

B6.129S7-*Src*^{tm1Sor}/J

Stock No: 002277

Protocol 19300: Separated PCR Assay - *Src*<tm1Sor>alternate1

Version 2.0

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 = ~400 bp

Heterozygote = 375 bp and ~400 bp

Wild type = 375 bp

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
10791		CGC TTC CTC GTG CTT TAC GGT AT		Mutant	B	
14275		TCC TAA GGT GCC AGC AAT TC		Wild type	A	
oIMR3129		GAG TTG AAG CCT CCG AAG AG		Common	A, B	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH ₂ O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTPS-kapa	0.26 mM
14275	0.50 uM
oIMR3129	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	--	-1.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles
6	94.0	--	
7	50.0	--	
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.

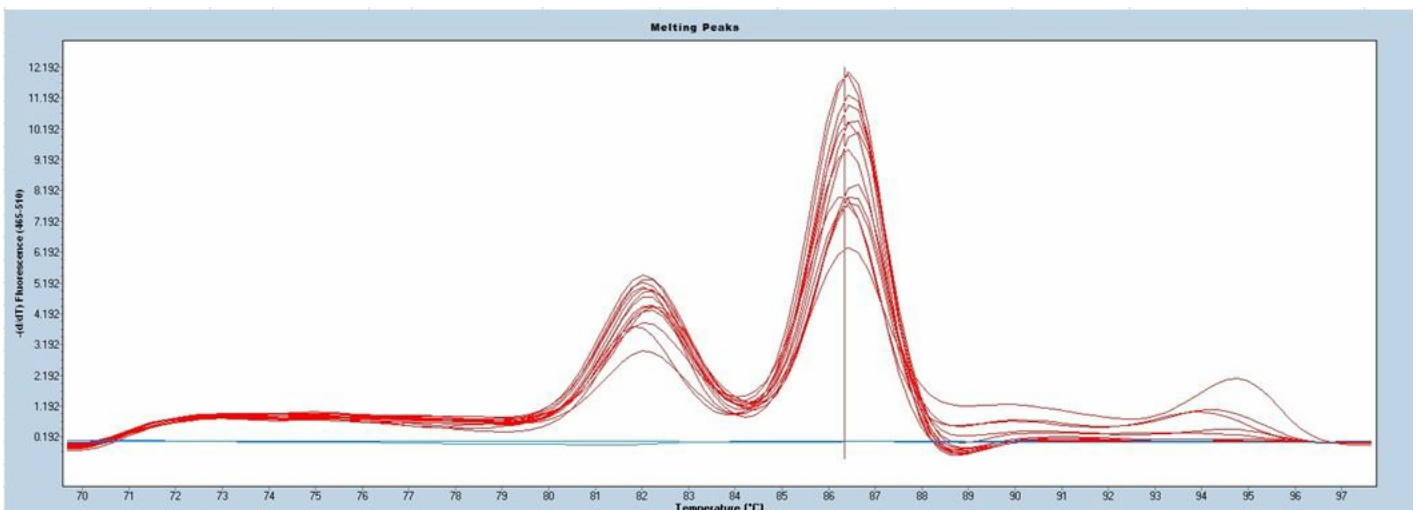
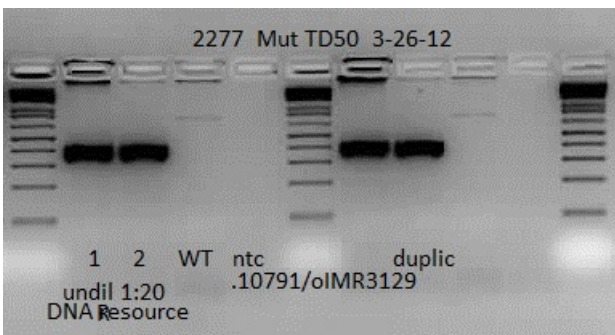
Reaction B

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTPS-kapa	0.26 mM
10791	0.50 uM
oIMR3129	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	--	-1.5 C per cycle decrease
4	68.0	--	
5		--	repeat steps 2-4 for 10 cycles
6	94.0	--	
7	50.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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Melting Peaks

