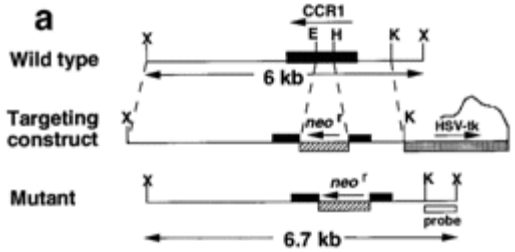
11.09.15 MLS

Genotyping Protocol: **MMRRC 37392**

Assay Type: PCR- can distinguish heterozygous animals from homozygous animals.

DNA Extraction: DNA from tail snips was extracted using the protocol provided with EMD Millipore's KOD Xtreme Hot Start DNA Polymerase Kit (Cat#71975-3). Kit directions were followed for amplification from mouse tails.

Strain Description: A neomycin cassette replaced the open reading frame that corresponds to 33% of the protein, from the third to the fifth transmembrane segment, of the chemokine (C-C motif) receptor 1 gene (*Ccr1*). Details can be found in Gao et al (1997) J Exp Med. 185(11):1959-68.



Primer Information:

1) Name: m3xRT3	Sequence: 5'-TTT GAC CTT CTT CTC ACT GGG TCT TC-3'
2) Name: m3xs300	Sequence: 5'-GCT GTC TCT GAT CTG GTC TTC CTT-3'
2) Name: m3xs600	Sequence: 5'-GAG TTC ACT CAC CGT ACC TGT AGC-3'
2) Name: ANeo	Sequence: 5'-TGG GTG GAG AGG CTT TTT GCT TCC TCT TGC-3'

Primer location: m3xRT3, m3xs300 and m3xs600 are located in Exon 1 of *Ccr1* on Chromosome 9. ANeo is located in the neo cassette.

Assay name: Ccr1 KO PCR

MUT PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
KOD Xtreme Buffer	Millipore	2X	10
KOD Xtreme dNTPs	Millipore	2mM	4
ANeo	Sigma	25µM	0.3
m3xs300	Sigma	25µM	0.3
KOD Xtreme *Taq*	Millipore (Cat# 71975-3)	1 U/µl	0.4
sterile water			3

PCR Setup:
Final Reaction: 18µl master mix & 2µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

1)	95°C	4 minutes
2)	94°C	30 seconds
3)	68.5°C	30 seconds
4)	72°C	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	7 minutes
7)	4°C	hold until refrigerate product

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WT PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
KOD Xtreme Buffer	Millipore	2X	10
KOD Xtreme dNTPs	Millipore	2mM	4
Calb1iCre.F	Sigma	25µM	0.3
Calb1iCre.R	Sigma	25µM	0.3
KOD Xtreme *Taq*	Millipore (Cat# 71975-3)	1 U/µl	0.4
sterile water			3

PCR Setup:
Final Reaction: 18µl master mix & 2µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

1)	95°C	4 minutes
2)	94°C	30 seconds
3)	68.5°C	30 seconds
4)	72°C	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	7 minutes
7)	4°C	hold until refrigerate product

Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

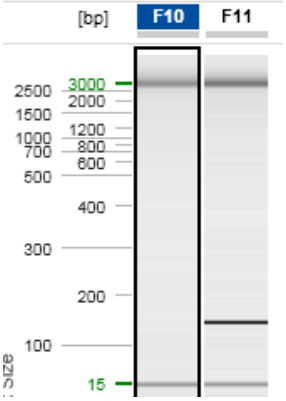
Alignment Marker: QX Alignment Marker 15bp/3kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100-3kb (Cat# 929553)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected products:
Mut allele: 150bp roduct
WT allele: 180bp product

Genotyping results:
Heterozygous: 150bp product from MUT PCR, 180bp product from WT PCR
Homozygous: 150bp product from MUT PCR, no product from WT PCR
Wild-type: no product from MUT PCR, 180bp product from WT PCR

Example Gels:

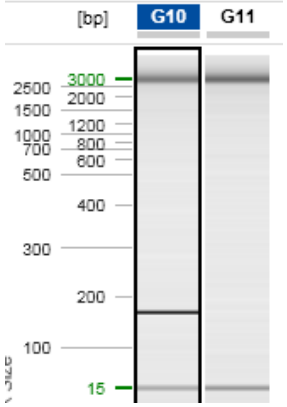
Mut gel:



Lane F10 displays a sample negative for the MUT allele (no product on MUT gel).
Lane F11 displays a sample positive for the MUT allele (150bp product on MUT gel).

Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.

WT gel:



Lane G10 displays a sample positive for the WT allele (180bp product on WT gel).
Lane G11 displays a sample negative for the WT allele (no product on WT gel).

Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.