

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

Stock No: 032127

Protocol 35246: Probe Assay - Ccr2<sup>tm2.1Ifc</sup> Probe

Version 1.0

Notes

Taqman qPCR protocols are run on a real time PCR instrument. Use an appropriate instrument specific Fluorophore/Quencher combination.

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

>[chr9:124020345+124020465](#) 121bp GCAAGGCTATTTGGATTAAGGA TATGCCGTGGATGAACTGAG

Mutant= 116 bp

Wild Type = 121 bp

Sequence

Wt Sequence:

```
GCAAGGCTATTTGGATTAAGGAATTTGGCATTGTGTTACA
AATAATCATTTTGTCTCTGACCACAGAATCAAAGgaAATG
GAAGACAATAATATGTTACCTCAGTTCATCCACGGCATA
```

Mutant Sequence:

```
GCAAGGCTATTTGGATTAAGGAATTTGGCATTGTGTTACA
AATAATCATTTTGTCTCTGACCACAGAATCAAAGGccatg
cttctccgaggacgtcatcaaggagtcatgc
```

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
45609		GCA AGG CTA TTT GGA TTA AGG A		Common	A	
45610		GCA TGA ACT CCT TGA TGA CG		Mutant Reverse	A	RFP
45611		TAT GCC GTG GAT GAA CTG AG		Wild type Reverse	A	
45612	Fluorophore-1	AGA ATC AAA GGC CAT GGC TTC	Quencher-1	MUT Probe		
45613	Fluorophore-2	CAA AGG AAA TGG AAG ACA ATA ATA TGT T	Quencher-2	WT Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
45609	0.40 uM
45610	0.40 uM
45611	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

