

Genotyping Protocol: **MMRRC 31748**

Assay Type: PCR- can distinguish between heterozygous and 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 carries a Cre transgene inserted into the wingless-related MMTV integration site 3A gene (*Wnt3a*) on Chromosome 11. The transgene is inserted into exon 4 after the stop codon.

Primer Information:

- | | |
|-----------------------|--|
| 1) Name: M31748 Mut F | Sequence: 5'-GTG ACT TTG TGG TGT GGC TGG GT-3' |
| 2) Name: M31748 R | Sequence: 5'-GAT GGG AGC TGG ATG GCC TGC T-3' |
| 3) Name: M31748 WT F | Sequence: 5'-GCA TAA CGC GCG CAC TGA GC-3' |

Primer Location: M31748 Mut F is located in the transgene. M31748 R is located in exon 4 of *Wnt3a*, after the stop codon. M31748 WT F is located in exon 4 of *Wnt3a* before the stop codon.

Assay Name: Wnt3a-Cre PCR

MUT PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
10X Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega (Cat#U1515)	1.25mM	3.2
M31748 Mut F	Sigma	25µM	0.3
M31748 R	Sigma	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
10X Buffer with MgCl ₂ (green cap)	Roche	10X	2
dNTPs	Promega	1.25mM	3.2
M31748 WT F	Sigma	25µM	0.3
M31748 R	Sigma	25µM	0.3
Faststart <i>Taq</i>	Roche	5 U/µl	0.2
sterile water			13

For Both MUT and WT Master Mixes:

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 Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:

- 1) 95°C 3 minutes
- 2) 94°C 30 seconds
- 3) 70°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
- 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 50-800bp (Cat# 929561)

Method: AM320

Injection: 10s at 5KV

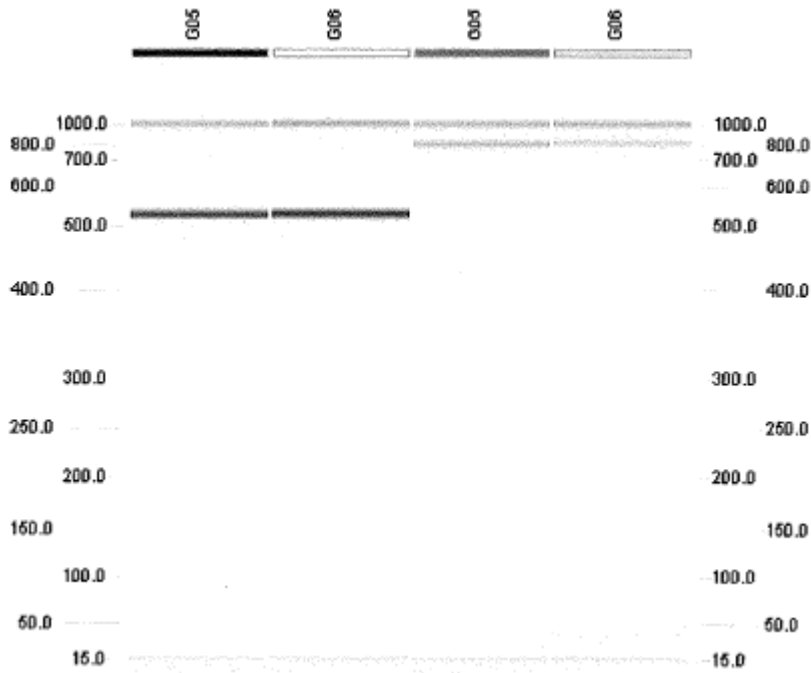
Separation: 320s at 6KV

Expected Products:

Wild type (WT): 752bp

Mutant allele (Mut): 528bp

Example of Gel:



Lanes G05 and G06 on the left display samples positive for the mutant allele (528bp band).
Lanes G05 and G06 on the right display samples positive for the WT allele (752bp band).

Please note: the 15bp and 1000bp bands are reference markers specific to the QIAxcel method and do not represent expected products.