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Genotyping Protocol: **MMRRC line 30088**

Assay Type: PCR - can distinguish heterozygous animals from homozygous animals

DNA Extraction: DNA from tail snips was extracted using Sigma's RedExtract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for animal tissues were performed with a few minor modifications as follows: Use only 50 µl of Extraction Solution, 12.5 µl Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Mutation Information: In this line most of exon 1 of the *Zip4* gene (*Slc39a4*, a gene involved in zinc absorption) has been replaced with EGFP. EGFP was inserted in-frame.

Primer Information:
1) Name: mZip4WT(as) Sequence: 5'-GAA TGG AGC TGT GTG TCC CAG G-3'
2) Name: mZip4WT(s) Sequence: 5'-CAC AGG GAC TTG TGT TGG CTG TG-3'
3) Name: mZip4Mut(s) Sequence: 5'-TCA CTG CAT TCT AGT TGT GGT TTG TCC-3'

Primer location: mZip4WT(as) binds to intron 1 of *Zip4*. mZip4WT(s) binds to exon 1 of *Zip4*. mZip4Mut(s) binds to the EGFP open reading frame.

Assay Name: Zip4 KO PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
mZip4WT(as)	IDT or Sigma	25µM	0.3
mZip4WT(s)	IDT or Sigma	25µM	0.3
mZip4Mut(s)	IDT or SigmA	25µM	0.3
sterile water			5.1

PCR Setup:
Final Reaction: 16µl master mix & 4µl DNA template (10-20 ng/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:
1) 94°C 3 minutes
2) 94°C 1 minute
3) 66°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

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining

Expected products:
Wild-type: 375 bp
Heterozygous: 375 bp and 277 bp
Homozygous: 277 bp

Control DNA: positive and negative animals

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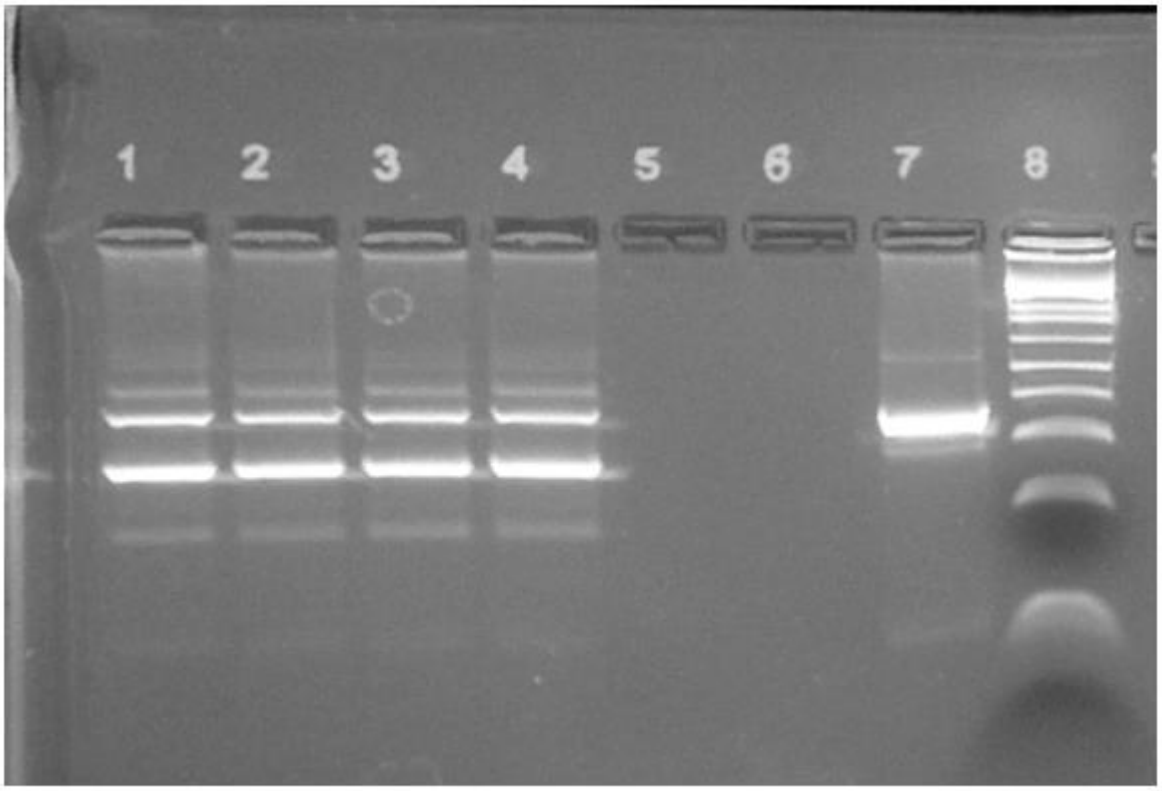
Example of Gel:



Wells 1-4 are heterozygous. Well 7 is a wild-type control. Well 8 is 1 Kb Plus DNA ladder (Invitrogen Cat. # 10787-018).