05.16.11 MS
01.07.14 MLS

Genotyping Protocol: **MMRRC 32780**

Assay Type: PCR to detect transgene positive animals- cannot distinguish hemizygous 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-ERT2 driven by the human doublecortin gene (*DCX*) promoter.

Primer Information:
1) Name: hDCX.F   Sequence: 5'-TGA ATG TCG GAT AGC TGC AC-3'
2) Name: Cre.R   Sequence: 5'-GCA AAC GGA CAG AAG CAT TT-3'
3) Name: ERT2-F   Sequence: 5'-GAT TGG TCT CGT CTG GCG CTC C-3'
4) Name: ERT2-R   Sequence: 5'-ACG GCT AGT GGG CGC ATG T-3'

Primer location: hDCX.F is located in the promoter region of the human *DCX* gene which is contained in the transgene. Cre.R is located in the *Cre* gene. ERT2-F and ERT2-R bind to the human estrogen receptor T2 (ERT2) gene designed in the pCAG-ERT2-Cre-ERT2 vector.

Assay name: M32780 PCR

hDCX-Cre 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
hDCX.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   1 minute
3)   64°C   1 minute
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

Expected product:
Positive: 899bp
Negative: no product

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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)
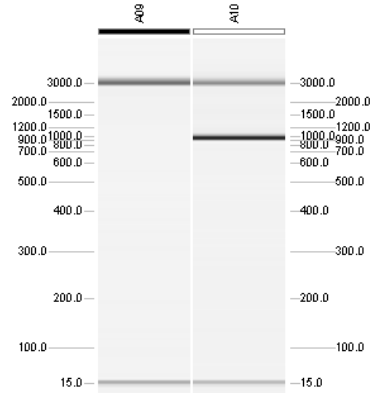   Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)
      Method: AM320   Injection: 10s at 5KV
                                Separation: 320s at 6KV

Example gel:

Lane A09 displays a transgene negative sample (no product).
Lane A10 displays a transgene positive sample (899bp product).

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

ERT2 PCR:

Note: it is not necessary to run this assay in addition to the first unless confirmation of the presence of the tamoxifen inducible element is desired.

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
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

Expected product: 502 bp

Product Analysis:
Products may be analyzed on the Qiaxcel.

For analysis on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004):
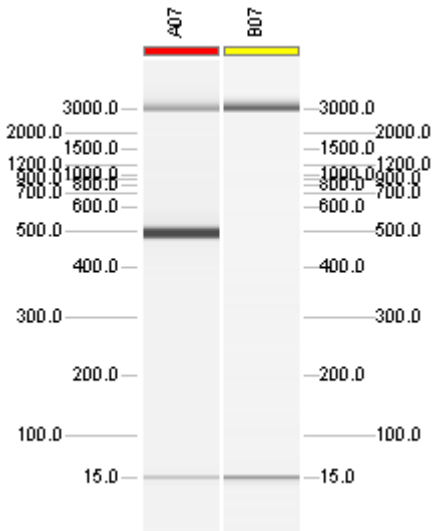   Alignment Marker: QX Alignment Marker 15bp/3kb (Cat# 929522)
      Size Marker: QX DNA Size Marker 100bp-3kb (Cat# 929553)
      Method: AH320   Injection: 20s at 2kV
                                 Separation: 320s at 6kV

Example gel:

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

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

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