Genotyping Protocol: **MMRRC 31753**

Assay Type: PCR- 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 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'

Primer location: hDCX.F is located in the promoter region of the human *DCX* gene. Cre.R is located in the cre gene.

Assay name: hDCX-Cre PCR

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
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)

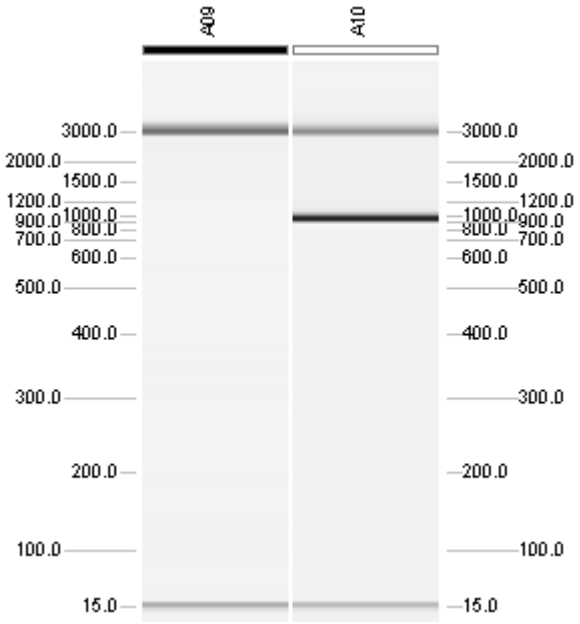
All reactions were performed in 200µl thin walled PCR tubes and were run in Perkin Elmer 2400 thermocycler or Applied Biosystems 2700 thermocycler.

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

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

Expected product:
Transgene positive: 899bp
Transgene negative: no product

Example gel:



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

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