

B6N.129P2-Cd19^{tm1(cre)}Cgn/J

Stock No: 018958

Protocol 30980: Separated PCR Assay - Cd19<tm1(cre)Cgn> Alternate2

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 = ~200 bp

Heterozygote = ~200 bp and 280 bp

Wild type = 280 bp

 >[chr7:133557730-133558009](#) 280bp GACTCCTCACCTGTCTCTTCTGA AGAGCTCCTCTTTACCTCCACA

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
35897		GAC TCC TCA CCT GTC TCT TCT GA		Common	A, B	
35898		AGA GCT CCT CTT TAC CTC CAC A		Wild type Reverse	A	
oIMR8990		CAG GTT CTT GCG AAC CTC AT		Mutant Reverse	B	

Reaction A

COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl ₂	2.60 mM
dNTPS-kapa	0.26 mM
35897	0.50 uM
35898	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.

Reaction B

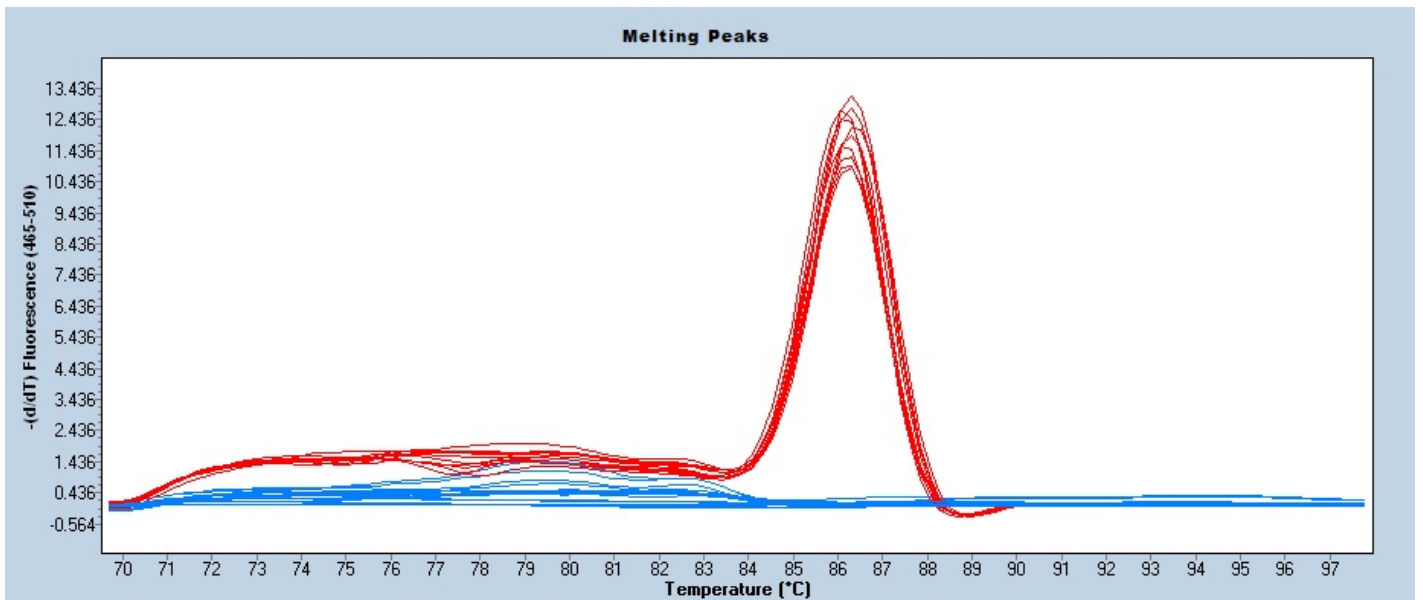
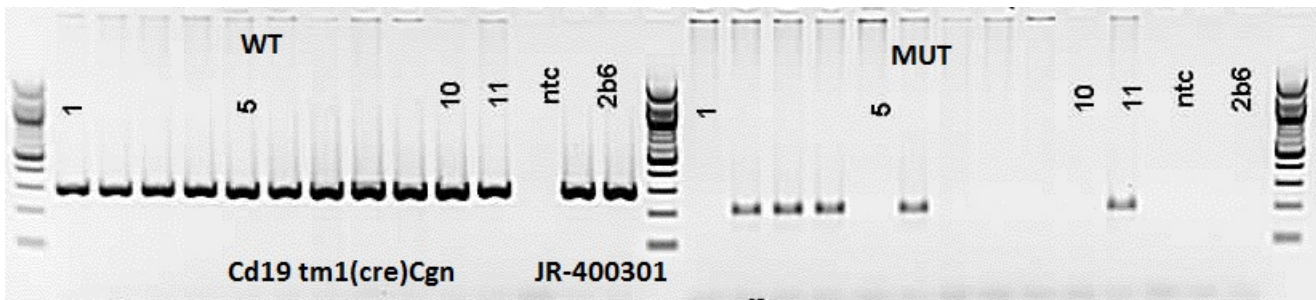
COMPONENT	FINAL CONCENTRATION
ddH2O	
Kapa 2G HS buffer	1.30 X
MgCl2	2.60 mM
dNTPS-kapa	0.26 mM
35897	0.50 uM
oIMR8990	0.50 uM
Glycerol	6.50 %
Dye	1.00 X
Kapa 2G HS taq polym	0.03 U/ul
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	94.0	--	
2	94.0	--	
3	65.0	--	-0.5 C per cycle decrease
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7	60.0	--	
8	72.0	--	
9		--	repeat steps 6-8 for 28 cycles
10	72.0	--	
11	10.0	--	hold

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Melting Peaks

