

B6;129-Pak6^{tm1Amin} Pak5^{tm1Amin}/J

Stock No: 015825

Protocol 29351: Standard PCR Assay - Pak6^{tm1Amin}

Version 2.2

Notes

This assay will not work without the use of 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 = 711 bp

Heterozygote = 711 bp and 607 bp

Wild type = 607 bp

Looks best when run on a 3% gel.

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
11927		GAG GAA ACC CCA GGT CAT ATA CCT			A	
11928		TCA GTT ATC AGC TCC AAC ACC CTG			A	
9115		GCT ACC GGT GGA TGT GGA ATG TGT			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
11927	0.50 uM
11928	0.50 uM
9115	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.

