06.03.11 MS
03.29.12 MS

Genotyping Protocol: **MMRRC 32779**

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 with Cre-ERT2 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; ERT2 PCR

Cux2-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°C 3 minutes
2) 94°C 30 seconds
3) 64°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

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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

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

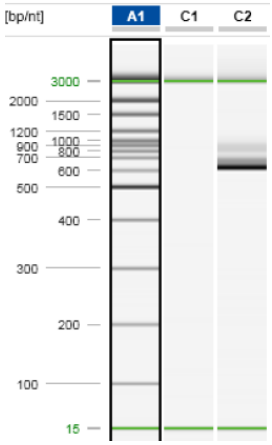
Product Analysis (Both PCRs):
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

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

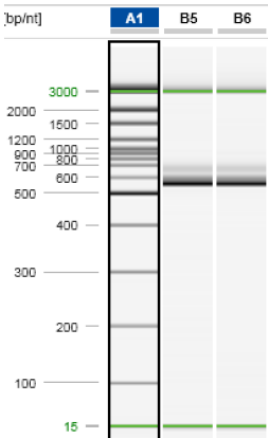
Example gels: (*Please note: the 15bp and 3kb bands are reference markers specific to the Qiaxcel method and do not represent expected products.*)

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)

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ERT2 PCR:

Primer Information:
1) Name: ERT2-F Sequence: 5'-GAT TGG TCT CGT CTG GCG CTC C-3'
2) Name: ERT2-R Sequence: 5'-ACG GCT AGT GGG CGC ATG T-3'

Primer Location: Primer set binds to the human estrogen receptor T2 (ERT2) gene designed in the pCAG-ERT2-Cre-ERT2 vector.

Assay Name: ERT2 PCR

ERT2 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
ERT2-F	IDT	25µM	0.3
ERT2-R	IDT	25µM	0.3
FastStart *Taq*	Roche (Cat# 12032953001)	5 U/µl	0.2
sterile water			13

PCR Setup:
Final Reaction: 19 µl master mix & 1 µl extracted DNA (10-20ng/µl).

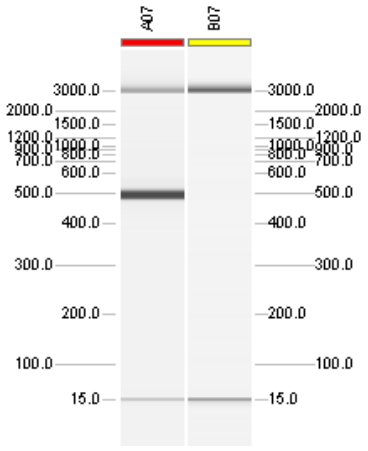
All reactions were performed in 200 µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

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:
For analysis 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 50bp-800bp (Cat# 929556)
Method: AH320 Injection: 20s at 2kV
Separation: 320s at 6kV

Expected product: 502 bp

Example gel:



Lane	Sample
A07	pCAG-ERT2-Cre-ERT2 (+)
B07	C57BL/6 (neg Control)

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