

Genotyping Protocol: **MMRRC 31751**

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 has an IRES-Cre transgene inserted after exon 4 of the developing brain homeobox 1 gene (*Dbx1*) on Chromosome 7.

Primer Information:

- | | |
|-----------------------|--|
| 1) Name: M31751wt.F2 | Sequence: 5'-AAT CAC CTT GAG GGC AGC GCC-3' |
| 2) Name: M31751 Mut F | Sequence: 5'-GAG CAG CCT TAG AGG GTG GGG G-3' |
| 3) Name: M31751 R | Sequence: 5'-TGG CTG ACT GAT ACC CCC TGC AG-3' |

Primer location: M31751 Mut F is located in the 3' end of the inserted transgene. M31751 R is located in exon 4 after the stop codon. M31751wt.F2 is located in intron 3 of *Dbx1*.

Assay name: Dbx1-Cre PCR**MUT 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
M31751 Mut F	Sigma	25µM	0.3
M31751 R	Sigma	25µM	0.3
FastStart <i>Taq</i>	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) | 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/1Kb (Cat# 929521)

Size Marker: QX DNA Size Marker 50bp-1Kb (Cat# 929556)

Method: AM320 Injection: 10s at 5KV
 Separation: 320s at 6KV

Expected product: MUT: 538bp

WT 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
M31751 Mut F	Sigma	25µM	0.3
M31751 R	Sigma	25µM	0.3
FastStart <i>Taq</i>	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) 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)

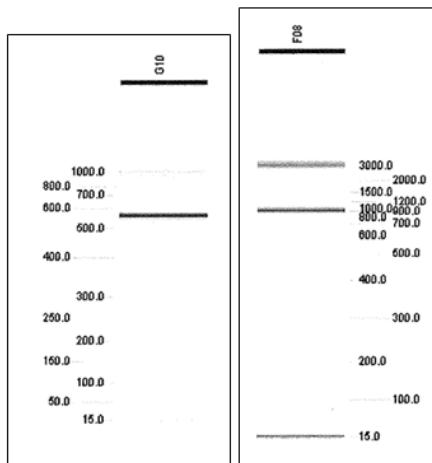
Size Marker: QX DNA Size Marker 100-3Kb (Cat# 929553)

Method: AM320 Injection: 10s at 5KV
Separation: 320s at 6KV

Expected product:

WT: 894bp

Example gels:



Lane G10 displays the 538bp product from the MUT PCR.
Lane F08 displays the 894bp product from the WT PCR.

Please note: the 15bp and 3000bp bands seen in Lane F08 and the faint 1000 bp band seen in Lane G10 are reference markers specific to the Qiaxcel method and do not represent amplification products