

B6.129P2-H2-K1^{tm1Bpe} H2-D1^{tm1Bpe}/DcrJ

Stock No: 019995

Protocol 26847: Standard PCR Assay - H2-K1<tm1Bpe>alternate2

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 = ~220 bp

Heterozygote = ~220 bp and 250 bp

Wild type = 250 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
21082		CCT GGC TCC GAC TCA GAC		Wild type Forward	A	
21083		GCG GTG ACG AAA TAC CTC AG		Wild type Reverse	A	
21219		TGC TGA CCT GCT GGA TTA CA		Mutant Forward	A	HPRT mini
21220		CCT GAC CAA GGA AAG CAA AG		Mutant Reverse	A	HPRT mini

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
21082	0.50 uM
21083	0.50 uM
21219	0.50 uM
21220	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.

