

Genotyping Protocol: **MMRRC 36498**

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 has loxP sites inserted into Intron 5 and Exon 12 of the *Slc39a4* gene (also called *Zip4*). The loxP site in Exon 12 is followed by a MC1-neomycin cassette. Details can be found in Geiser et al (2012) PLoS Genet 8(6): e1002766.

Primer Information:

- 1) Name: M36498 Zip4 F Sequence: 5'-AGG AGG AAG AGT AGT GGA TTT CAA GG-3'
 2) Name: M36498 Zip4 WT R Sequence: 5'-CGA GCC ATA GAG ATA CCC TGT GG-3'
 3) Name: M36498 Zip4 MUT R Sequence: 5'-CTT TTC TGG ATT CAT CGA CTG TGG-3'

Primer Location: M36498 Zip4 F is located in Intron 5 of *Slc39a4* and M36498 Zip4 WT R is located at the beginning of Exon 6. These two primers are on either side of the loxP site in Intron 5. M36498 MUT R is located in the inserted neomycin cassette.

Assay Name: Zip4 flox 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
M36498 Zip4 F	Sigma	25µM	0.3
M36498 Zip4 WT R	Sigma	25µM	0.3
M36498 Zip4 MUT R	Sigma	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.7

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.

Cycle Parameters:

- 1) 95°C 3 minutes
 2) 94°C 30 seconds
 3) 68°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

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Expected products:

WT allele: 187bp

Mutant allele: 227bp

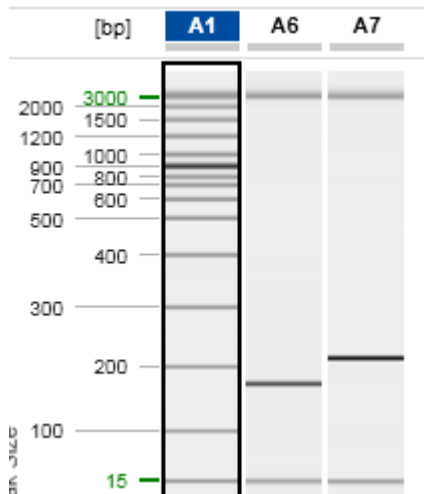
Genotype Interpretation:

Wild-type: 187bp product only

Heterozygous: 187bp product and 227bp product

Homozygous mutant: 227bp product only

Example of Gel:



Lane A1 displays 15bp-3kb size marker.

Lane A6 displays a wild type sample (187bp product)

Lane A7 displays a homozygous mutant sample (227bp product)

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