

B6.129-Ucp1^{tm1Kz}/J

Stock No: 003124

Protocol 18834: Standard PCR Assay - Ucp1^{tm1Kz} Alternate4

Version 1.0

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 = 206 bp

Heterozygote = 206 bp and 279 bp

Wild type = 279 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
11931		CTT CCT GAC TAG GGG AGG AGT		Mutant Reverse	A	
26819		TCG TCA TCA ATA AGG GGA AAC		Wild type Forward	A	
27695		CTT CTT CCC TGA TGC TCC AT		Wild type Reverse	A	
27696		GAT CCC CCG GGC AAT TCT		Mutant Forward	A	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
11931	0.50 uM
26819	0.50 uM
27695	0.50 uM
27696	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.

