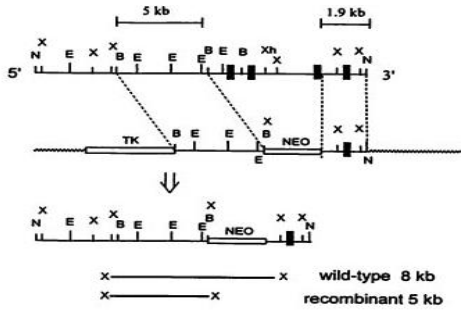
07.21.11 MS

Genotyping Protocol: **MMRRC 30201**

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

DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). 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.

Mutation Information: This strain carries a knock-out allele of the CD55 antigen. Exons 1-3 of this gene were replaced by a *neo* gene cassette.



Primer Information:

WT PCR
1) Name: M30201 WT F
Sequence: 5'-AGC AGC TAC CGG GTG TCT TA-3' (forward primer binds to exon 2 of CD55 gene)

2) Name: M30201 WT R
Sequence: 5'-TGC TCA GCA AAC TTG GAG TG-3' (reverse primer binds to intronic region 2-3 of CD55 gene)

KO PCR
1) Name: Neo F Sequence: 5'-CATTCGACCACCAAGCGAAACATC-3'
2) Name: Neo R Sequence: 5'-ATATCACGGGTAGCCAACGCTATG-3'

Both primers bind to Neo gene.

Assay Name: CD55 KO PCR

WT PCR:

WT PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
10X Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
M30201 WT F	Sigma	25 µM	0.3
M30201 WT R	Sigma	25 µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/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) 95°C 3 minutes
2) 94°C 30 seconds
3) 63°C 30 seconds
4) 72°C 30 seconds
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

KO PCR:

KO PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
10X Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
Neo F	IDT	25 µM	0.3
Neo R	IDT	25 µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:
Final Reaction: 19µl master mix & 1µl DNA template (10-20ng/µl)

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

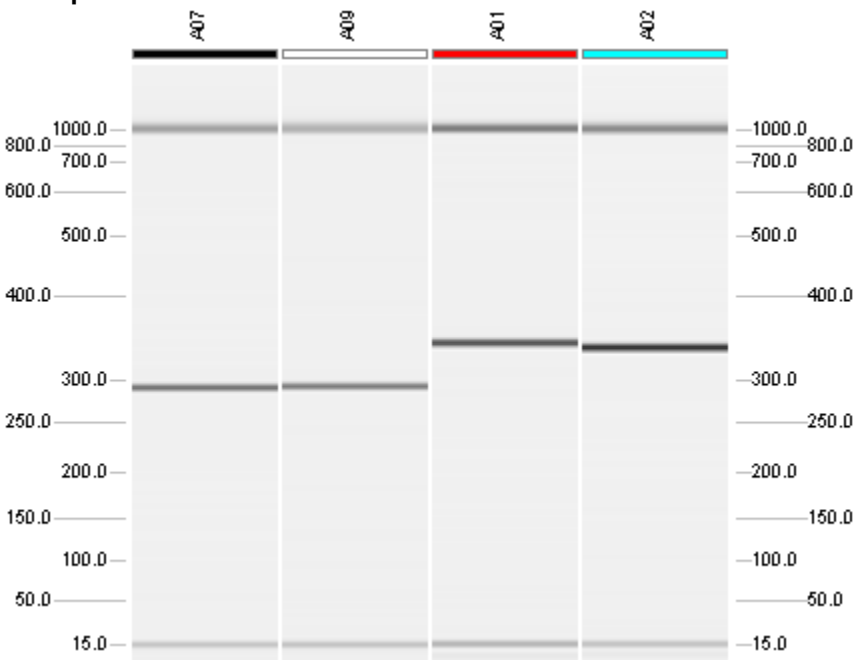
Cycle Parameters:
1) 95°C 5 minutes
2) 94°C 30 seconds
3) 68°C 30 seconds
4) 72°C 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72°C 10 minutes
7) 4°C hold until refrigerate product

Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).
Alignment Marker: QX Alignment Marker 15bp/1000bp (Cat# 929521)
Size Marker: QX DNA Size Marker 50-800bp (Cat# 929556)
Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Expected Products:
WT: 342bp
KO: 289bp

Genotype	KO PCR	WT PCR
Wild type	no product	342 bp
Heterozygous	289 bp	342 bp
Homozygous	289 bp	no product

Example of Gel:



Lanes A07 and A09 display samples positive for the KO allele (289bp band).
Lanes A01 and A02 display samples positive for the WT allele (342bp band).

Please note: the bands appearing at 1000bp and 15bp are reference bands specific to the QIAxcel method and do not represent expected products.