Name of Mouse model or mutation:

Scn9a-DEL713-EM1-B6
Scn9a-DEL790-EM2-B6

Description:

Exon deletion generated using CRISPR/Cas9 reagents.

Type of mutation:

Exon deletion of ENSMUSE00001003218.

Sequence details:

WT

AGCTGCCTCCCTAGTGATTTCAATTACTTAGGCATATAGCATTTGGAGATCGACCCAGTGTAAATTCT
GGGTCTGCTACATTGTGTAACCAACAAGCAGCAAATACTAGTTGATCAAAATAAAAGTAAAGTGATT
TTTATTCATAGTTTTTAGTATTTTATGTCTCGAAACACTTTGCAATATTTATTATCTTTGCCAATAAACT
TTAAAGATTACTGGCTGTTTGATGTTTCTATGAAATCTAGTAAAGATATATTTATAAACAATGGAAAC
ACAATAAAATCAATATGACTCCAGTATGATCACTTTTACAATTTTATTTGGAAATTAAGGGAATTCAT
TCAGTCATTTACTTGGTGACTTGCCCTATCAATCTGAGATCAATCACTGCCTATGATAAAGTCCTTTAT
ACATTACTTGAAGATTTTGTGTGTATACAGTGTGAGCACGTACTTATTGCTGAATATGTGTACTTCTG
AAATACTTTTTCCAGTCCACATTCAACTGGAAATAATCAATGTTACTGTCAACATGCTATTGACTTTTT
CTGCAGGTAAATGCACAACCAATATATGAATACAACCTCTACATGTACATTTATTTTGTCATCTTCATC
ATCTTTGGCTCATTCTTCACTTTGAACTTGTTCATTGGTGTCATCATCGATAATTTCAACCAACAGAAG
AAGAAGATAAGTATTTTAAGTGTTTCCTACATTGCACTGAAAAAGAATGAACTTCATATAAATGGATT
ATTTTGCATTTATTTTTCAAAATAATTGGTCAACTATGTAATTGTATATATGTATAATCTTATTGTTTTG
TTCTGTAATTTTAAATGTTATATCAATAGTTTTAATTAAATTATTACATTATATTATATATAGTTACATA
TTATTATCTATTATATTTATTATCATTTATTTATAGTATTATTTATTCATATACTACTATATATTAAAATT
TATTAAAATATACTTATAATTAAACACAATAGTCTTAAACATTATATTAATTCTCTGATTTCTCTGATGT
TGCCTATAGAGGACTGAATAATAAATATACCACTAGCTCAAAGCAGTAAGGTACTTTTTTCAAAGTA
GTTTTATTTATTTATTTTCAGAGGTATTCTTTTTTTCCTGCTAACCATCATTCTGGGGTATGTGTTATGA
TGGAAGTTAAGTGACAGTTACTTATAATATGGCTGCTGAACTCATGTCACTTATGTTATAGCATAAGA
AATAATACTACTGAAAGTAAGACCTGCTGAGTGTGTGTGTCAATCAC

*Red text indicates extremity of largest deletion i.e. EM2.

Mutant

Scn9a-DEL713-EM1-B6

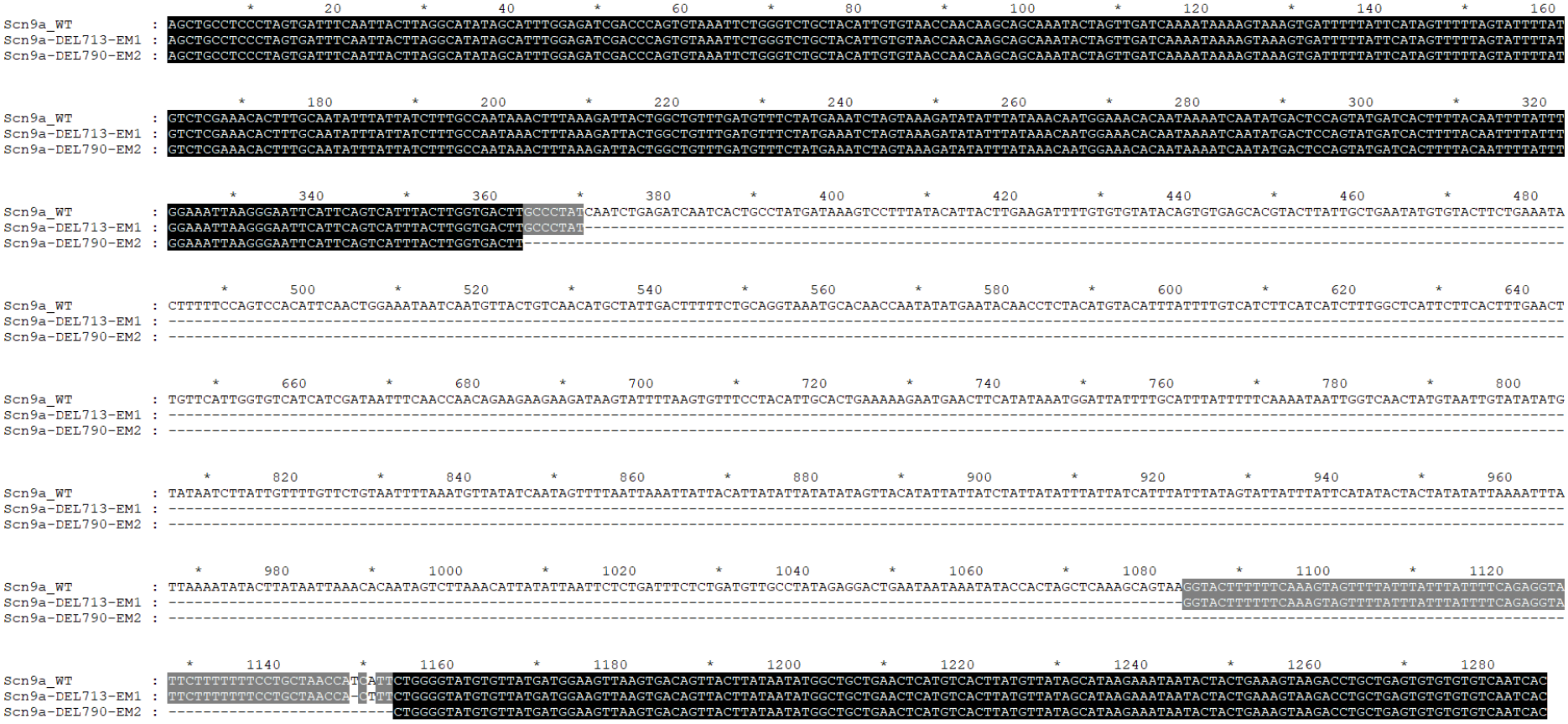
AGCTGCCTCCCTAGTGATTTCAATTACTTAGGCATATAGCATTTGGAGATCGACCCAGTGTAAATTCT
GGGTCTGCTACATTGTGTAACCAACAAGCAGCAAATACTAGTTGATCAAAATAAAAGTAAAGTGATT
TTTATTCATAGTTTTTAGTATTTTATGTCTCGAAACACTTTGCAATATTTATTATCTTTGCCAATAAACT

TTAAAGATTACTGGCTGTTTGATGTTTCTATGAAATCTAGTAAAGATATATTTATAAACAATGGAAAC
ACAATAAAATCAATATGACTCCAGTATGATCACTTTTACAATTTTATTTGGAAATTAAGGGAATTCAT
TCAGTCATTTACTTGGTGACTTGCCCTAT**[713_nt_del]**GGTACTTTTTTCAAAGTAGTTTTATTTATTT
ATTTTCAGAGGTATTCTTTTTTTCCTGCTAACCA**[3_nt_replaced_by_2_nt_(CT)]**TTCTGGGGTATG
TGTTATGATGGAAGTTAAGTGACAGTTACTTATAATATGGCTGCTGAACTCATGTCACTTATGTTATA
GCATAAGAAATAATACTACTGAAAGTAAGACCTGCTGAGTGTGTGTGTCAATCAC

Scn9a-DEL790-EM2-B6

AGCTGCCTCCCTAGTGATTTCAATTACTTAGGCATATAGCATTTGGAGATCGACCCAGTGTAAATTCT
GGGTCTGCTACATTGTGTAACCAACAAGCAGCAAATACTAGTTGATCAAAATAAAAGTAAAGTGATT
TTTATTCATAGTTTTTAGTATTTTATGTCTCGAAACACTTTGCAATATTTATTATCTTTGCCAATAAACT
TTAAAGATTACTGGCTGTTTGATGTTTCTATGAAATCTAGTAAAGATATATTTATAAACAATGGAAAC
ACAATAAAATCAATATGACTCCAGTATGATCACTTTTACAATTTTATTTGGAAATTAAGGGAATTCAT
TCAGTCATTTACTTGGTGACTT**[790_nt_del]**CTGGGGTATGTGTTATGATGGAAGTTAAGTGACAGT
TACTTATAATATGGCTGCTGAACTCATGTCACTTATGTTATAGCATAAGAAATAATACTACTGAAAGT
AAGACCTGCTGAGTGTGTGTGTCAATCAC

Nucleotide 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:

Scn9a_F1449V_F7 primer (5'-3')	AGCTGCCTCCCTAGTGATTTC
Scn9a_F1449V_R7 primer (5'-3')	GTGATTGACACACACACTCAGC
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	61
Elongation time (min)	1.5
WT product size (bp)	1286
Mutant product size (bp)	550
Notes	Primers used for sequencing: Geno_Scn9a_F6 (5'-3'): AGCATTTGGAGATCGACCCAG Geno_Scn9a_R6 (5'-3'): TGACACACACACTCAGCAGG

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.

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	Scn9a-F1449V-UNI1
Forward Primer (5'-3')	TCAGTGCAATGTAGGAAACACTTA
Reverse Primer (5'-3')	CATCTTCATCATCTTTGGCTCATTCTTC
Probe (5'-3')	CTTATCTTCTTCTTCTGTTGG
Label	FAM-BHQ1

The Scn9a-F1449V-UNI1 ddPCR assay recognises both the WT and the intended F1449V alleles of the gene. WT controls and correct mutants are expected to call at 2 copies. Deletion mutants are expected to call at 1 copy for F1 (HET) animals. Random insertions of the donor are expected to call 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.

Scn9a-DEL713

Allele Description

This is a CRISPR/Cas9 induced mutation deleting 713 nucleotides of the *Scn9a* gene. 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 wildtype loss of allele (WT-LOA) 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:

- Universal probe and Universal primer designed 5' of the deleted region.
- Wildtype specific primer situated within the deleted region.
- Mutant specific primer that either bridges the junction designed for the CRISPR mutant allele or is 3' of the junction

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

Scn9a-DEL713

Scn9a-DEL713-WT1 assay (FAM labelled)

TTAAAGA**TTACTGGCTGTTTGATGTTTCTATGAAA**TCTAGTAAAGATATATTTATAAACAATGGAAA
CACAATAAAAT**CAATATGACTCCAGTATGATCACTTTTACA**ATTTTATTTGGAAATTAAGGGAATTCA
TTCAGTCATTTACTTGGTGACTTgccctat**caatctgagatcaatcactgcctatg**ataaagtcctttatacattacttgaa

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

Oligo Scn9a-DEL713	5' label	Sequence 5' → 3'	3' label	Oligo Type
Scn9a-DEL713-UNI_F	n/a	**TTACTGGCTGTTTGATGTTTCTATGAAA**	n/a	Universal Forward
Scn9a-DEL713-UNI_PROBE	FAM	**CAATATGACTCCAGTATGATCACTTTTACA**	BHQ	Universal Probe
Scn9a-DEL713-WT_R	n/a	**CATAGGCAGTGATTGATCTCAGATTG**	n/a	WT Reverse

Scn9a-DEL713-MUT1 assay (FAM labelled)

TTAAAGA**TTACTGGCTGTTTGATGTTTCTATGAAA**TCTAGTAAAGATATATTTATAAACAATGGAAA
CACAATAAAAT**CAATATGACTCCAGTATGATCACTTTTACA**ATTTTATTTGGAAATTAAGGGAATTCA
TTCAGTCATTTACTTGGTGACTTG**CCCTATGGTACTTTTTTCAAAGTAGTTTT**ATTTATTTATTTTCAG

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

Oligo Scn9a-DEL713	5' label	Sequence 5' → 3'	3' label	Oligo Type
Scn9a-DEL713-UNI_F	n/a	**TTACTGGCTGTTTGATGTTTCTATGAAA**	n/a	Universal Forward
Scn9a-DEL713-UNI_PROBE	FAM	**CAATATGACTCCAGTATGATCACTTTTACA**	BHQ	Universal Probe
Scn9a-DEL713-MUT_R	n/a	**AAAACTACTTTGAAAAAAGTACCATAGGG**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

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

Oligo Scn9a-DEL713	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

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 5µM & primers 15µM 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. Non-template controls are also run.

qPCR cycling conditions

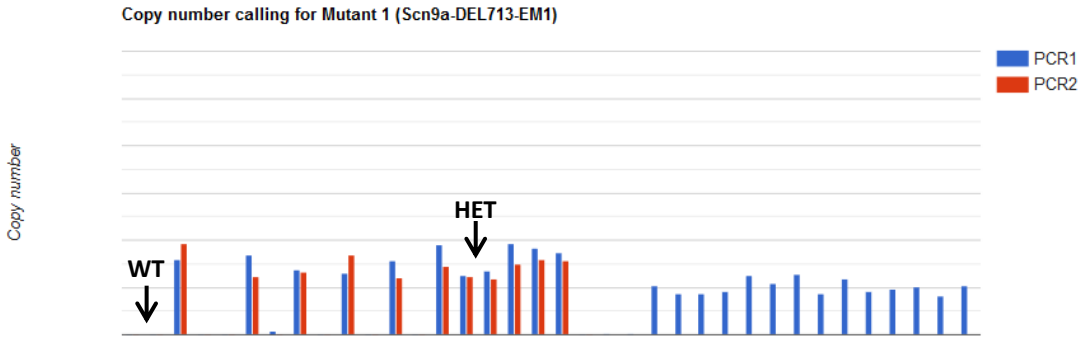
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

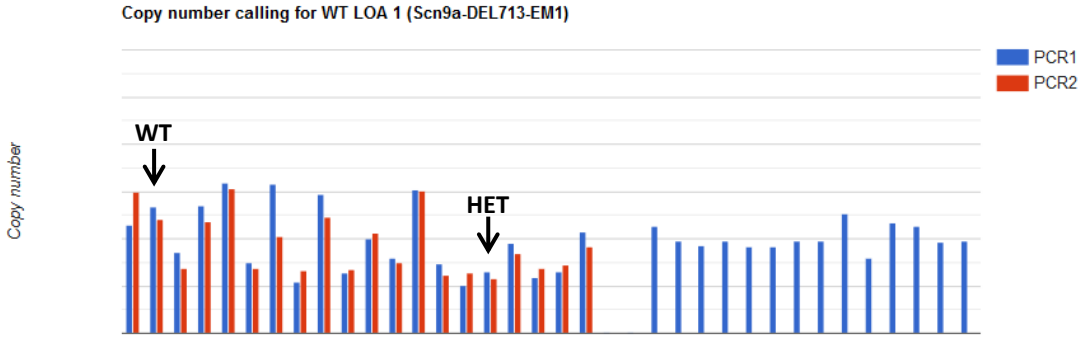
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Scn9a-DEL713-WT1 and Scn9a-DEL713-MUT1 copy called result, image showing copy number chart for WT and Mutant assays (Task 259492 results)





Version No.	1
Date:	22/06/2020
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman