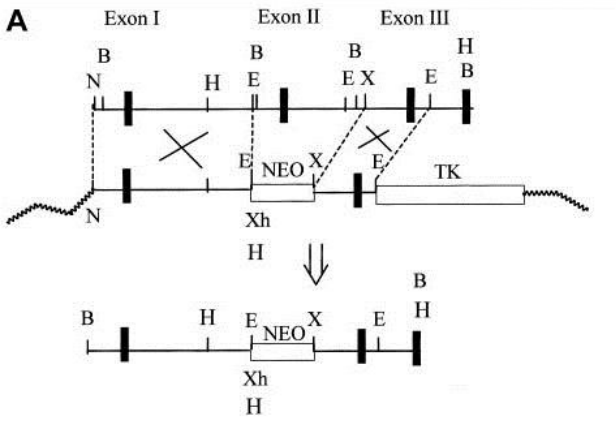
03.01.12 MS

Genotyping Protocol: **MMRRC 30200**

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 CD59 antigen. Exon 2 of this gene was replaced by a *neo* gene cassette. Details can be found in Miwa T. et al (2002) Blood 99(10):3707-16.



Primer Information:

WT PCR
1) Name: M30200 WT F
Sequence: 5'- CTGCTTCTGGCTGTGTTCTG -3' (forward primer binds to exon 2)
2) Name: M30200 WT R
Sequence: 5'- CCCTCATAGCCCACACCTAA -3' (reverse primer binds to intronic region 2-3)

KO PCR
1) Name: M30200 KO F
Sequence: 5'- CGTTGGCTACCCGTGATATT -3' (forward primer binds to *neo* gene cassette)
2) Name: M30200 KO R
Sequence: 5'- TCCTCACCTTTGGAAGGAAA -3' (reverse primer binds to intronic region 2-3)

Assay Name: CD59 KO PCR

PCR Master Mix Components:

WT PCR

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
M30200 WT F	IDT	25 µM	0.3
M30200 WT 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:
PCR
1) 95°C 5 minutes
2) 94°C 1 minute
3) 65°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

Expected Product: WT: 332bp

KO PCR

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
M30200 KO F	IDT	25 µM	0.3
M30200 KO R	IDT	25 µM	0.3
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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

Expected product: KO: ~1000bp

Product Analysis:

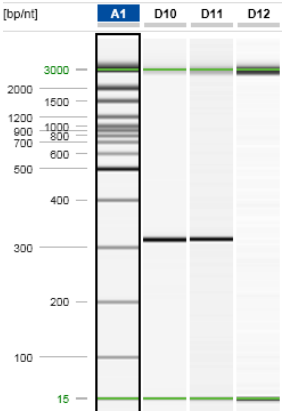
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

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

Genotype	KO PCR	WT PCR
WT	no product	332bp
Heterozygous	~1000bp	332bp
Homozygous	~1000bp	no product

Example gels:

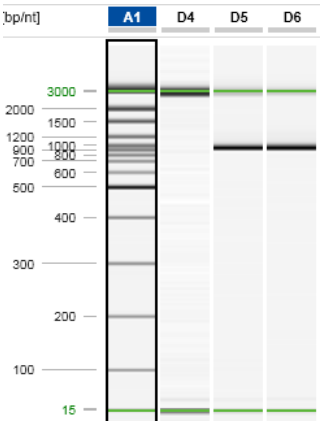
WT PCR:



Lane A1 displays a 15bp-3kb size marker
Lane D10 displays a WT sample (332 bp product)
Lane D11 displays a heterozygous sample (332 bp product)
Lane D12 displays a homozygous sample (no product)

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

KO PCR:



Lane A1 displays a 15bp-3kb size marker
Lane D4 displays a WT sample (no product)
Lane D5 displays a heterozygous sample (~1000bp product)
Lane D6 displays a homozygous sample (~1000bp product)

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