12.23.11 MS
01.13.14 MLS

Genotyping Protocol: **MMRRC 34574**

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: This strain has a lentiviral vector transgene construct containing an EGFP/cre fusion gene under control of the mouse Thy1.2 minimal promoter inserted into intron 2 of the RIKEN cDNA 2810474O19 gene (*2810474O19Rik*).

Primer Information:
1) Name: M34574 Common R Sequence: 5'-TTT CTT GGC ACC AGA AGA CC-3'
2) Name: M34574 MUT 2F Sequence: 5'-TCC CTC AGA CCC TTT TAG TCA-3'
3) Name: M34574 WT F Sequence: 5'-TGG CAG CAA ACT TCT TTT CC-3'

Primer location: M34574 Common R is located in intron 2 of the *2810474O19Rik* gene. M34574 MUT 2F is located in the lentiviral construct. M34574 WT F is located in at the end of intron 2 of the *2810474O19Rik* gene, upstream of the lentiviral insertion point.

Assay Name: M34574 PCR

Mut 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
M34574 Common R	Sigma	25µM	0.3
M34574 MUT F	Sigma	25µM	0.3
FastStart *Taq*	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 3 minutes
2) 94°C 30 seconds
3) 63°C 30 seconds
4) 72°C 30 seconds
5) Repeat steps 2-3 34 times for a total of 35 cycles
6) 72°C 10minutes
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 100bp-3kb (Cat# 929553)

Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Expected Product: 215bp

WT 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
M34574 Common R	Sigma	25µM	0.3
M34574 WT F	Sigma	25µM	0.3
FastStart *Taq*	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:
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Product Analysis:
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Alignment Marker: QX Alignment Marker 15bp/3kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100bp-3kb (Cat# 929553)
Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Expected Product: 221bp

Genotype	Mut PCR	WT PCR
WT	no product	221bp
Heterozygous	215bp	221bp
Homozygous	215bp	no product

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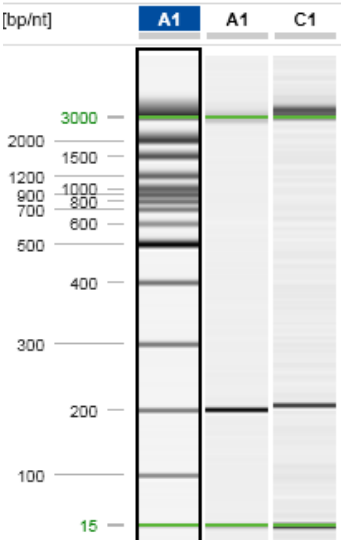
Example Gel:



Lane A1 displays a 15bp-3kb QIAxcel size ladder.

Lane A1 displays a positive sample for the MUT PCR (215bp band).

Lane C1 displays a positive sample for the WT PCR (221bp band).

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