

10.09.13 MLS

12.01.17 MLS

Genotyping Protocol: **MMRRC 36956**

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 carries a deletion of exons 8 and 9 of the cytochrome P450, family 4, subfamily f, polypeptide 18 gene (*Cyp4f18*). The exons are flanked by loxP sites and deleted by Cre-recombination.

Primer Information:

1) Name: M36956 KO F	Sequence: 5'-AAG CCC ATG CCT ACA ATG AC-3'
2) Name: M36956 KO R	Sequence: 5'-GTG GTT GCC TGT GAA TTC CT-3'
3) Name: M36956 WT F	Sequence: 5'-GTG CTG TCC ATT TGT TGG TG-3'
4) Name: M36956 WT R	Sequence: 5'-GAG CAG GAA AGG AGC TCA GA-3'

Primer Location: M36956 KO F and R flank both exons 8 and 9 of *Cyp4f18*. M36956 WT F and R flank exon 9 and lie in the deleted region.

Assay Name: *Cyp4f18* KO PCR

KO 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
M36956 KO F	Sigma or IDT	25µM	0.3
M36956 KO R	Sigma or IDT	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

WT 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
M36596 WT F	Sigma or IDT	25µM	0.3
M36956 WT R	Sigma or IDT	25µM	0.3
FastStart <i>Taq</i>	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup (both KO and WT):

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 (both KO and WT):

- 1) 95°C 3 minutes
- 2) 94°C 30 seconds
- 3) 66°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

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7) 4°C hold until refrigerate product

Product Analysis (both KO and WT):

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:

KO PCR:

Mutant: 415bp

Wild-type: no product

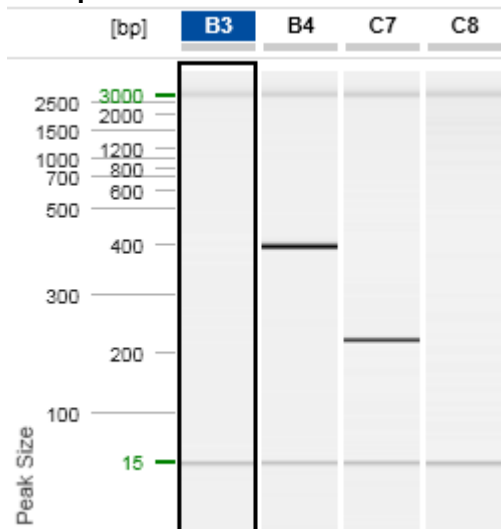
WT PCR:

Mutant: no product

Wild-type: 238bp

Genotype:	KO PCR Result	WT PCR result
<i>Wild Type</i>	No product	238bp
<i>Heterozygous</i>	415bp	238bp
<i>Homozygous mutant</i>	415bp	No product

Example of Gels:



Lane B3 displays a wild-type sample (no product) and Lane B4 displays a homozygous mutant sample (415bp product) from the KO PCR.

Lane C7 displays a wild-type sample (238bp product) and Lane C8 displays a homozygous mutant sample (no product) from the WT PCR.

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