08.03.16 MLS

Genotyping Protocol: **MMRRC 36790**

Assay Type: PCR; Can distinguish heterozygous animals from homozygous animals.

DNA Extraction: DNA from tail snips was extracted using the protocol provided with EMD Millipore's KOD Xtreme Hot Start DNA Polymerase Kit (Cat#71975-3). Kit directions were followed for amplification from mouse tails.

Strain Description: A *lox*P site was inserted into Intron 4 of *Zip5* (also called *Slc39a5*). A second *lox*P site and a neomycin cassette were inserted downstream from Exon 12.

Primer Information:
1) Name: M36790 Zip5 I4 F Sequence: 5'-ATC CAG GGA AGT ATC TCA GGG TTA GG -3'
2) Name: M36790 Zip5 I4 R Sequence: 5'-CAT GCC ACC TGA TCA CAG GGT C-3'
Primer Location: M36790 Zip5 I4 F and R are located in Intro 4 of *Zip5*, on either side of the *lox*P site in Intron 4. They detect the presence of the *lox*P site.

Assay Name: Zip5 flox PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
KOD Xtreme Buffer	Millipore	2X	10
KOD Xtreme dNTP	Millipore	2mM	4
M36790 Zip5 I4 F	Sigma	25µM	0.3
M36790 Zip5 I4 R	Sigma	25µM	0.3
KOD Xtreme *Taq*	Millipore (Cat# 71975-3)	1 U/µl	0.4
sterile water			3

PCR Setup:
Final Reaction: 18µl master mix & 2µl DNA template (10-20ng/µl)
All reactions were performed in 200µl thin walled PCR tubes and were run in an Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 95°C 3 minutes
2) 94°C 20 seconds
3) 68°C 25 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:
WT: 157bp
Floxed: 197bp

Example of Gel:

Lane C5 displays a WT sample (157bp product)
Lane C2 displays a sample heterozygous for the flox allele (157bp and 197bp products)
Lane B10 displays a sample homozygous for the floxed allele (197bp product)