5.02.11 MS
01.31.14 MLS

# Genotyping Protocol: **MMRRC 31778**

**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 cut-like homeobox 2 gene (*Cux2*) on Chromosome 5. The transgene is inserted into the final exon of *Cux2*.

**Primer Information**:
1) Name: M31778 Mut F Sequence: 5'-GCA GCC AGG GCT TAC CTG TAC AC-3'
2) Name: M31778 WT F Sequence: 5'-TGC AGC GGC GGC ATG AGA AA-3'
3) Name: M31778 R Sequence: 5'-CTG TGT CTG CTC CAC GAC AGC AG-3'

**Primer Location:** M31778 Mut F is located in the end of the inserted transgene. M31778 WT F is located before the transgene insertion site. M31778 R is located after the transgene in the last exon of *Cux2*.

**Assay Name: Cux2-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 |
| M31778 Mut F | Sigma | 25µM | 0.3 |
| M31778 R | 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 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 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 100-3000bp (Cat# 929553)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV
**Expected Product**: 734bp MUT product

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**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 |
| M31778 WT F | Sigma | 25µM | 0.3 |
| M31778 R | Sigma | 25µM | 0.3 |
| Faststart *Taq* | Roche | 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 Eppendorf Master Cycler or Applied Biosystems 2700 thermocycler.

**Cycle Parameters**:

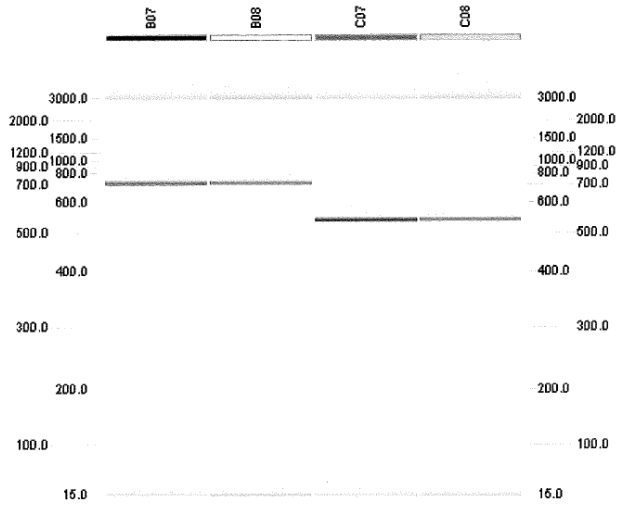
| | | |
|---|---|---|
| 1) | 95°C | 3 minutes |
| 2) | 94°C | 30 seconds |
| 3) | 70°C | 30 seconds |
| 4) | 72°C | 30 seconds |
| 5) | Repeat steps 2-4 34 times for a total of 35 cycles | |
| 6) | 72°C | 10 minutes |
| 7) | 4°C | hold until refrigerate product |

**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/3kb (Cat# 929522)
Size Marker: QX DNA Size Marker 100-3000bp (Cat# 929553)
Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

**Expected Product**: 553bp WT product

**Example of Gels:**




Lanes B07 and B08 display samples positive for the mutant allele (734bp band).
Lanes C07 and C08 display samples positive for the WT allele (553bp band).

*Please note: the 15bp and 3000bp bands seen in all lanes are reference markers specific to the Qiaxcel method and do not represent amplification products*