

Genotyping Protocol: MMRRC 30290

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

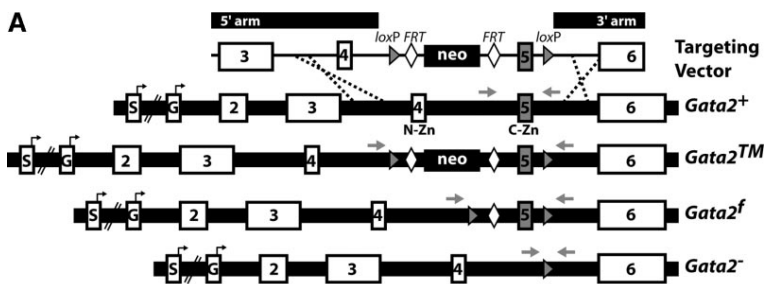
DNA Extraction: DNA from tail snips was extracted using Qiagen's DNeasy Blood and Tissue kit (Cat# 69506). Kit directions for animal tissues were performed with a few minor modifications as follows: repeat AW1 and AW2 wash steps one time, elute in 200µl of AE buffer once.

Strain Characteristics: This strain has a Neo cassette flanked by FRT sites cloned upstream of exon 5 of the *Gata2* gene. See Charles *et al.*, 2006. *Molecular Endocrinology* 20(6):1366-1377.

Primer Information:

1) Name: M30290 A Sequence: 5'- GCC TGC GTC CTC CAA CAC CTC TAA -3'
 2) Name: M30290 B Sequence: 5'- TCC GTG GGA CCT GTT TCC TTA C -3'

Primer location: Genotyping primers in intronic sequence spanning floxed exon 5.



From Charles *et al.*, 2006. *Molecular Endocrinology* 20(6):1366-1377.

Assay Name: Gata2 PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat# U1515)	1.25mM	3.2
M30290 A	Sigma	25µM	0.3
M30290 B	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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

1) 95°C 5 minutes
 2) 94°C 1 minute
 3) 60°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 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:

Floxed allele: 844 bp

WT allele: 717 bp

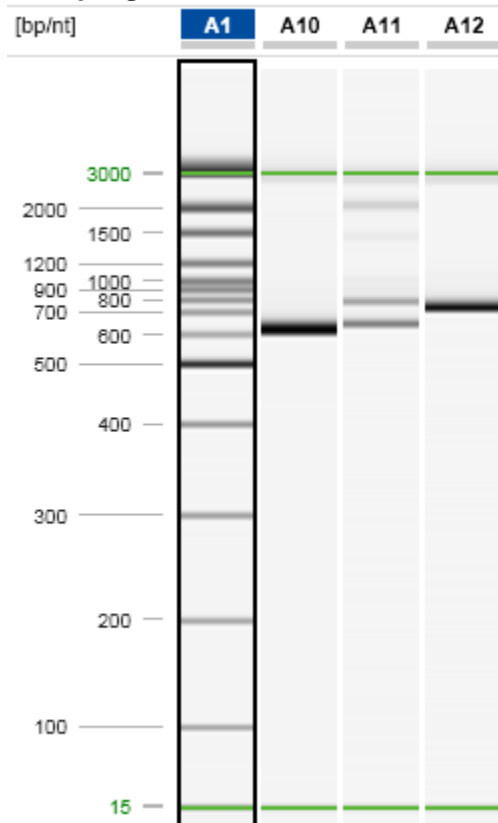
Interpretation:

Homozygous for floxed allele: 844 bp

Heterozygous: 844 bp & 717 bp

Wild-type: 717 bp

Example gel:



Lane A1 displays a 15 bp-3 kb size marker
Lane A10 displays a WT sample (717 bp product)
Lane A11 displays a heterozygous sample (717 bp and 844 bp product)
Lane A12 displays a homozygous sample (844 bp product)

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