STOCK *Vip*$^{tm1(cre)Zjh}$/J

Stock No: 010908
Protocol 29677: Standard PCR Assay - Vip<tm1(cre)Zjh>
Version 2.2

Notes

This assay does not work well without the use of a Hotstart Taq polymerase.
Changed expected mutant band size from ~250 to ~350, JAK 2/18/13.

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 ~350 bp
Heterozygote = ~350 bp and 175 bp
Wildtype = 175bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
9527		CCC CCT GAA CCT GAA ACA TA		Mutant Forward	A	
9984		GGA CAC AGT AAG GGC ACA CA		Common	A	
9985		TCC TTG GAA CAT TCC TCA GC		Wild type Forward	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTP KAPA	0.26 mM
9527	0.50 uM
9984	0.50 uM
9985	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.