

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

Stock No: 032127

Protocol 32483: Probe Assay - Cx3cr1<tm1Litt> Probe Alternate1

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

Mutant= 85 bp

Wild Type = 110 bp

>[chr9:119961380-119961489](#) 110bp CGTGATCTGGTTTCTCCTTCC CCAATAACAGGCCTCAGCA

Sequence

Wt Sequence:

```
tccttaaggctcacgtgatctggtttctcctcccctccagGACCTCACCATGT
CCACCTCCTTCCCTGAACTGGATCTAGAGAAATTTGAGTA
TGACGATTCTGCTGAGGCCTGTTATTTGGGCGACATTGTG
GCCTTTGGAACCATCTTCTGTCCGTCTTCTA
```

Mutant Sequence:

```
GGACCTGCTTACTGCATGCAGCCAGTGAGAACTACAATC
CTTTAAGGCTCACGTGATCTGGTTTCTCCTTCCCTCCAG
GACCTCccgcgggcccggtccaccggtgccaccatggtgagcaagggc
gaggagctgtcaccggggtggtgccatcctggtcgagctggacggcgac
```

JAX Protocol

Protocol Primers

PRIMER	5' LABEL	SEQUENCE 5' → 3'	3' LABEL	PRIMER TYPE	REACTION	NOTE
31017		CGT GAT CTG GTT TCT CCT TCC		Common	A	
32856		CTC CTC GCC CTT GCT CAC		Mutant Reverse	A	EGFP
39210		CCA AAT AAC AGG CCT CAG CA		Wild type Reverse	A	
39211	Fluorophore-1	CCA GGA CCT CCC GCG GG	Quencher-1	MUT Probe		
39212	Fluorophore-2	CCA GGA CCT CAC CAT GTC CAC C	Quencher-2	WT Probe		

Reaction A

COMPONENT	FINAL CONCENTRATION
Kapa Probe Fast QPCR	1.00 X
ddH2O	
31017	0.40 uM
32856	0.40 uM
39210	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

