

09.22.10 MS
08.02.16 MLS

Genotyping Protocol: **MMRRC 15979**

Assay Type: PCR (can distinguish heterozygous animals from homozygous animals). This is a double knockout strain assay.

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 Characteristics: *Zip1* and *Zip3* double knockout mouse.

Zip1 KO: An EGFP reporter and *loxP*-flanked neomycin cassette were inserted into Exon 2 of *Zip1* (also called *Slc39a1*) via homologous recombination. Homozygous KO mice were crossed with Cre mice. Details can be found in Dufner-Beattie et al (2006) Genesis 44:239–251.

Zip3 KO: An EGFP reporter and *loxP*-flanked neomycin cassette were inserted into Exon 2 of *Zip3* (also called *Slc39a3*) via homologous recombination. Homozygous KO mice were crossed with Cre mice. Details can be found in Dufner-Beattie et al (2005) Mol. Cell Biol. 25(13): 5607–5615.

Zip1 KO Primer Information:

- 1) Name mZip1WT(as) Sequence: 5'- TCC GAT GCG ACT GCT TCT GG -3'
- 2) Name mZip1WT(s) Sequence: 5'- AGA TCT ATA TTG GCC TTC GCG TGC -3'
- 3) Name mZip1MUT(as) Sequence: 5'- AAC TTC AGG GTC AGC TTG CCG TAG -3'

Zip3 KO Primer Information:

- 1) Name mZip3WT(s) Sequence: 5'- CAT CAG ATC CTC TGG AAC TGG AGT TAC A -3'
- 2) Name mZip3WT(as) Sequence: 5'- AAC ACA CAG AGT ATG GAT TCT CAG AAC CC -3'
- 3) Name mZip3Mut(s) Sequence: 5'- TCA CTG CAT TCT AGT TGT GGT TT GTC C -3'
- 4) Name mZip3Mut(as) Sequence: 5'- TTA AGA GGG TGG ATC AGC CTG TAA AGT AC -3'

Assay Names: Zip1 KO PCR; Zip3 KO PCR

Zip1 KO PCR:

Master Mix Components:

Component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
mZip1WT(as)	IDT	25 µM	0.3
mZip1WT(s)	IDT	25 µM	0.3
mZip1Mut(as)	IDT	25 µM	0.3
Sterile water			5.1

PCR Setup:

Final Reaction: 16µl master mix & 4µl 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 3 minutes
- 2) 94°C 1 minute \
- 3) 64°C 1 minute 35 cycles
- 4) 72°C 1 minute /
- 6) 72°C 10 minutes
- 7) 4°C hold until refrigerate product

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Product Analysis:

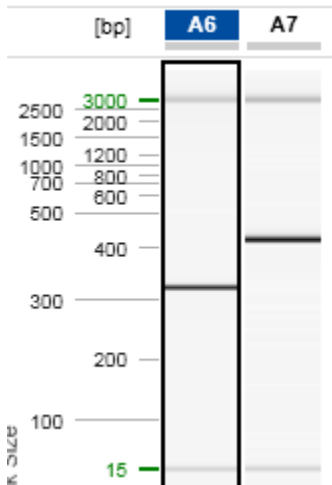
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

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:

Hom KO= 433 bp product
 WT = 328 bp product
 Het= 328 bp and 433 bp products

Example gel:



Lane A6 displays a WT sample (328bp product)
 Lane A7 displays a homozygous sample (433bp product)

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

Zip3 KO PCR

Zip3 KO WT PCR Master Mix Components:

Component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
mZip3WT(as)	IDT	25 µM	0.3
mZip3WT(s)	IDT	25 µM	0.3
Sterile water			5.4

Zip3 KO Mut PCR Master Mix Components:

Component	manufacturer	concentration	µl/rxn
Extract-N-Amp PCR Reaction Mix	Sigma (Cat#XNAT2R)	2X	10
mZip3Mut(as)	IDT	25 µM	0.3
mZip3Mut(s)	IDT	25 µM	0.3
Sterile water			5.4

PCR Setup:

Final Reaction: 16µl master mix & 4µl DNA template (10-20ng/µl)

All reactions were performed in 200µl thin walled PCR tubes and were run in Applied Biosystems 2700 thermocycler.

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Cycle Parameters:

Zip3 WT PCR

- | | | |
|----|------|--------------------------------|
| 1) | 94°C | 3 minutes |
| 2) | 94°C | 1 minute \ |
| 3) | 68°C | 1 minute 35 cycles |
| 4) | 72°C | 1 minute / |
| 6) | 72°C | 10 minutes |
| 7) | 4°C | hold until refrigerate product |

Zip3 KO PCR

- | | | |
|----|------|--------------------------------|
| 1) | 94°C | 3 minutes |
| 2) | 94°C | 1 minute \ |
| 3) | 61°C | 1 minute 35 cycles |
| 4) | 72°C | 1 minute / |
| 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:

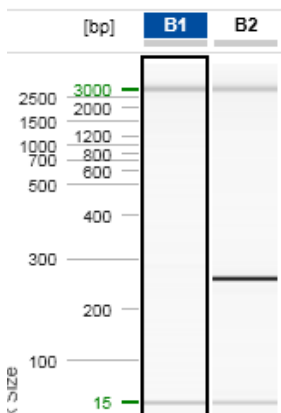
Hom KO: 267 bp product

WT: 461 bp product

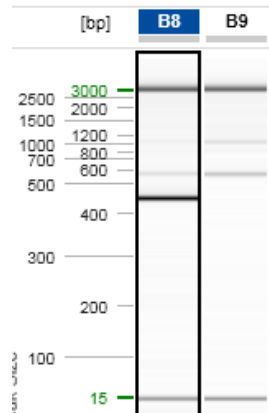
Het: 267 and 461 bp product

Example of Gel:

Mut Gel:



WT Gel:



Lane B1 displays a WT sample (no product)
Lane B2 displays a homozygous sample (267bp product)

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

Lane B8 displays a WT sample (461bp product)
Lane B9 displays a homozygous sample (no product)
there are two nonspecific bands with this PCR, ~550bp and ~1kb

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