

B6.129S4-Cd80^{tm1Shr}/J

Stock No: 003611

Protocol 26909: Standard PCR Assay - Cd80<tm1Shr>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

Run TD 65-60

Meltcurve- Genescanning analysis

Mutant = ~380 bp

Heterozygote = ~380 bp and 179 bp

Wild type = 179 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
20125		GCT GTG AAC ATG GGT CTG C		Wild type Reverse	A	
oIMR2060		CAC GAG ACT AGT GAG ACG TG		Mutant Reverse	A	
oIMR6791		CAG TGG CTT CCA GAT CCT TC		Common	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
20125	0.50 uM
oIMR2060	0.50 uM
oIMR6791	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.

