by gel electrophoresis on a 1.5% agarose gel.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
oIMR7617		GCT CAG GAC TCG TGT TCT CC		Common	A	
oIMR7618		CCA AAG AAG GAA TTG GTG GA		Wild type Reverse	A	
oIMR8162		TGG ATG TGG AAT GTG TGC GAG		Mutant	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR7617	0.50 uM
oIMR7618	0.50 uM
oIMR8162	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.