C57BL/6NJ

Stock No: 005304
Protocol 21935: End Point Analysis Assay - Crb1<rd8>End Point
Version 3.0

Notes

Mutant = -/- B6N (mutation fixed on this background)
Heterozygote = C/-
Wild type = C/C B6J
Use controls from DNA Resorces jr 3392
Mutation is a deleted C

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 = -/-
Heterozygote = C/-
Wild type = C/C
Mutation is a deleted C

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
16217		TTG CAT GGA GGA AAC TGT GA		Forward	A	
16218		TGT ATC CAG GCT CAC ACC TG		Reverse	A	
16219	Fluorophore-1	CAG TTC TTA TCG GTG TGC CTG	Quencher-1	WT Probe		
16220	Fluorophore-2	CAG TTC TTA TGG TGT GCC TG	Quencher-2	MUT Probe		

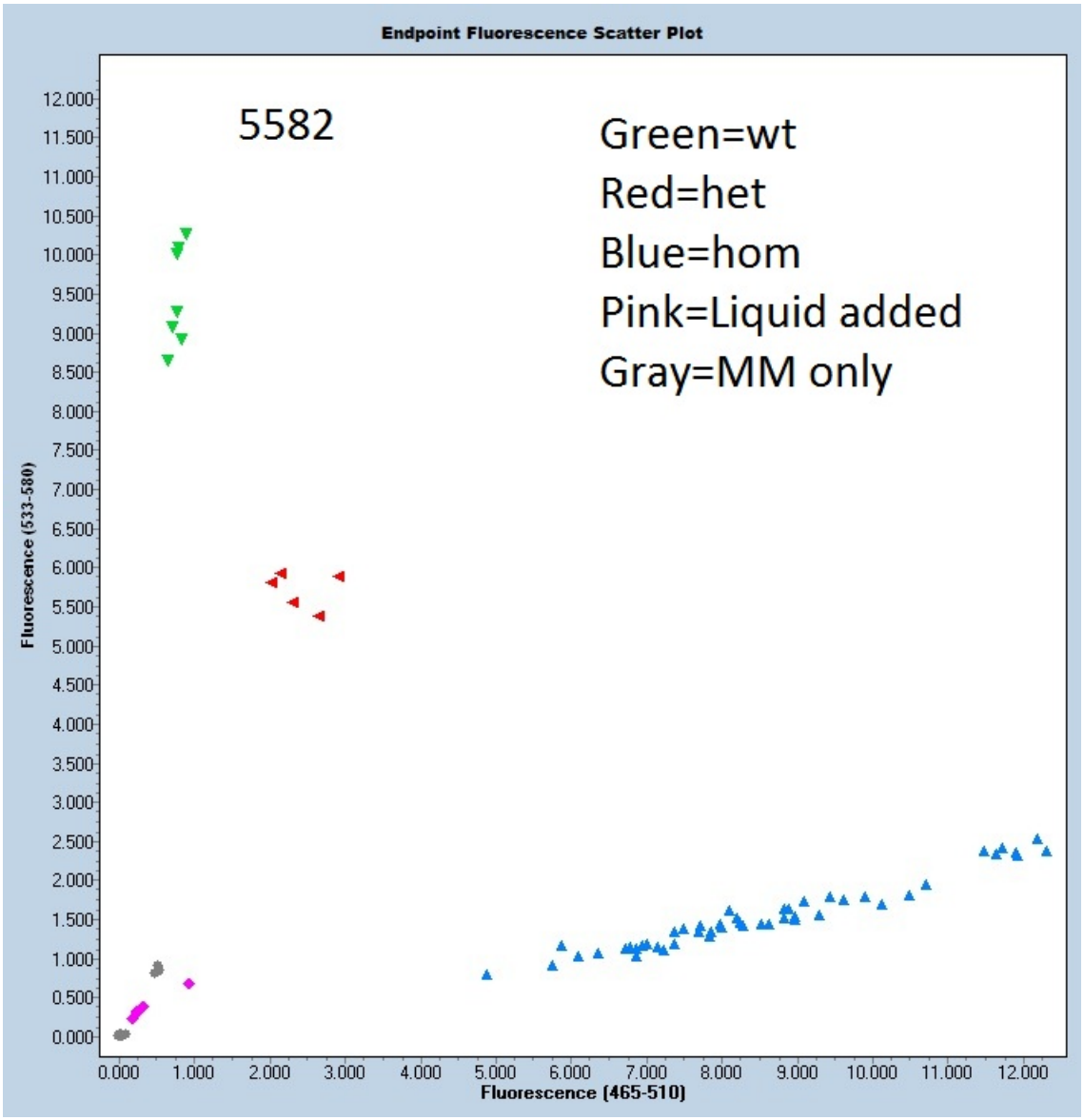
Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
16217	0.40 uM
16218	0.40 uM
Wt Probe	0.15 uM
Mutant Probe	0.15 uM
DNA	

Cycling

STEP	TEMP °C	TIME	NOTE
1	95.0	--	
2	95.0	--	
3	60.0	--	
4		--	repeat steps 2-3 for 40 cycles
5	4.0	--	Forever

JAX uses a very high speed Taq (~1000 bp/sec), use cycling times recommended for your reagents.


Endpoint Fluorescence Scatter Plot
5582
Green=wt
Red=het
Blue=hom
Pink=Liquid added
Gray=MM only
Fluorescence (533-580)
Fluorescence (465-510)
12.000
11.500
11.000
10.500
10.000
9.500
9.000
8.500
8.000
7.500
7.000
6.500
6.000
5.500
5.000
4.500
4.000
3.500
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