B6.129S4-*Pdgfra*$^{tm11(EGFP)Sor}$/J

Stock No: 007669
Protocol 23020: Standard PCR Assay - Pdgfra<tm11(EGFP)Sor>
Version 1.3

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 = 242 bp
Heterozygote = 242 bp and 451 bp
Wild type = 451 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
oIMR7801		CCC TTG TGG TCA TGC CAA AC		Wild type Forward	A	
oIMR7802		GCT TTT GCC TCC ATT ACA CTG G		Wild type Reverse	A	
oIMR7919		ACG AAG TTA TTA GGT CCC TCG AC		Mutant Reverse	A	

Reaction A

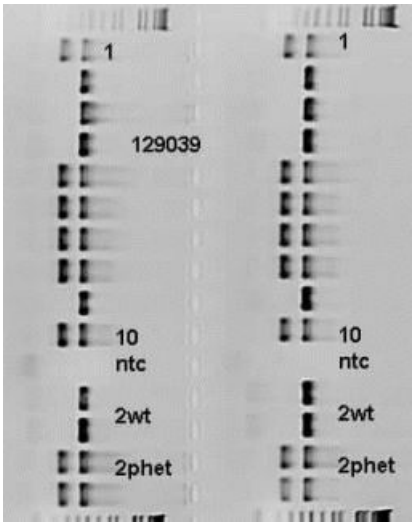
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
oIMR7801	0.50 uM
oIMR7802	0.50 uM
oIMR7919	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

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.


1
129039
10
ntc
2wt
2phet
1
10
ntc
2wt
2phet