06.03.11 MS
10.11.13 MLS

Genotyping Protocol: **MMRRC 32778**

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 carries a transgene which contains Cre recombinase driven by the mouse cut-like homeobox 2 gene (*Cux2*) promoter.

Primer Information:
1) Name: Cux2.F Sequence: 5'-AAG ACC TAC CAT GCC ACA CC-3'
2) Name: Cux2.R Sequence: 5'-CTG CCC CAA GTG TAA TGT CA-3'
3) Name: Cre.R Sequence: 5'-GCA AAC GGA CAG AAG CAT TT-3'
Primer location: Cux2.F is located just before exon 1, and Cux2.R is located just after exon 1 of the *Cux2* gene. Cre.R is located in the *Cre* gene.

Assay name: Cux2-Cre PCR

MUT 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
Cux2.F	Sigma	25µM	0.3
Cre.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 Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

Cycle Parameters:
1) 95^oC 3 minutes
2) 94^oC 30 seconds
3) 64^oC 30 seconds
4) 72^oC 1 minute
5) Repeat steps 2-4 34 times for a total of 35 cycles
6) 72^oC 10 minutes
7) 4^oC hold until refrigerate product

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
Cux2.F	Sigma	25µM	0.3
Cux2.R	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

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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)	60°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

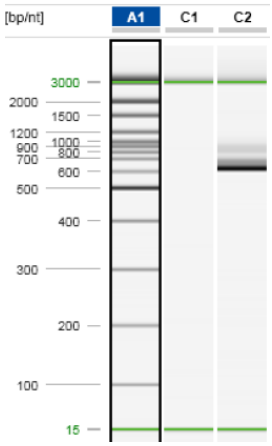
Product Analysis (Both PCRs):
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

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:
WT: 592bp
MUT: 650bp

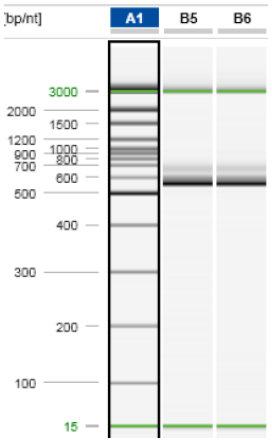
Example gels: (*Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.*)

MUT PCR Gel:



Lane A1 displays a 15bp-3kb size ladder
Lane C1 displays a sample negative for the mutant allele (no product)
Lane C2 displays a sample positive for the mutant allele (650bp product)

WT PCR Gel:



Lane A1 displays 15bp-3kb size ladder
Lanes B5 and B6 display samples positive for the WT allele (592bp product)