MRL/MpJ

Stock No: 000486
Protocol 21974: End Point Analysis Assay - H2<k>rs8249979 Alternate 1-EP
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

Sequence

Mut = T H2<k> Jrs: 646, 656, 465, 4804
WT= C other haplotypes
tcccaccaTGAGCCTCTACCCTCTAGGAATTCG(c/t)CAGGGA
CCAGTCAGGTC
ACTCAGCTggggcttct

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
21918	Fluorophore-1	TAG GAA TTC GTC AGG GAC CAG	Quencher-1	MUT Probe		
21919	Fluorophore-2	AGG AAT TCG CCA GGG ACC	Quencher-2	WT Probe		
22189		ATC CCA CCA TGA GCC TCT AC		Forward	A	
22190		CAG CTG AGT GAC CTG ACT GG		Reverse	A	

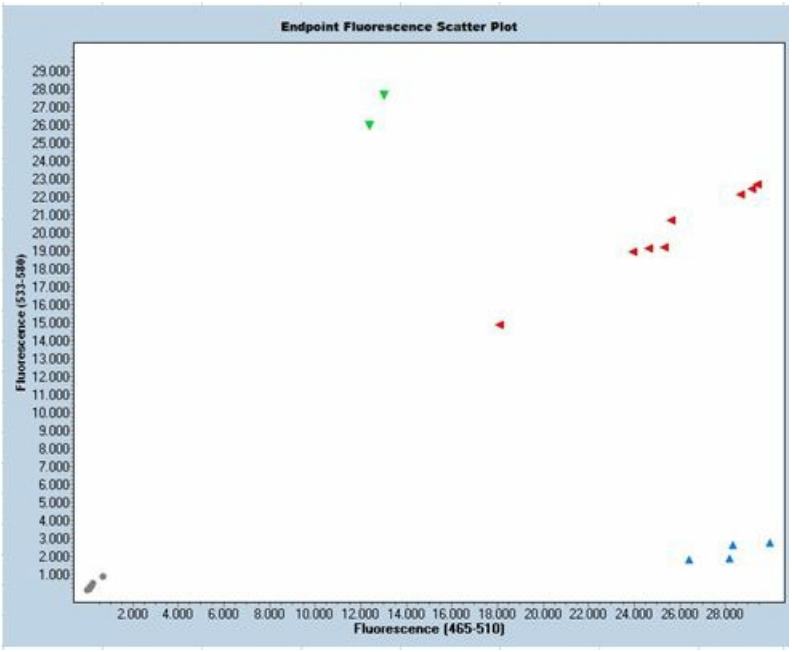
Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
22189	0.40 uM
22190	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
Fluorescence (533-580)
29.000
28.000
27.000
26.000
25.000
24.000
23.000
22.000
21.000
20.000
19.000
18.000
17.000
16.000
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2.000 4.000 6.000 8.000 10.000 12.000 14.000 16.000 18.000 20.000 22.000 24.000 26.000 28.000
Fluorescence (465-510)