

**Name of Mouse model or mutation:****FOXN1-DELTA550-EM1-B6****FOXN1-DELTA550-EM2-B6****Description:**

Series of point mutations made by CRISPR/Cas9 gene editing.

**Type of mutation:**

One nucleotide deletion (c.1370delA) and extend the reading frame by mutating a stop (c.1515 -1516 GA&lt;AC) to mimic a clinical variant.

**Delivery method:**

Pronuclear injection into 1-cell stage embryo

**Genetic Background:**

C57BL/6J

**Nuclease:**

Cas9 mRNA

**sgRNAs:**

Protospacer sequence	PAM sequence
GGCCACCCAGTAGGTCCTGC	<b>AGG</b>
GAACCCCCTGCAGGACCTAC	<b>TGG</b>
CAGCCACGGTGCCAAGCTGA	<b>TGG</b>
CACGATACTCTACTGCCAGA	<b>TGG</b>

**IssDNA donor sequence (5'-3'):**

AGGGAGGGGGGTCTGGGCTGGGAGGCTACAAAGGAGAGAGGCCTCATGGTGTTTTCTTTGGGCC  
 TTTGCAGAAGAGCTGGACAGCCTCATTGGAGACAAAAGGGAAAACTGGGCTCTCCGCTGCTGGGC  
 TGCCACCCCCTGGGCTGGCAGGCCAGGTCCCATCCGGCCATGGCACCATCAGCTGGTCTTTCCC  
 AGCCTCTGCACCCAATGCATCCAGCTCCAGGCCCCATGCCTGGCAAGAACCtCTGCAGGACCTACTc  
 GGTGGCCATGCTCCCTCCTGCTATGGGCAGACCTACCCACCCTTTCCCCAGCCTGGCCCCTTCTGGA  
 CACtAGtAGCCATTGTTCCACAGCCAGATGGGCATCTTGAGCTGCAGGCCAGCCAGGCACCCCCC  
 AGGACTCACCTCTACCTGCCACACACCACCCAGCCACGGTGCCAAGCTacTGGCTGAGCCTTCTCA  
 GCCAGGACCATGCACGATACTCTAtTgtCAGATGGAGACCTTGGGACTGACCTGGATGCTATCAACC  
 CTTCTCACTGACTTCCAGGGTGAGCTGGGAGCTGGGAGCTGGGAAGGGTGGGACAGG  
 ACTAGAGAGGTGCTCTTGGCTGCGTGGCCTAGCCTCTTCATGTCTCTAGGCTGCCACCTGCTGGCTCC

TGAGTTTCTTGTCACCTGAGGCGGATGACTAGGAATGTTAAATTCGCATTTGCTCCCTGCCCTCCAGA  
TCATGAGCTA

### **Microinjection mixes:**

Microinjection buffer (MIB; 10 mM Tris-HCl, 0.1 mM EDTA, 100 mM NaCl, pH7.5) was prepared and filtered through a 2 nm filter and autoclaved. Cas9 mRNA, sgRNAs and lssDNA donor were diluted and mixed in MIB to the working concentrations of 100 ng/μl, 50 ng/μl each and 50 ng/μl, respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F<sub>0</sub> progeny.

### **Sequence details**

#### **WT**

TGTTCTGCTCTGGTAGACTGCTCCTGATGGCTGGAAGAATTCTGTTCCGCCATAACCTGTCCCTCAACA  
AGTGCTTTGAGAAGGTGGAGAATAAATCCGGAAGTTCCTCTCGAAAGGGCTGTCTGTGGGCCCTCA  
ATCCTTCCAAAATCGACAAGATGCAGGAAGAAGTGCAGAAGTGAAGAGGAAAGACCCCATTGCTG  
TGCGCAAAGCATGGCCAAACCAGGTGAGGCTGTCAGGCCTGTGTGAGAAAGGCCAAGGGACCTG  
GGTACCAGAATGAAGAAGAGCAGAGCCTGGGGAGAGAGGGATACAGGGAGGGGGGTCTGGGCT  
GGGAGGCTACAAAGGAGAGAGGCCTCATGGTGTTCCTTTGGGCCTTTCAGAAAGAGCTGGACAG  
CCTCATTGGAGACAAAAGGGAAAAACTGGGCTCTCCGCTGCTGGGCTGTCCACCCCCTGGGCTGGC  
AGGCCAGTCCCATCCGGCCCATGGCACCATCAGCTGGTCTTCCAGCCTCTGCACCCAATGCATC  
CAGCTCCAGGCCCATGCCTGGCAAGAACCCCCTGCAGGACCTACTGGGTGGCCATGCTCCCTCCTG  
CTATGGGCAGACCTACCCACACCTTCCCCAGCCTGGCCCTTCTGGACACCAGCAGCCATTGTTCC  
CACAGCCAGATGGGCATCTTGAGCTGCAGGCCAGCCAGGCACCCCCAGGACTCACCTCTACCTGC  
CCACACACCACCCAGCCACGGTGCCAAGCTgaTGCTGAGCCTTCCTCAGCCAGGACCATGCACGAT  
ACTCTACTGCCAGATGGAGACCTTGGGACTGACCTGGATGCTATCAACCCTTCTCTCACTGACTTCGA  
CTTCCAGGGTGAGCTGGGAGCTGGGAGCTGGGAAGGGTGGGACAGGACTAGAGAGGTGCTCTTG  
GCTGCGTGGCCTAGCCTCTTCATGTCTCTAGGCTGCCACCTGCTGGCTCCTGAGTTTCTTGTCACCTG  
AGGCGGATGACTAGGAATGTTAAATTCGCATTTGCTCCCTGCCCTCCAGATCATGAGCTAAAAGGCT  
CTTCCCCCATCTCCCCCTCCTGGTACTCTGATCATGTACCAGATGGCTTCATCATCAAACCGCTCAT  
CTGACAAAGCTGGGAAAAGCCGGAAGTTCATTGGTGTACTTGACTGTGAGGGACTGGGAGGCTA  
AACGTTCCAAAATCATTGCGTTTTATCAGAATTTAGCTTTCCTCAGGAGACAGACTTTCTATGGA  
ATGGAAGACCTCAGAGCTAAGCATGTCCTAGAGAGATTTGAGGGTACAAGCCCAGAGAGGTGAATC  
TTTACATTCTCCTCCATCTTGCCCGCTCCATCCTACAGGCAGCCAAGCCCCACCCTCTACCCTTCTAAG  
CCCCGCCCTTCCCGTTTGAAGTGTAGTGTACCGAGCAAGCCTTTTTGGAGG

#### **FOXN1-DELTA550-EM1-B6 or FOXN1-DELTA550-EM2-B6**

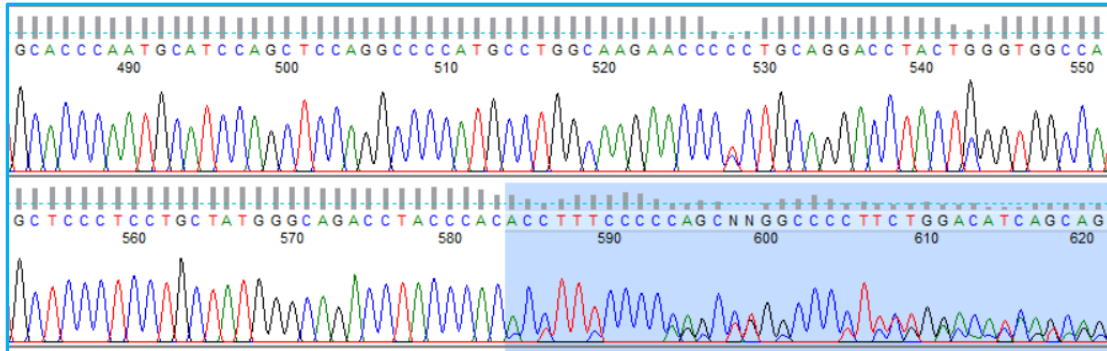
TGTTCTGCTCTGGTAGACTGCTCCTGATGGCTGGAAGAATTCTGTTCCGCCATAACCTGTCCCTCAACA  
AGTGCTTTGAGAAGGTGGAGAATAAATCCGGAAGTTCCTCTCGAAAGGGCTGTCTGTGGGCCCTCA  
ATCCTTCCAAAATCGACAAGATGCAGGAAGAAGTGCAGAAGTGAAGAGGAAAGACCCCATTGCTG  
TGCGCAAAGCATGGCCAAACCAGGTGAGGCTGTCAGGCCTGTGTGAGAAAGGCCAAGGGACCTG

GGTACCAGAATGAAGAAGAGCAGAGCCTGGGGAGAGAGGGATACAGGGAGGGGGTCTGGGCT  
GGGAGGCTACAAAGGAGAGAGGCCTCATGGTGTTCCTTTGGGCCTTGCAGAAGAGCTGGACAG  
CCTCATTGGAGACAAAAGGGAAAAACTGGGCTCTCCGCTGCTGGGCTGTCCACCCCTGGGCTGGC  
AGGCCAGGTCCCATCCGGCCCATGGCACCATCAGCTGGTCTTTCCAGCCTCTGCACCCAATGCATC  
CAGCTCCAGGCCCATGCCTGGCAAGAACCCTCTGCAGGACCTACTCGGTGGCCATGCTCCCTCCTG  
CTATGGGCAGACCTACCA~~CC~~CTTTCCCCCAGCCTGGCCCCTTCTGGACACTAGTAGCCATTGTTCCC  
ACAGCCAGATGGGCATCTTGAGCTGCAGGCCAGCCAGGCACCCCCAGGACTCACCTCTACCTGCC  
CACACACCACCCAGCCACGGTGCCAAGCTACTGGGCTGAGCCTTCTCAGCCAGGACCATGCACGATA  
CTCTATTGTCAGATGGAGACCTTGGGACTGACCTGGATGCTATCAACCCTTCTCTCACTGACTTCGAC  
TTCCAGGGTGAGCTGGGAGCTGGGAGCTGGGAAGGGTGGGACAGGACTAGAGAGGTGCTCTTGG  
CTGCGTGGCCTAGCCTCTTCATGTCTCTAGGCTGCCACCTGCTGGCTCCTGAGTTTCTGTACCTGA  
GGCGGATGACTAGGAATGTTAAATTCGCATTTGCTCCCTGCCCTCCAGATCATGAGCTAAAAGGCTC  
TTCCCCCATCTCCCCCTCTGGTACTCTGATCATGTACCAGATGGCTTCATCATCAAACCGCTCATC  
TGACAAAGCTGGGAAAAGCCGGAAGTTCCATTGGTGTACTTGACTGTGAGGGACTGGGAGGCTAA  
ACGTTCCAAAATCATTGCGTTTTATCAGAATTTAGCTTTCACCTCAGGAGACAGACTTTCTATGGAA  
TGGAAGACCTCAGAGCTAAGCATGTCCTAGAGAGATTTGAGGGTACAAGCCCAGAGAGGTGAATCT  
TTACATTCTCCTCCATCTTGCCCGCTCCATCCTACAGGCAGCCAAGCCCCACCCTCTACCCTTCTAAGC  
CCCGCCCCTTCCGTTTGAAGTGTAGTGTACCGAGCAAGCCTTTTTGGAGG

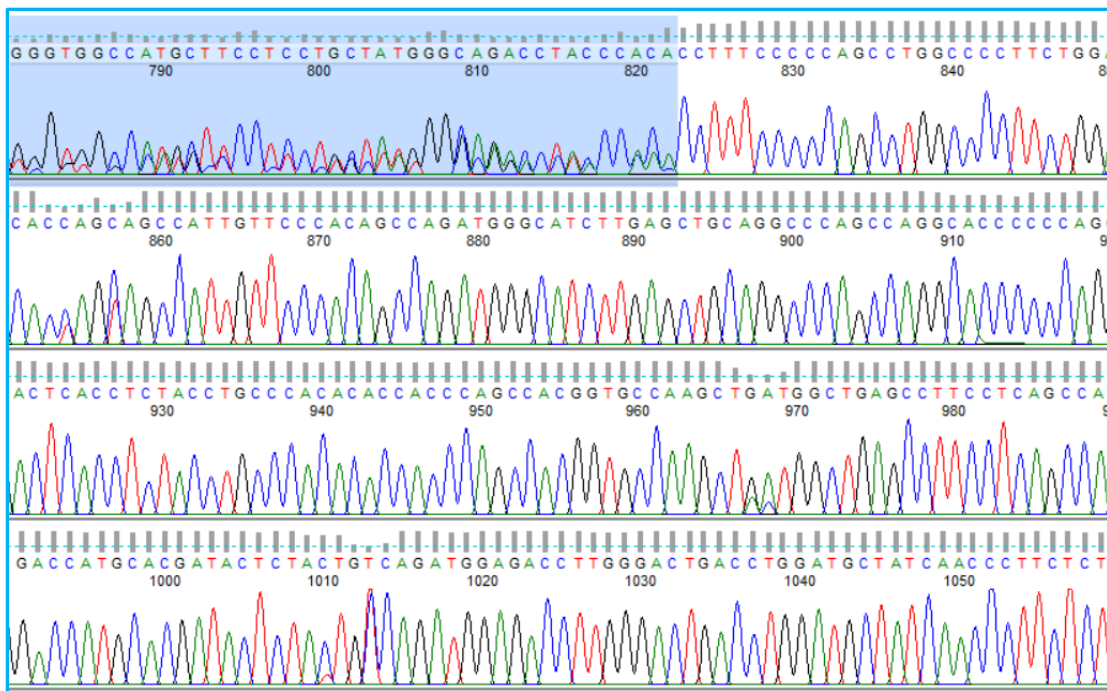
\*Silent changes in red, red highlight indicates c.1370delA, red and underlined indicates c.1515 -1516 GA<AC, yellow highlight indicates introduction of SpeI restriction site for genotyping as requested.

**FOXN1-DELTA550-EM1-B6 heterozygous F1 animal sequence trace:**

**Forward:**



**Reverse (has been reverse complemented):**



Blue indicates where sequencing trace has reached the 1 nt deletion and so becomes out of frame.

\*Please note EM1 and EM2 have the same sequence but are transmitted from two different founders.

## Nucleotide Alignment:

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*          20          *          40          *          60          *          80          *          100
Foxn1_WT : TGTTCGCTCTGGTAGACTGCTCCTGATGGCTGGAGAAATTCGTTCGGCATAACCTGTCCCTCAACAAGTGCCTTTGAGAGAGTGGAGAATAAATCCGGA
Foxn1_EM1 : TGTTCGCTCTGGTAGACTGCTCCTGATGGCTGGAGAAATTCGTTCGGCATAACCTGTCCCTCAACAAGTGCCTTTGAGAGAGTGGAGAATAAATCCGGA
TGTTCGCTCTGGTAGACTGCTCCTGATGGCTGGAGAAATTCGTTCGGCATAACCTGTCCCTCAACAAGTGCCTTTGAGAGAGTGGAGAATAAATCCGGA

*          120         *          140         *          160         *          180         *          200
Foxn1_WT : AGTTCCTCTCGAAAGGGCTGTCTGTGGGCCCTCAATCCTTCCAAAATCGACAAGATGCAGGAAGAACTGCAGAAGTGGAGAGGAAAGACCCCATTCCTG
Foxn1_EM1 : AGTTCCTCTCGAAAGGGCTGTCTGTGGGCCCTCAATCCTTCCAAAATCGACAAGATGCAGGAAGAACTGCAGAAGTGGAGAGGAAAGACCCCATTCCTG
AGTTCCTCTCGAAAGGGCTGTCTGTGGGCCCTCAATCCTTCCAAAATCGACAAGATGCAGGAAGAACTGCAGAAGTGGAGAGGAAAGACCCCATTCCTG

*          220         *          240         *          260         *          280         *          300
Foxn1_WT : TGGCGAAAAGCATGGCCAAACAGGTGAGGCTGTCAGGCCCTGTGTGAGAAAGGCCAAGGGACCTGGGTACCAGAATGAAGAAGAGCAGAGCCTGGGGAGA
Foxn1_EM1 : TGGCGAAAAGCATGGCCAAACAGGTGAGGCTGTCAGGCCCTGTGTGAGAAAGGCCAAGGGACCTGGGTACCAGAATGAAGAAGAGCAGAGCCTGGGGAGA
TGGCGAAAAGCATGGCCAAACAGGTGAGGCTGTCAGGCCCTGTGTGAGAAAGGCCAAGGGACCTGGGTACCAGAATGAAGAAGAGCAGAGCCTGGGGAGA

*          320         *          340         *          360         *          380         *          400
Foxn1_WT : GAGGGATACAGGGAGGGGGTCTGGGCTGGGAGGCTACAAGGAGAGAGGCCCTCATGGTGTTCCTTTGGGCCTTTGCAGAAGAGCTGGACAGCCTCAT
Foxn1_EM1 : GAGGGATACAGGGAGGGGGTCTGGGCTGGGAGGCTACAAGGAGAGAGGCCCTCATGGTGTTCCTTTGGGCCTTTGCAGAAGAGCTGGACAGCCTCAT
GAGGGATACAGGGAGGGGGTCTGGGCTGGGAGGCTACAAGGAGAGAGGCCCTCATGGTGTTCCTTTGGGCCTTTGCAGAAGAGCTGGACAGCCTCAT

*          420         *          440         *          460         *          480         *          500
Foxn1_WT : TGGAGACAAAAGGGAAAAAATCGGGCTCTCCGCTGCTGGGCTGTCCACCCCTGGGCTGGCAGGCCAGGTCCCACCGGGCCATGGCACCATCAGCTGGT
Foxn1_EM1 : TGGAGACAAAAGGGAAAAAATCGGGCTCTCCGCTGCTGGGCTGTCCACCCCTGGGCTGGCAGGCCAGGTCCCACCGGGCCATGGCACCATCAGCTGGT
TGGAGACAAAAGGGAAAAAATCGGGCTCTCCGCTGCTGGGCTGTCCACCCCTGGGCTGGCAGGCCAGGTCCCACCGGGCCATGGCACCATCAGCTGGT

*          520         *          540         *          560         *          580         *          600
Foxn1_WT : CTTTCCACGCTCTGCACCCAAATGCATCCAGCTCCAGGCCCATGGCTGGCAAGAACCCCTGCAGGACCTACTGGTGGCCATGCTCCCTCCTGCTATG
Foxn1_EM1 : CTTTCCACGCTCTGCACCCAAATGCATCCAGCTCCAGGCCCATGGCTGGCAAGAACCCCTGCAGGACCTACTGGTGGCCATGCTCCCTCCTGCTATG
CTTTCCACGCTCTGCACCCAAATGCATCCAGCTCCAGGCCCATGGCTGGCAAGAACCCCTGCAGGACCTACTGGTGGCCATGCTCCCTCCTGCTATG

*          620         *          640         *          660         *          680         *          700
Foxn1_WT : GGCAGACCTACCCACCTTTCCCCAGCCTGGGCCCTTCTGGACACAGGCCATTGTTCCACAGCCAGATGGGCATCTTGAGCTGAGGCCCCAGCC
Foxn1_EM1 : GGCAGACCTACCCACCTTTCCCCAGCCTGGGCCCTTCTGGACACAGGCCATTGTTCCACAGCCAGATGGGCATCTTGAGCTGAGGCCCCAGCC
GGCAGACCTACCCACCTTTCCCCAGCCTGGGCCCTTCTGGACACAGGCCATTGTTCCACAGCCAGATGGGCATCTTGAGCTGAGGCCCCAGCC

*          720         *          740         *          760         *          780         *          800
Foxn1_WT : AGGCACCCCCAGGACTCACCTCTACCTGCCACACACCACCCAGCCAGGGTGCAGCTGGGCTGAGCCTTCCACAGCCAGGACCATGCACGATAGT
Foxn1_EM1 : AGGCACCCCCAGGACTCACCTCTACCTGCCACACACCACCCAGCCAGGGTGCAGCTGGGCTGAGCCTTCCACAGCCAGGACCATGCACGATAGT
AGGCACCCCCAGGACTCACCTCTACCTGCCACACACCACCCAGCCAGGGTGCAGCTGGGCTGAGCCTTCCACAGCCAGGACCATGCACGATAGT

*          820         *          840         *          860         *          880         *          900
Foxn1_WT : CTAATGCAGATGGAGACCTTGGGACTGACCTGGATGCTATCAACCTTCTCTCACTGACTTCGACTTCCAGGGTGAAGCTGGGAGCTGGGAGTGGGAAG
Foxn1_EM1 : CTAATGCAGATGGAGACCTTGGGACTGACCTGGATGCTATCAACCTTCTCTCACTGACTTCGACTTCCAGGGTGAAGCTGGGAGCTGGGAGTGGGAAG
CTAATGCAGATGGAGACCTTGGGACTGACCTGGATGCTATCAACCTTCTCTCACTGACTTCGACTTCCAGGGTGAAGCTGGGAGCTGGGAGTGGGAAG

*          920         *          940         *          960         *          980         *          1000
Foxn1_WT : GGTGGGACAGGACTAGAGAGGTGCTTGGCTGGCTGGCTAGCCTCTCATGCTCTTAGGCTGCCACCTGCTGGCTCCGAGTTCCTTGTACCTGAGG
Foxn1_EM1 : GGTGGGACAGGACTAGAGAGGTGCTTGGCTGGCTGGCTAGCCTCTCATGCTCTTAGGCTGCCACCTGCTGGCTCCGAGTTCCTTGTACCTGAGG
GGTGGGACAGGACTAGAGAGGTGCTTGGCTGGCTGGCTAGCCTCTCATGCTCTTAGGCTGCCACCTGCTGGCTCCGAGTTCCTTGTACCTGAGG

*          1020        *          1040        *          1060        *          1080        *          1100
Foxn1_WT : CGGATGACTAGGAATGTTAAATTCGCATTTGCTCCCTGCCCTCCAGATCATGAGCTAAAAGGCTCTTTCCCCCATCTCCCCCTCCTGGTACTCTGATCA
Foxn1_EM1 : CGGATGACTAGGAATGTTAAATTCGCATTTGCTCCCTGCCCTCCAGATCATGAGCTAAAAGGCTCTTTCCCCCATCTCCCCCTCCTGGTACTCTGATCA
CGGATGACTAGGAATGTTAAATTCGCATTTGCTCCCTGCCCTCCAGATCATGAGCTAAAAGGCTCTTTCCCCCATCTCCCCCTCCTGGTACTCTGATCA

*          1120        *          1140        *          1160        *          1180        *          1200
Foxn1_WT : TGTACCAGATGGCTTTCATCATCAAAACCGCTCATCTGACAAAGCTGGGAAAAGCCGGAAGTCCATTTGGTGTACTTGACTGTGAGGACTGGGAGGCTAAA
Foxn1_EM1 : TGTACCAGATGGCTTTCATCATCAAAACCGCTCATCTGACAAAGCTGGGAAAAGCCGGAAGTCCATTTGGTGTACTTGACTGTGAGGACTGGGAGGCTAAA
TGTACCAGATGGCTTTCATCATCAAAACCGCTCATCTGACAAAGCTGGGAAAAGCCGGAAGTCCATTTGGTGTACTTGACTGTGAGGACTGGGAGGCTAAA

*          1220        *          1240        *          1260        *          1280        *          1300
Foxn1_WT : CGTTCCAAATCATTTCGTTTTATCAGAATTTAGCTTTCACTTCAGGAGACAGACTTCTATGGAATGGAAGACCTCAGAGCTAAGCATGTCTAGAGA
Foxn1_EM1 : CGTTCCAAATCATTTCGTTTTATCAGAATTTAGCTTTCACTTCAGGAGACAGACTTCTATGGAATGGAAGACCTCAGAGCTAAGCATGTCTAGAGA
CGTTCCAAATCATTTCGTTTTATCAGAATTTAGCTTTCACTTCAGGAGACAGACTTCTATGGAATGGAAGACCTCAGAGCTAAGCATGTCTAGAGA

*          1320        *          1340        *          1360        *          1380        *          1400
Foxn1_WT : GATTTGAGGGTACAAGCCAGAGAGGTGAATCTTTACATTTCTCTCCATCTTGCCCGCTCCATCCTACAGGCAGCCAGCCCAACCTCTACCTCTCTAA
Foxn1_EM1 : GATTTGAGGGTACAAGCCAGAGAGGTGAATCTTTACATTTCTCTCCATCTTGCCCGCTCCATCCTACAGGCAGCCAGCCCAACCTCTACCTCTCTAA
GATTTGAGGGTACAAGCCAGAGAGGTGAATCTTTACATTTCTCTCCATCTTGCCCGCTCCATCCTACAGGCAGCCAGCCCAACCTCTACCTCTCTAA

*          1420        *          1440        *
Foxn1_WT : GCCCCGCCCTTCCCGTTTGAAGTGTAGTGTACCGAGCAAGCCTTTTTGGAGG
Foxn1_EM1 : GCCCCGCCCTTCCCGTTTGAAGTGTAGTGTACCGAGCAAGCCTTTTTGGAGG
GCCCCGCCCTTCCCGTTTGAAGTGTAGTGTACCGAGCAAGCCTTTTTGGAGG

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## Predicted Protein Alignment:

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*          20          *          40          *          60          *          80          *          100
Foxn1_WT : ELDSLIGDKREKLSPLLGCPPLGAGPPIRPMAPSAGLSQFLHPMHPAPGPMGKPNLQDLLGGHAPSICYGQTYEHLSPSLAPSGHQPLFPQPDGHL
Foxn1_EM1 : ELDSLIGDKREKLSPLLGCPPLGAGPPIRPMAPSAGLSQFLHPMHPAPGPMGKPNLQDLLGGHAPSICYGQTYEFPFPAWPLLDTSSSHCSHSMQGLI

*          120         *          140         *          160         *          180         *          200
Foxn1_WT : ELQAQPGTQDPSPLPAHTPPSHGAKLMAEPSSARTMHDLLPDGLDGLDLDAINPSLTDLDFQGNLWEQLKDDSLALDPLVLVTSSTSSMLPPPPAAH
Foxn1_EM1 : SCRPSQAPRPTHLYLPHHPATVPSYWLSPQPGPCTILYQMETLGLTWMLSTLLSLTSTSRREICGSS*-----

*          220         *          240         *          260         *
Foxn1_WT : CFPPPGCLAETGNEAGELAPPGGSGALGDMHLSLYSAFVELESTPSSAAAGPAVYLSPGSKPLALA*
Foxn1_EM1 : -----

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### QC strategy employed at Harwell to check the edited allele:

Genomic DNA was extracted from ear clip biopsies and amplified in a PCR reaction using the following conditions/primer sequences:

Geno_Foxn1_F7 primer (5'-3')	AGGATCAACGATTCTGCCCA
Geno_Foxn1_R7 primer (5'-3')	AGGTTGATTTGCCGCCTACA
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	58
Elongation time (min)	1.25
WT product size (bp)	2276
Mutant product size (bp)	2275
Notes	Amplicons sequenced with: Geno_Foxn1_F1 (5'-3': AGGAAAGACCCCATTGCTGT) Geno_Foxn1_R1 (5'-3': TTCCGGCTTTTCCCAGCTTT) Geno_Foxn1_F6 (5'-3': TGTTCTGCTCTGGTAGACTGC) Geno_Foxn1_R6 (5'-3': CCTCCAAAAAGGCTTGCTCG)

All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site. F1 sequences should be heterozygous unless on sex chromosome.

### Off-target site with $\leq 2$ mismatches for guide(s) used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
<a href="#">16:93164540-93164562</a>	CAGCCA <b>G</b> GGTGCCAAGCTGT <b>G</b> GG	Inetrgenic	Geno_Foxn1_OT1_F1 primer (ACTTGCATGTTCTTCACAGTCTTC) Geno_Foxn1_OT1_R1 primer (CTAGCAACACGGACAACAAGC)

All amplicons were sent for Sanger sequencing. No evidence of off-target activity was detected.

### Additional integrations of the donor sequence

Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to copy count the donor sequence compared against a VIC-labelled reference assay for Dot1l:

Assay name	Foxn1-delta550-donor-UNIV1
Forward Primer (5'-3')	GGGCTGGGAGGCTACAAAG
Reverse Primer (5'-3')	TGAGGCTGTCCAGCTCTTCTG
Probe (5'-3')	AGGCCTCATGGTGTTCCTTTGGGC
Label	FAM-BHQ1

The ddPCR assay is universal to both the WT and DELTA550 mutant alleles of the Foxn1 gene. Therefore, WT animals and correct mutants will call at 2 copies. Heterozygous deletion mutants are expected to call at 1 copy and random integrants at >2 copies.

Reference Assay Name	Dot1l
Forward primer (5'-3')	GCCCCAGCACGACCATT
Reverse primer (5'-3')	TAGTTGGCATCCTTATGCTTCATC
Probe (5'-3')	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

VIC-labelled reference assay for Dot1l gene.



## Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; c.1370delA and c.1515 - 1516 GA<AC in *Foxn1*. The stock was generated at MRC Harwell via microinjection of CRISPR/Cas9 reagents into 1-cell stage embryos.

## qPCR Copy Counting Genotyping Strategy

The genotyping strategy presented here has been optimized for reagents and conditions used by the Genotyping Core at MRC Harwell. To genotype animals, we recommend researchers validate the assay independently. PCR cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Samples are genotyped using qPCR copy counting with both a wild type and a mutant assay against a known reference assay (*Dot1l* on chromosome 10; 2 copies present). Samples for this line are genotyped using the following primers and probe:

- Wild type (WT) assay with probe and reverse primer binding to the WT bases mutated in the mutant allele.
- Mutant assay with probe and reverse primer binding to the G601R, F606Y and R609H point mutations.

For autosomal genes that have been targeted, the following results would be expected:

Genotype of the Modified allele	WT Assay	Mutant Assay
Wildtype	2	0
Heterozygous	1	1
Homozygous mutant	0	2





# Foxn1-DELTA550

## Foxn1-DELTA550-WT1 assay (FAM labelled)

CTAT**GGGCAGACCTACCCACA**CCTTTCCCCAGCCTGGCCCCTTCTGGACACcAgc**AGCCATTGTTCC**  
**CACAGCCAGAT**GGGCATCTTGAGCTGCAGGCCAGCCAGGCCACCCCCAGGACTCACCTCTACCTG  
 CCCACACACCACCCAGCCACGGTGCCAAGCTga**TGGCTGAGCCTTCCTC**AGCCAGGACCATGCACGA

Lower case letters denote bases changed in the mutant allele.  
 Probe sequence is in bold and shaded grey.  
 Primer sequences are in bold and underlined.

Oligo Foxn1-DELTA550	5' label	Sequence 5' → 3'	3' label	Oligo Type
Foxn1-DELTA550-WT_F	n/a	<b><u>GGGCAGACCTACCCACA</u></b>	n/a	Wild type Forward
Foxn1-DELTA550-WT_PROBE	FAM	<b><u>AGCCATTGTTCCCACAGCCAGAT</u></b>	ZEN/IBFQ	Wild type Probe
Foxn1-DELTA550-WT_R	n/a	<b><u>GAGGAAGGCTCAGCCATC</u></b>	n/a	Wild type Reverse

## Foxn1-DELTA550-MUT1 assay (FAM labelled)

TAT**GGGCAGACCTACCCACC**CCTTTCCCCAGCCTGGCCCCTTCTGGACACtAGt**AGCCATTGTTCCCA**  
**CAGCCAGAT**GGGCATCTTGAGCTGCAGGCCAGCCAGGCCACCCCCAGGACTCACCTCTACCTGCC  
 ACACACCACCCAGCCACGGTGCCAAGCTac**TGGCTGAGCCTTCCTC**AGCCAGGACCATGCACGATAC

Lower case letters denote bases changed in the mutant allele.  
 Probe sequence is in bold and shaded grey.  
 Primer sequences are in bold and underlined.

Oligo Foxn1-DELTA550	5' label	Sequence 5' → 3'	3' label	Oligo Type
Foxn1-DELTA550-MUT_F	n/a	<b><u>GGGCAGACCTACCCACC</u></b>	n/a	Mutant Forward
Foxn1-DELTA550-MUT_PROBE	FAM	<b><u>TAGCCATTGTTCCCACAGCCAGAT</u></b>	ZEN/IBFQ	Mutant Probe
Foxn1-DELTA550-MUT_R	n/a	<b><u>GAGGAAGGCTCAGCCAGT</u></b>	n/a	Mutant Reverse



## Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**  
**CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAAGATGAAGCATAAGGATGCCAACTACTAACA  
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT  
CACCTATGA

Oligo Foxn1-DELTA550	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	<b><u>GCCCCAGCACGACCATT</u></b>	n/a	WT Forward
Dot1l_Probe	VIC	<b>CCAGCTCTCAAGTCG</b>	BHQ	WT Probe
Dot1l_Reverse	n/a	<b><u>TAGTTGGCATCCTTATGCTTCATC</u></b>	n/a	WT Reverse

Probe sequence is in bold and shaded grey  
Primer sequences are in bold and underlined

## DNA extraction method

DNA is extracted from ear clips using Applied Biosystems Taqman Sample-to-SNP Kit and qPCR run using 1:10 dilution from the crude preparation.

## qPCR master mix 1X

Applied Biosystems GTX Taqman master mix	5 µl
Dot1l_Forward (20 µM)	0.225 µl
Dot1l_Reverse (20 µM)	0.225 µl
Dot1l_Probe (5 µM)	0.2 µl
FAM Assay (probe 5 µM & primers 15 µM each)	0.3 µl
ddH2O	1.55 µl
DNA (1:10 dilution of ABI Sample-to-SNP prep)	2.5 µl

Each sample is ran in technical duplicate. Seven WT and/or mutant controls are also included in duplicate along with non-template controls.

## qPCR cycling conditions

qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

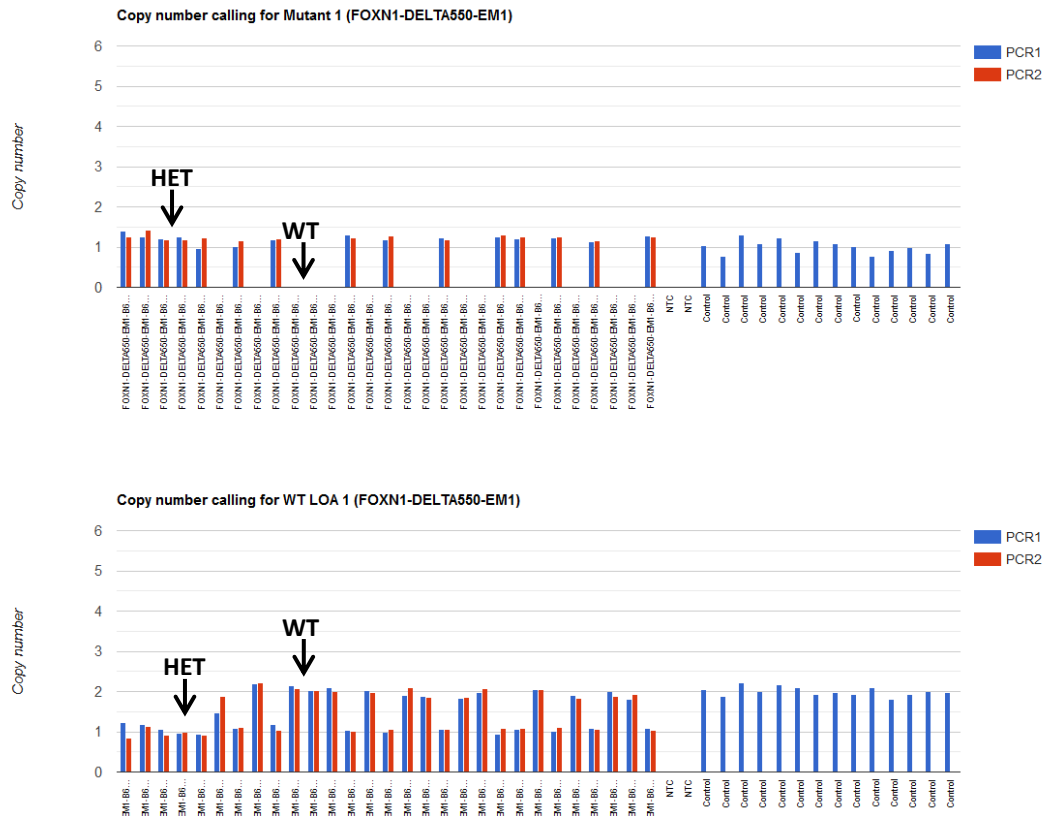
95°C for 20 sec  
Then 40 cycles of;  
95°C for 3 sec  
60°C for 30 sec



## Analysis

The results are analysed using CopyCaller software v2.0 from Applied Biosystems or in-house software that is based on CopyCaller v2.0.

Foxn1-DELTA550-WT1 and Foxn1-DELTA550 -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 298847 results)



Version No. 1  
Date: 05/08/2020  
Created/Updated by: Daniel Ford  
Approved by: Rumana Zaman