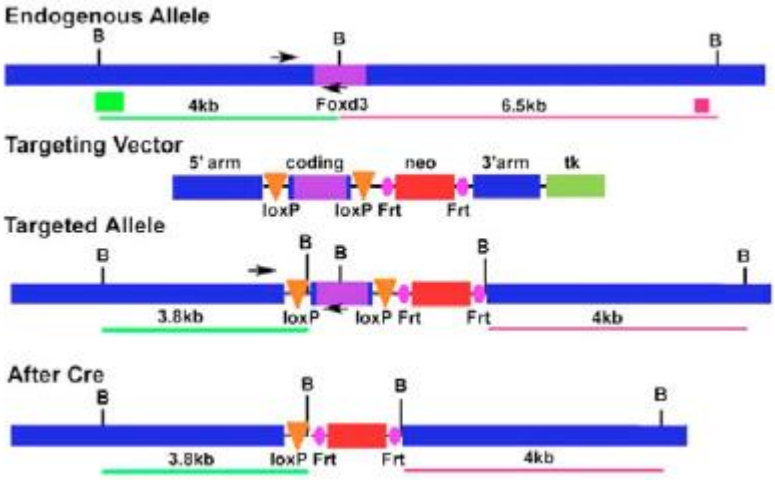
10.10.12 MLS
05.01.15 MLS

Genotyping Protocol: **MMRRC 36674**

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.

Mutation Information: This is a conditional allele of *Foxd3***.** The entire coding region of the forkhead box D3 (*Foxd3*) gene (Exon 1) is surrounded by loxP sites. Details can be found in Teng L et al., Development 2008 May;135(9):1615-24.



Primer Information:
1) Name: Foxd3 F Sequence: 5'-CGG CTT TCT TTC GGG GGA C-3'
2) Name: Foxd3 R Sequence: 5'-ACA TAT CGC TGG CGC TGC CG-3'

Primer Location: Foxd3 F is located just upstream of the *Foxd3* start codon. Foxd3 R is located just downstream of the *Foxd3* start codon. The primers detect the presence of a loxP site.

Assay Name: Foxd3 conditional PCR

PCR Master Mix Components:

component	manufacturer	concentration	µl/rxn
Buffer with $MgCl_2$ (green cap)	Roche	10X	2
dNTP	Promega (Cat# U1515)	1.25mM	3.2
Foxd3 F	Sigma	25µM	0.3
Foxd3 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 an Applied Biosystems 2700 thermocycler.

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Cycle Parameters:

1)	95°C	3 minutes
2)	94°C	20 seconds
3)	68°C	25 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

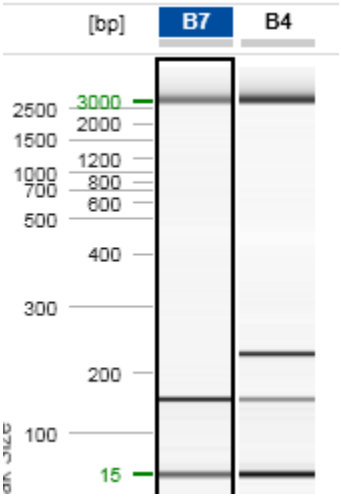
Product Analysis:
All products were analyzed on the Qiaxcel (instrument and all supplies from Qiagen) with the Qiaxcel DNA Screening Kit (Cat# 929004).

Alignment Marker: QX Alignment Marker 15bp/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 allele: 151bp
Conditional allele: 230bp

Genotypes:
WT: 151bp band only
Heterozygous for conditional allele: 151bp and 230bp bands
Homozygous for conditional allele: 230bp band only.

Example of Gel:



Lane B7 displays a WT sample (151bp product)
Lane B4 displays a heterozygous sample (151bp and 230bp products)

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