09.02.10 MS
08.03.16 MLS

Genotyping Protocol: **MMRRC 11562**

Assay Type: PCR can distinguish between wild type allele and mutant allele *Fxyd2*.

DNA Extraction: DNA from tail snips was extracted using Sigma's Extract-N-Amp Tissue PCR Kit (Cat#XNAT2R). Kit directions for fresh or frozen tails were performed with a few minor modifications as follows: use 50 µl of Extraction Solution and 12.5 µl of Tissue Preparation Solution and 50 µl of Neutralization Solution B.

Strain Description: Most of Exon 4 and part of Intron 4 of the mouse *Fxdy2* gene is replaced by a LacZ/Neo cassette. Details can be found in Jones et al (2005) J Biol Chem 280(19):19003-11.

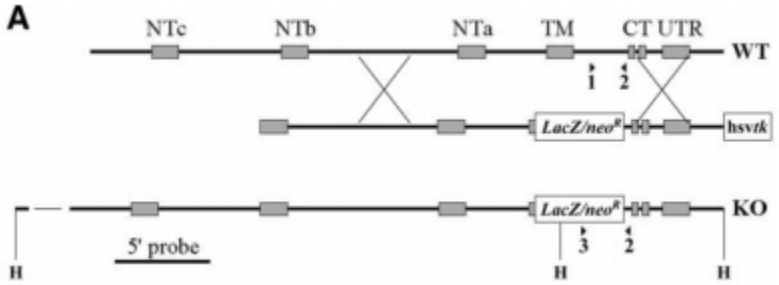

Image from Jones et al (2005) J Biol Chem 280(19):19003-11.

Primer Information:

1) Name: M11562 F:	Sequence: 5' ACC GCT TCT TTC AGT GTG CC 3'
2) Name: M11562 R:	Sequence: 5' TGG GAC CAG AGT TCG TGA TG 3'
3) Name: Fxyd2 G3	Sequence: 5' CTG TGC TGG ACT GGG GAC AT 3'
4) Name: Neo 5-1	Sequence: 5' GCT TGC CGA ATA TCA TGG TGG A 3'

Assay Name: Fxdy2 PCR

PCR Master Mix Components:

Master Mix for WT allele:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25 mM	3.2
M11562 F	Sigma	25µM	0.3
M11562 R	Sigma	25µM	0.3
FastStart *Taq*	Roche (Cat#12032953001)	5 U/µl	0.2
sterile water			13

Master Mix for Mutant allele:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$	Roche	10X	2
dNTP	Promega	1.25 mM	3.2
Fxyd2 G3	IDT	25µM	0.3
Neo 5-1	IDT	25µM	0.3
FastStart *Taq*	Roche	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 Applied Biosystems 2700 thermocycler.

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Cycle Parameters (for both WT and MUT PCRs):

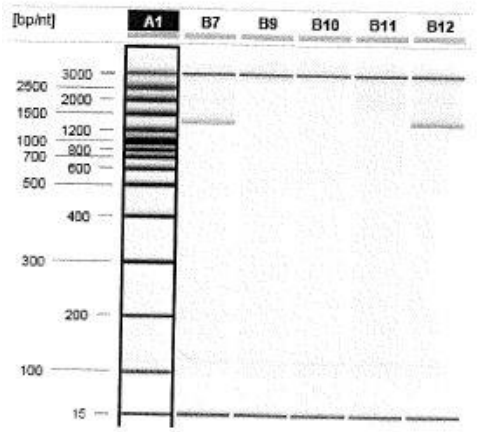
1)	94°C	5 minutes
2)	94°C	30 seconds
3)	68°C	30 seconds
4)	72°C	1 minute
5)	Repeat steps 2-4 34 times for a total of 35 cycles	
6)	72°C	7 minutes
7)	4°C	hold until refrigerate product

Product Analysis:
All products were analyzed on a 3% agarose gel with ethidium bromide staining

Expected products:
WT: 819bp product from WT PCR, no product from MUT PCR
Heterozygous: 819bp product from WT PCR, 1205bp product from MUT PCR
Homozygous mutant: no product from WT PCR, 1205bp product from MUT PCR

Example Gels:

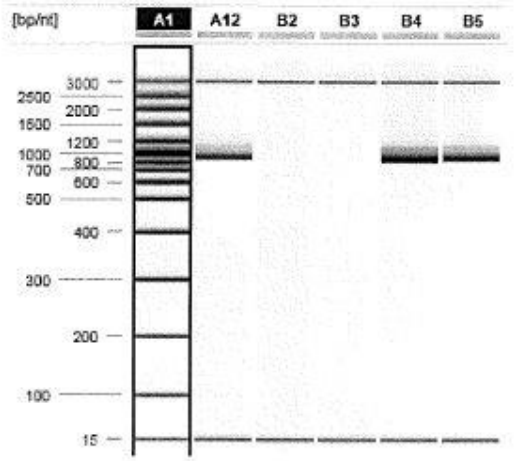
Mut Gel:



Lane A1 displays a 15bp-3kb QIAxcel size ladder (Cat#929522)
Lane B7 displays a sample positive for the Mutant allele (1205bp product)
Lanes B9 and B10 are extraction and PCR blanks, respectively.
Lane B11 is a WT control (no product on Mut PCR)
Lane B12 is a heterozygous control (1205bp product on Mut PCR)

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

WT Gel:



Lane A1 displays a 15bp-3kb QIAxcel size ladder (Cat#929522)
Lane A12 displays a sample positive for the WT allele (819bp product)
Lanes B2 and B3 are extraction and PCR blanks, respectively.
Lane B4 is a WT control (819bp product on WT PCR)
Lane B5 is a heterozygous control (819bp product on WT PCR)

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