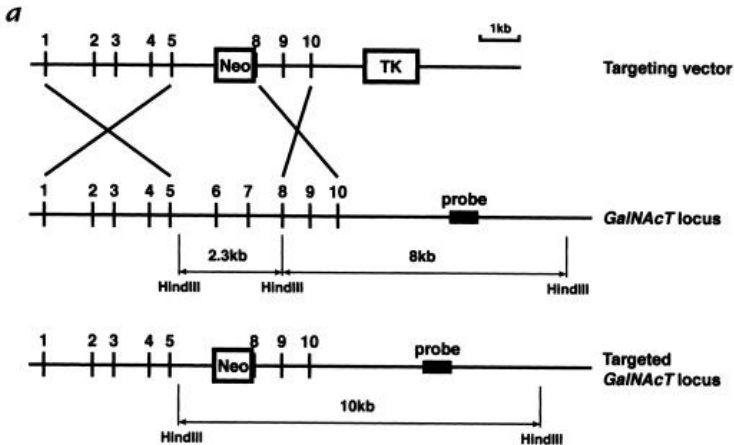


Genotyping Protocol: **MMRRC 36**

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.

Strain Description: A neo cassette is inserted into the beta-1,4-N-acetyl-galactosaminyl transferase 1 gene (*B4galnt1*), resulting in the deletion of exons 6, 7 and part of 8. Details can be found in Liu et al (1999) J Clin Invest 103(4):497-505.



Primer Information:

- | | |
|----------------------|---|
| 1) Name: L36-exon7 S | Sequence: 5'-ACA CGT GGA GCA CTA CTT CAT G-3' |
| 2) Name: L36-exon8AS | Sequence: 5'-AGG TCC AGG GGC GTC TTC T-3' |
| 3) Name: L36-neo AS | Sequence: 5'-TGC AAT CCA TCT TGT TCA ATG-3' |

Assay Name: GalNAcT PCR

PCR Master Mix Components:

Master Mix: WT

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25 mM	3.2
L36-exon7 S	IDT	25 µM	0.4
L36-exon8AS	IDT	25 µM	0.4
FastStart Taq	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			12.8

Master Mix: KO

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25 mM	3.2
L36-neo AS	IDT	25 µM	0.4
L36-exon8AS	IDT	25 µM	0.4
FastStart Taq	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			12.8

08.05.10 MS
11.12.13 MLS

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 Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 94°C 10 minutes
- 2) 94°C 1 minute
- 3) 64°C 30 seconds
- 4) 72°C 2 minutes
- 5) Repeat steps 2-4 44 times for a total of 45 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

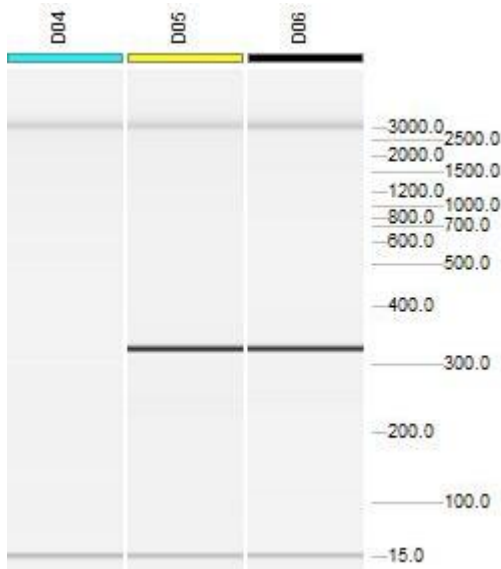
Injection: 10s at 5KV

Separation: 320s at 6KV

Expected products: WT 600 bp
KO 300 bp

Example gels:

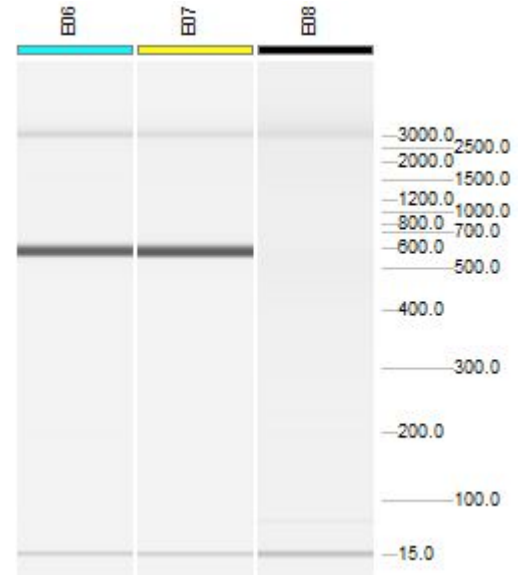
KO PCR:



Lane D04 displays a sample negative for the KO allele (no product)
Lanes D05 and D06 display samples positive for the KO allele (300bp products)

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

WT PCR:



Lanes E06 and E07 display samples positive for the WT allele (600bp product)
Lane E08 displays a samples negative for the WT allele (no product)

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