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<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	AGG
GAACCCCCTGCAGGACCTAC	TGG
CAGCCACGGTGCCAAGCTGA	TGG
CACGATACTCTACTGCCAGA	TGG

lssDNA donor sequence (5'-3'):
AGGGAGGGGGGTCTGGGCTGGGAGGCTACAAAGGAGAGAGGCCTCATGGTGTTTTCTTTTGGGCC
TTTGCAGAAGAGCTGGACAGCCTCATTGGAGACAAAAGGGAAAAACTGGGCTCTCCGCTGCTGGGC
TGTCCACCCCCTGGGCTGGCAGGCCCAGGTCCCATCCGGCCCATGGCACCATCAGCTGGTCTTTCCC
AGCCTCTGCACCCAATGCATCCAGCTCCAGGCCCCATGCCTGGCAAGAACCCtCTGCAGGACCTACTc
GGTGGCCATGCTCCCTCCTGCTATGGGCAGACCTACCCACCCTTTCCCCCAGCCTGGCCCCTTCTGGA
CACtAGtAGCCATTGTTCCCACAGCCAGATGGGCATCTTGAGCTGCAGGCCCAGCCAGGCACCCCCC
AGGACTCACCTCTACCTGCCCACACACCACCCAGCCACGGTGCCAAGCTacTGGCTGAGCCTTCCTCA
GCCAGGACCATGCACGATACTCTAtTGtCAGATGGAGACCTTGGGACTGACCTGGATGCTATCAACC
CTTCTCTCACTGACTTCGACTTCCAGGGTGAGCTGGGAGCTGGGAGCTGGGAAGGGTGGGACAGG
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_0 progeny.

Sequence details

WT

TGTTCTGCTCTGGTAGACTGCTCCTGATGGCTGGAAGAATTCTGTTCGCCATAACCTGTCCCTCAACA
AGTGCTTTGAGAAGGTGGAGAATAAATCCGGAAGTTCCTCTCGAAAGGGCTGTCTGTGGGCCCTCA
ATCCTTCCAAAATCGACAAGATGCAGGAAGAACTGCAGAAGTGGAAGAGGAAAGACCCCATTGCTG
TGCGCAAAAGCATGGCCAAACCAGGTGAGGCTGTCAGGCCTGTGTGAGAAAGGCCAAGGGACCTG
GGTACCAGAATGAAGAAGAGCAGAGCCTGGGGAGAGAGGGATACAGGGAGGGGGGTCTGGGCT
GGGAGGCTACAAAGGAGAGAGGCCTCATGGTGTTTTCTTTTGGGCCTTTGCAGAAGAGCTGGACAG
CCTCATTGGAGACAAAAGGGAAAAACTGGGCTCTCCGCTGCTGGGCTGTCCACCCCCTGGGCTGGC
AGGCCCAGGTCCCATCCGGCCCATGGCACCATCAGCTGGTCTTTCCCAGCCTCTGCACCCAATGCATC
CAGCTCCAGGCCCCATGCCTGGCAAGAACCCCCTGCAGGACCTACTGGGTGGCCATGCTCCCTCCTG
CTATGGGCAGACCTACCCACACCTTTCCCCCAGCCTGGCCCCTTCTGGACACCAGCAGCCATTGTTCC
CACAGCCAGATGGGCATCTTGAGCTGCAGGCCCAGCCAGGCACCCCCCAGGACTCACCTCTACCTGC
CCACACACCACCCAGCCACGGTGCCAAGCTgaTGGCTGAGCCTTCCTCAGCCAGGACCATGCACGAT
ACTCTACTGCCAGATGGAGACCTTGGGACTGACCTGGATGCTATCAACCCTTCTCTCACTGACTTCGA
CTTCCAGGGTGAGCTGGGAGCTGGGAGCTGGGAAGGGTGGGACAGGACTAGAGAGGTGCTCTTG
GCTGCGTGGCCTAGCCTCTTCATGTCTCTAGGCTGCCACCTGCTGGCTCCTGAGTTTCTTGTCACCTG
AGGCGGATGACTAGGAATGTTAAATTCGCATTTGCTCCCTGCCCTCCAGATCATGAGCTAAAAGGCT
CTTTCCCCCATCTCCCCCCTCCTGGTACTCTGATCATGTACCAGATGGCTTCATCATCAAACCGCTCAT
CTGACAAAGCTGGGAAAAGCCGGAAGTTCCATTGGTGTACTTGACTGTGAGGGACTGGGAGGCTA
AACGTTCCAAAATCATTTGCGTTTTATCAGAATTTAGCTTTCACTTCAGGAGACAGACTTTCTATGGA
ATGGAAGACCTCAGAGCTAAGCATGTCCTAGAGAGATTTGAGGGTACAAGCCCAGAGAGGTGAATC
TTTACATTCTCCTCCATCTTGCCCGCTCCATCCTACAGGCAGCCAAGCCCCACCCTCTACCCTTCTAAG
CCCCGCCCCTTCCCGTTTGAACTGTAGTGTACCGAGCAAGCCTTTTTGGAGG

FOXN1-DELTA550-EM1-B6 or FOXN1-DELTA550-EM2-B6

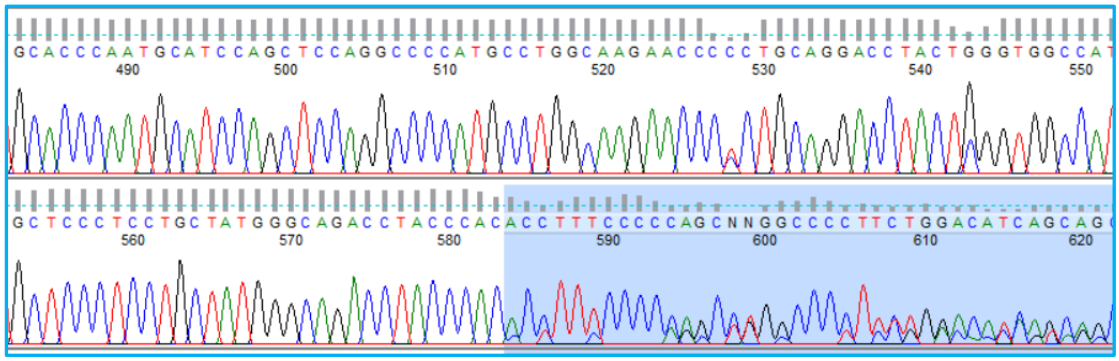
TGTTCTGCTCTGGTAGACTGCTCCTGATGGCTGGAAGAATTCTGTTCGCCATAACCTGTCCCTCAACA
AGTGCTTTGAGAAGGTGGAGAATAAATCCGGAAGTTCCTCTCGAAAGGGCTGTCTGTGGGCCCTCA
ATCCTTCCAAAATCGACAAGATGCAGGAAGAACTGCAGAAGTGGAAGAGGAAAGACCCCATTGCTG
TGCGCAAAAGCATGGCCAAACCAGGTGAGGCTGTCAGGCCTGTGTGAGAAAGGCCAAGGGACCTG

GGTACCAGAATGAAGAAGAGCAGAGCCTGGGGAGAGAGGGATACAGGGAGGGGGGTCTGGGCT
GGGAGGCTACAAAGGAGAGAGGCCTCATGGTGTTTTCTTTTGGGCCTTTGCAGAAGAGCTGGACAG
CCTCATTGGAGACAAAAGGGAAAAACTGGGCTCTCCGCTGCTGGGCTGTCCACCCCCTGGGCTGGC
AGGCCCAGGTCCCATCCGGCCCATGGCACCATCAGCTGGTCTTTCCCAGCCTCTGCACCCAATGCATC
CAGCTCCAGGCCCCATGCCTGGCAAGAACCCTCTGCAGGACCTACTCGGTGGCCATGCTCCCTCCTG
CTATGGGCAGACCTACCCACCCTTTCCCCCAGCCTGGCCCCTTCTGGACACTAGTAGCCATTGTTCCC
ACAGCCAGATGGGCATCTTGAGCTGCAGGCCCAGCCAGGCACCCCCCAGGACTCACCTCTACCTGCC
CACACACCACCCAGCCACGGTGCCAAGCTACTGGCTGAGCCTTCCTCAGCCAGGACCATGCACGATA
CTCTATTGTCAGATGGAGACCTTGGGACTGACCTGGATGCTATCAACCCTTCTCTCACTGACTTCGAC
TTCCAGGGTGAGCTGGGAGCTGGGAGCTGGGAAGGGTGGGACAGGACTAGAGAGGTGCTCTTGG
CTGCGTGGCCTAGCCTCTTCATGTCTCTAGGCTGCCACCTGCTGGCTCCTGAGTTTCTTGTCACCTGA
GGCGGATGACTAGGAATGTTAAATTCGCATTTGCTCCCTGCCCTCCAGATCATGAGCTAAAAGGCTC
TTTCCCCCATCTCCCCCCTCCTGGTACTCTGATCATGTACCAGATGGCTTCATCATCAAACCGCTCATC
TGACAAAGCTGGGAAAAGCCGGAAGTTCCATTGGTGTACTTGACTGTGAGGGACTGGGAGGCTAA
ACGTTCCAAAATCATTTGCGTTTTATCAGAATTTAGCTTTCACTTCAGGAGACAGACTTTCTATGGAA
TGGAAGACCTCAGAGCTAAGCATGTCCTAGAGAGATTTGAGGGTACAAGCCCAGAGAGGTGAATCT
TTACATTCTCCTCCATCTTGCCCGCTCCATCCTACAGGCAGCCAAGCCCCACCCTCTACCCTTCTAAGC
CCCGCCCCTTCCCGTTTGAACTGTAGTGTACCGAGCAAGCCTTTTTGGAGG

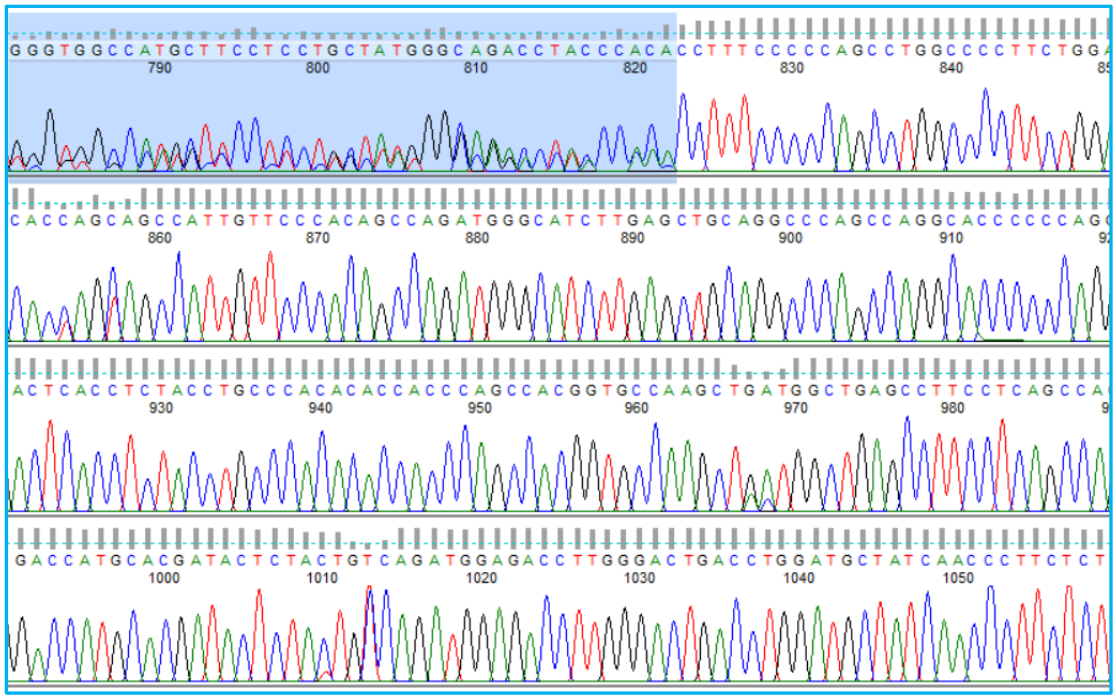
*Silent changes in red, red highlight indicates c.1370delA, red and underlined indicates c.1515 -1516 GA<AC, yellow highlight indicates introduction of SpeI restriction site for genotyping as requested.

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

Forward:



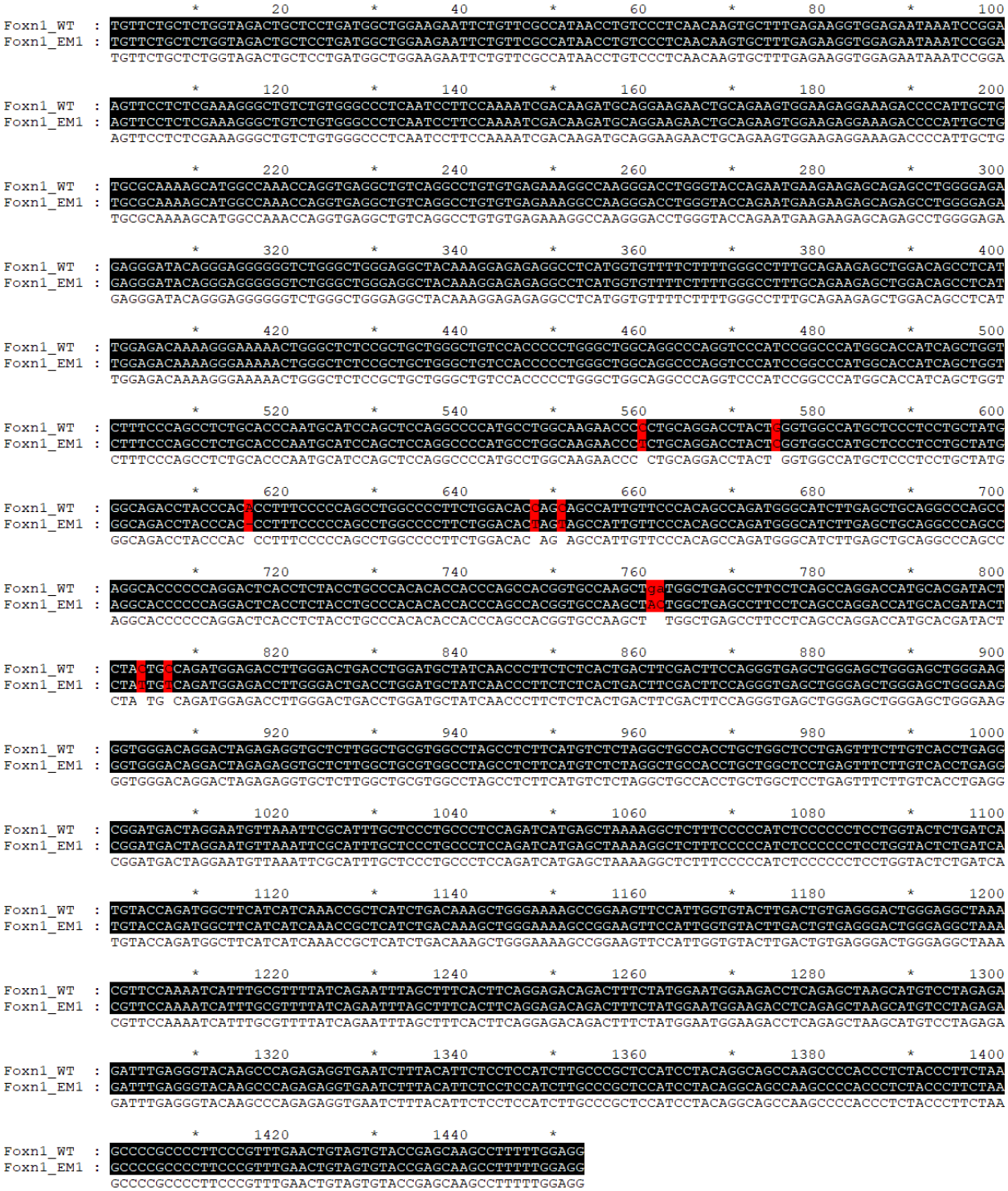
Reverse (has been reverse complemented):



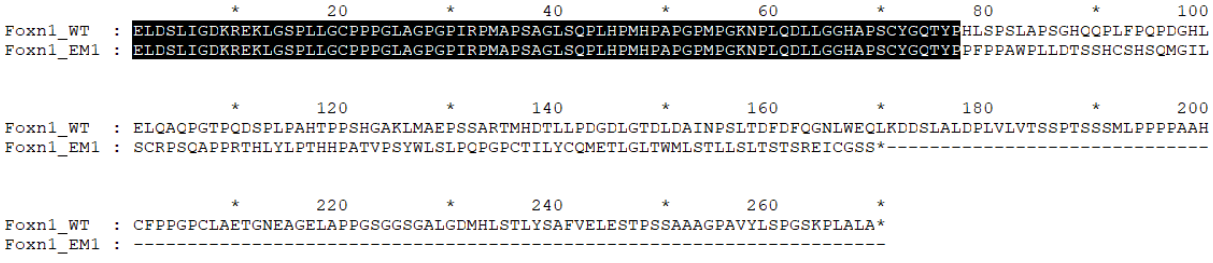
Blue indicates where sequencing trace has reached the 1 nt deletion and so becomes out of frame.

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

Nucleotide Alignment:



Predicted Protein Alignment:



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 ≤2 mismatches for guide(s) used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
16:93164540-93164562	CAGCCAGGGTGCCAAGCTGT GGG	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’)	AGGCCTCATGGTGTTTTCTTTTGGGC
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.

Foxn1-DELTA550

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**CCTTTCCCCCAGCCTGGCCCCTTCTGGACACcAGc**AGCCATTGTTCC**
CACAGCCAGATGGGCATCTTGAGCTGCAGGCCCAGCCAGGCACCCCCCAGGACTCACCTCTACCTG
CCCACACACCACCCAGCCACGGTGCCAAGCT**gaTGGCTGAGCCTTCCTC**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	**GGGCAGACCTACCCACA**	n/a	Wild type Forward
Foxn1-DELTA550-WT_PROBE	FAM	**AGCCATTGTTCCCACAGCCAGAT**	ZEN/IBFQ	Wild type Probe
Foxn1-DELTA550-WT_R	n/a	**GAGGAAGGCTCAGCCATC**	n/a	Wild type Reverse

Foxn1-DELTA550-MUT1 assay (FAM labelled)

TAT**GGGCAGACCTACCCACC**CTTTCCCCCAGCCTGGCCCCTTCTGGACACtAG**tAGCCATTGTTCCCA**
CAGCCAGATGGGCATCTTGAGCTGCAGGCCCAGCCAGGCACCCCCCAGGACTCACCTCTACCTGCCC
ACACACCACCCAGCCACGGTGCCAAGCT**acTGGCTGAGCCTTCCTC**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	**GGGCAGACCTACCCACC**	n/a	Mutant Forward
Foxn1-DELTA550-MUT_PROBE	FAM	**TAGCCATTGTTCCCACAGCCAGAT**	ZEN/IBFQ	Mutant Probe
Foxn1-DELTA550-MUT_R	n/a	**GAGGAAGGCTCAGCCAGT**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT CACCCTATGA

Oligo Foxn1-DELTA550	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	**GCCCCAGCACGACCATT**	n/a	WT Forward
Dot1l_Probe	VIC	**CCAGCTCTCAAGTCG**	BHQ	WT Probe
Dot1l_Reverse	n/a	**TAGTTGGCATCCTTATGCTTCATC**	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
ddH20	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

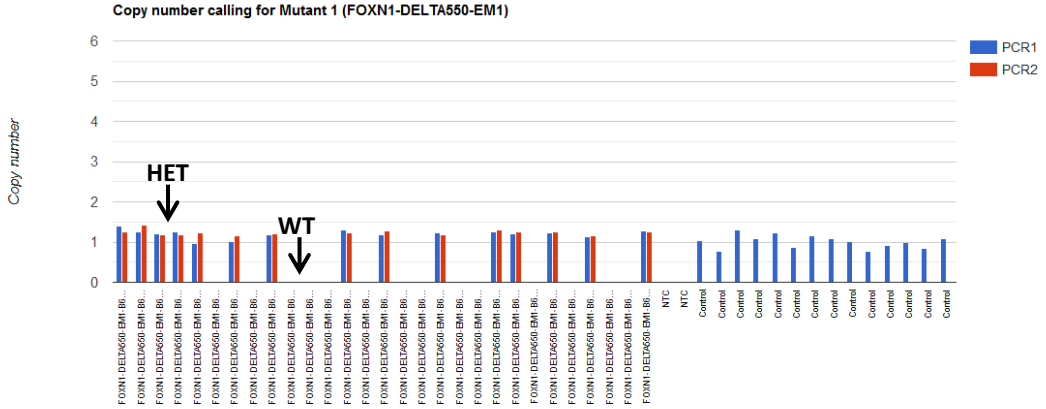
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

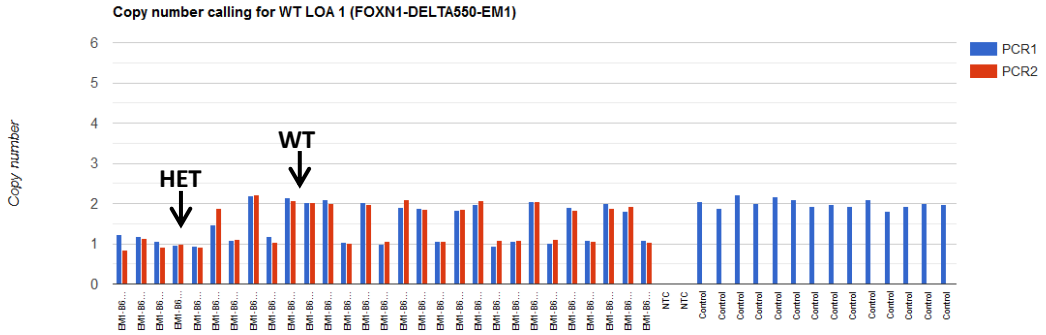
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

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)





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