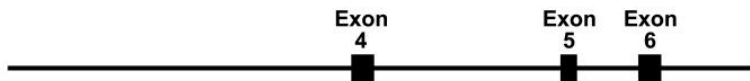
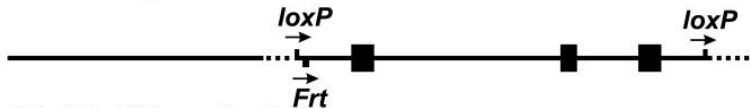


Genotyping Protocol: **MMRRC 36792**

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: A neomycin cassette, with loxP and Frt sites, was inserted into Intron 3 of the mouse solute carrier family 39 (metal ion transporter), member 8 gene (*Slc39a8*). The neo was later removed via FLP recombinase. Details can be found in Wang et al (2011) Biochem Biophys Res Commun 410(2): 289–294.

Slc39a8*(+) wild-type allele**Slc39a8*(neo) allele*****Slc39a8*(f) allele without neo****Primer Information:**

- 1) Name: Tg *Slc39a8* R Sequence: 5'-AAG CAT GTT TGT AGG GTC TGG-3'
 2) Name: M36792 flox F Sequence: 5'-GTC ATA GCA CAG GGC CAC TTA-3'

Primer Location: Both primers are located in Intron 3 of mouse *Slc39a8* just upstream of Exon 4. The primers flank the loxP and Frt sites upstream of Exon 4.

Assay Name: *Slc39a8* floxed PCR**PCR Master Mix Components:**

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
Tg <i>Slc39a8</i> R	Sigma	25µM	0.3
M36792 flox F	Sigma	25µM	0.3
FastStart <i>Taq</i>	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 an Applied Biosystems 2700 thermocycler.

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Cycle Parameters:

- 1) 95°C 3 minutes
- 2) 94°C 30 seconds
- 3) 64°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

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

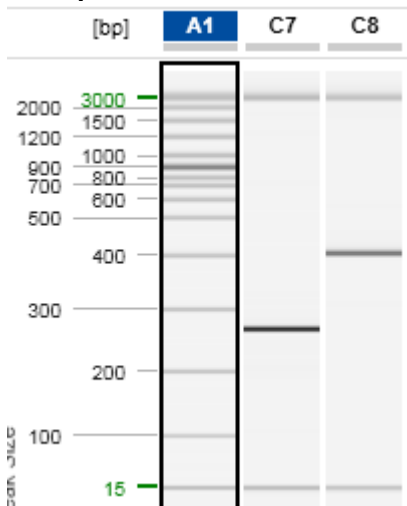
Separation: 320s at 6KV

Expected products:

Wild-type allele: 280bp

Floxed allele: 426bp

Example of Gel:



Lane A1 displays 15bp-3kb size marker.
Lane C7 displays a wild type sample (280bp product)
Lane C8 displays a homozygous floxed allele sample (426bp product)

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