02.18.19 MLS

Genotyping Protocol: **MMRRC 203**

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 Description: The 3rd and 4th exons of *Sod1* were replaced with a promoter-less neomycin resistance gene (CITE*neo*). Details can be found in Huang, TT *et al.* (1997) Arch Biochem Biophys 344(2):424-32.

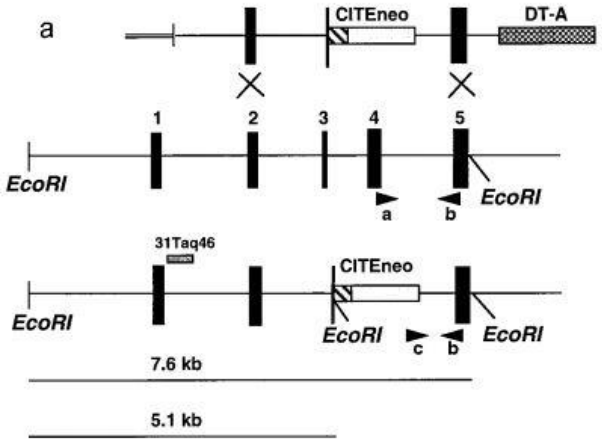


Image from Huang, TT *et al*. (1997) Arch Biochem Biophys 344(2):424-32.

Primer Information:

1) Name: M203 Mut F Sequence: 5'-CCG TCT TTT GGC AAT GTG AGG-3'
2) Name: M203 Mut R Sequence: 5'-TGG GGT ACC TTC TGG GCA TCC-3'
3) Name: M203 WT R Sequence: 5'-GGC GGA TGA AGA GAG GTG AGC-3'
4) Name: M203 WT R Sequence: 5'-ATT GGC CAC ACC GTC CTT TCC-3'

Primer location: M203 Mut F and M203 Mut R are located in the CITEneo construct. M203 WT F is located in Exon 3 of *Sod1* and M203 WT R is located in Exon 4 of *Sod1*.

Assay name: Sod1 KO 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
M203 Mut F	Sigma or IDT	25µM	0.3
M203 Mut R	Sigma or IDT	25µM	0.3
M203 WT F	Sigma or IDT	25µM	0.3
M203 WT R	Sigma or IDT	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			12.4

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:

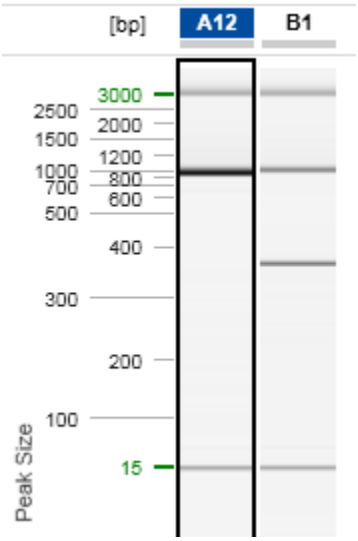
1)	95°C	4 minutes
2)	94°C	30 seconds
3)	66°C	30 seconds
4)	72°C	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	7 minutes
7)	4°C	hold until refrigerate product

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/1kb (Cat# 929521)
Size Marker: QX DNA Size Marker 50bp-800bp (Cat# 929556)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected products:
Wild type allele: 780bp product
Mutant allele: 350bp product

Example gel:



Lane A12 displays a wild-type sample (780bp product)

Lane B1 displays a heterozygous sample (780bp and 350bp products)

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