02.23.16 MLS

Genotyping Protocol: MMRRC 29593

Assay Type: PCR (can distinguish heterozygous animals from homozygous animals)

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy kit. Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Strain description: A neo cassette replaced all 3 exons of the platelet factor 4 gene (*Pf4*, also called *Cxcl4*). Details can be found in Eslin et al (2004) Blood 104(10):3173-80.

Primer Information:
WT allele:
Name: M29593 mPF4 FD Sequence: 5'- GGT ACC ACA CCG GCA GAT GAT AG -3'
Name: M29593 mPF4 Rev Sequence: 5'- CAC TAT GTT GAG CCC CCT TCC TG -3'

KO allele:
Name: M29593 Neo FD Sequence: 5'- TTT TGT CAA GAC CGA CCT GT -3'
Name: M29593 Neo Rev Sequence: 5'- TGC GCT GCG AAT CGG GAG CG -3'

M29593 mPF4 FD binds to intronic region 1-2 of the *Cxcl4* gene while M29593 mPF4 Rev binds downstream in exon 3 of the *Cxcl4* gene. M29593 Neo FD & M29593 Neo Rev bind to the neomycin gene replacing the entire *Cxcl4* gene (also referred to as *Pf4*= platelet factor 4).

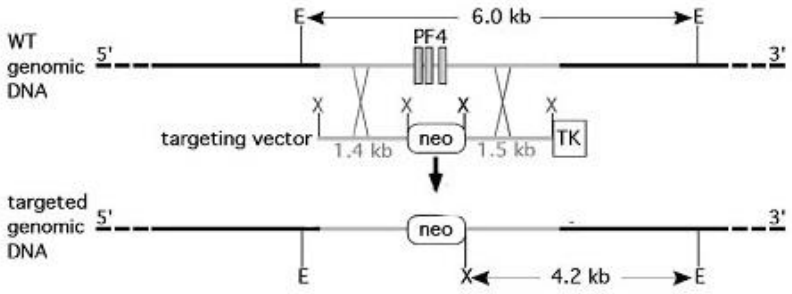


Image from Eslin et al (2004).

Assay Name: Cxcl4 KO PCR

MMRRC 29593 WT allele PCR
Master Mix Components

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
M29593 mPF4 FD	Sigma	25 µM	0.3
M29593 mPF4 Rev	Sigma	25 µM	0.3
sterile water			5.4

02.23.16 MLS

MMRRC 29593 KO allele PCR
Master Mix Components

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma	2X	10
M29593 Neo FD	Sigma	25 µM	0.3
M29593 Neo Rev	Sigma	25 µM	0.3
sterile water			5.4

PCR Setup:
Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

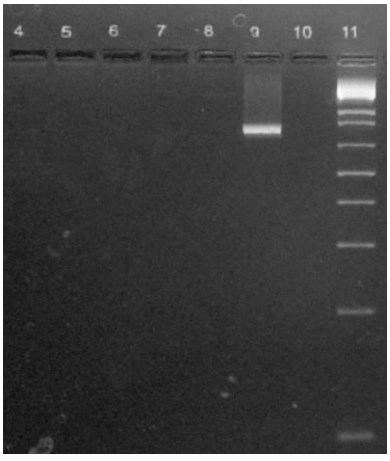
Cycle Parameters:
1) 94°C 3 minutes
2) 94°C 1 minute
3) 68°C 1 minute
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining.

Expected Products:
Wild-type: 751 bp on WT allele PCR & no product on KO allele PCR
Homozygous mutant: 620 bp on KO allele PCR & no product on WT allele PCR
Heterozygote: 620 bp on KO allele PCR & 751 bp on WT allele PCR

Example of Gel:
WT allele PCR

751 bp →

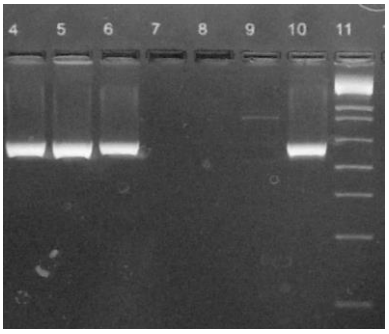


Wells 4-6 & 10 are homozygous mutant. Well 9 is wild-type. Wells 7 & 8 are negative controls with no DNA. Well 11 is 1 Kb Plus DNA ladder (Invitrogen Cat. # 10787-018).

02.23.16 MLS

KO allele PCR

620 bp →



Wells 4-6 & 10 are homozygous mutant. Well 9 is wild-type. Wells 7 & 8 are negative controls with no DNA. Well 11 is 1 Kb Plus DNA ladder (Invitrogen Cat. # 10787-018).