

B6.129(Cg)-Cx3cr1^{tm1Litt} Ccr2^{tm2.1Jfc}/JernJ

Stock No: 032127

Protocol 27927: Standard PCR Assay - Cx3cr1<tm1Litt>alternate1

Version 3.2

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 = ~500 bp

Heterozygote = 410 bp and ~500 bp

Wild type = 410 bp

Mutant T_m = 83 Area = 26 T_m = 89 Area = 36 (WT T_m = 81 Area = 7 T_m = 84 Area = 15)

Wild type T_m = 89 Area = 45

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
14276		GTC TTC ACG TTC GGT CTG GT		Wild type Forward	A	
14277		CCC AGA CAC TCG TTG TCC TT		Common	A	
14278		CTC CCC CTG AAC CTG AAA C		Mutant Forward	A	

Reaction A

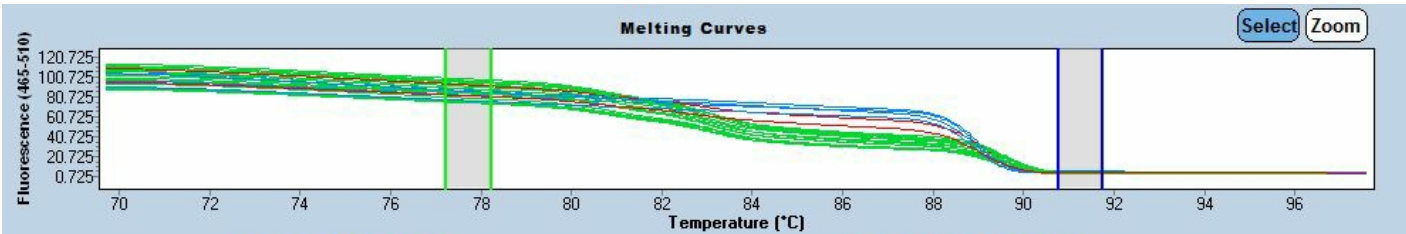
COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTP KAPA	0.26 mM
14276	0.50 uM
14277	0.50 uM
14278	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

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.



Pre-Melt Slider Settings

Low High

Post-Melt Slider Settings

Low High

