Name of Mouse model or mutation:
Gtf3c1-R1211W-EM1-B6N
Gtf3c1-R1211W-EM2-B6N

Description:
Point mutation made by CRISPR/Cas9 gene editing.

Type of mutation:
SNP: R1211W

Delivery method:
Electroporation into 1-cell stage embryo.

Genetic Background:
C57BL/6NTac

Nuclease:
Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
TCTTCAGCCGCTTCCGCTTC	TGG

ssODN donor sequence (5'-3'):
TGATGCTGAGCTCTGTGCTGACAAGGAAGAACAATTTGAACTGGACAGAGAACCTACCCCTGGC
AGGAACCGGAAAGTGCGTGGTGGGAAGtcaCAGAAGtGGAAGCGGCTGAAGAAGGAGCCTATAA
GGAAGACCAAGCGAAGAAGAAGAGGTCACCTGGGGATGTCGATGACCTGGGTCTTTGCATGGC
TGTGGCACA

Electroporation mixes:
Cas9 protein, sgRNAs and ssODNs were diluted and mixed in Electroporation buffer (EB; Gibco Opti-MEM I Reduced Serum Media – (Thermo Fisher Scientific)) to the working concentrations of 650 ng/µl, 130 ng/µl total and 400 ng/µl, respectively. Embryos were electroporated using the following conditions: 30 V, 3 ms pulse length, 100 ms pulse interval, 12 pulses. Electroporated embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Sequence details

Gtf3c1 WT

CTCCAGGAGGGGGTTACCTACAGGAACTGGGTGGCTATAGAGACCCATGGAAGTTCTCAGCTCACC
CCAGTGCTATGCTTACCTTTCAGAACAACACCTCCGAAAATGGGCTCACAGGAAGACTCCAGACCTT
CCTGTCTAAGCGCCCAATGCCCCTTGGGTCCGGAGGTATGTGTCGGACATCCACACAGCGAGCCAGT
ATGAGGCAGTTAAGACCAACACCACCGTGGGTTCTGCTGGCCTGTGTGACCTCTGCAGGGACTGTCC
ACAACAGCTGTGTCCTCTGGTTATTTCTAGGCAGTGGCCGTTTGCCTCTCTGGTCAGAGGGGAGAGC
TGATGCTGAGCTCTGTGCTGACAAGGAAGAACAATTTGAACTGGACAGAGAACCTACCCCTGGCAG
GAACCGGAAAGTGCGTGGTGGGAAGAGCCAGAAGCGGAAGCGGCTGAAGAAGGAGCCTATAAGG
AAGACCAAGCGAAGAAGAAGAGGTCACCTGGGGATGTCGATGACCTGGGTCTTTGCATGGCTGTG
GCACATTGCTGTGGTAGTCCCACAGGGCCTGGGTTCATCCTGCAAAATGTCCGTTTTCCCTGTCTGCA
GCCCTGACTTGGCTGTATCCCTGGCCAACCAAAGCCTGGGCTATTTCCCCAGTTTTCTCTGGCTGTGA
ACTTTGCCAGCCTTTCTCTTTAGCAACTTTCTGATGTAGCAAATTTTGGATTTCATCTCCACTGGAAAA
AACTCTTCCTAGCAAGATTCTTGTCATCTAAATGAAGTTCCTTTCTTCTCTTGGTTTGTCTTGTTTTGTC
TGCATCTGCTTCAAGCTTAGTGCTGTTGAAGTGAGACTAGGAGGAAGCTGGGAATGGCTTAAATCT
GCACTGTCCCTTCAGAACTAGCATAGCCTTCCTTGGTACTTTCTAGTCTGTGGTTCATCATCTGAACTG
CATTTCTAATGGAAGTGAGTGATGGCGGGCAGCTCCCTGAGTAAGTAGGCTATATGTTTAATATCAA
GCCTAGATGCAGGCTCTTGAGAAGAAAATGCTGATGCAGGAACCTACTTTATTGGTTTGTGTTGCTG
GCATTCATAATAAACAGCATCCCATCTCTTGATATAGCCCTTAACTTAGCCTGTTACAAATACGTGAA
AGACCAGAATTCCTCAGAGAAAATGGGGCGGCTGGATGTGTATATGT

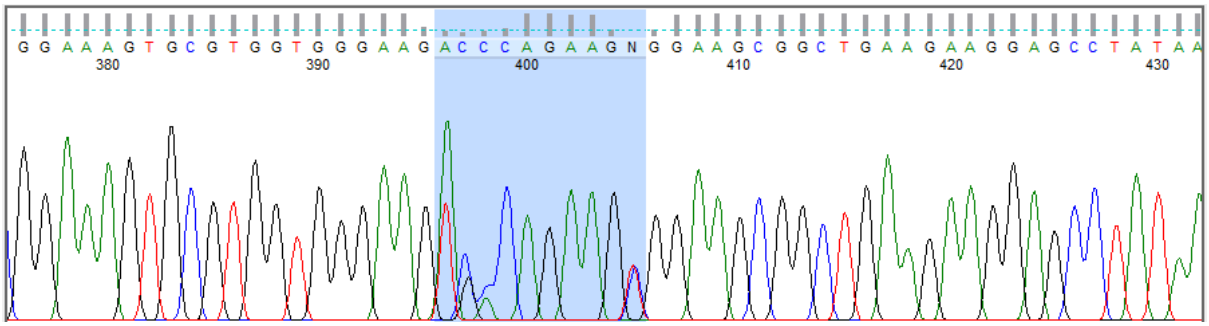
Gtf3c1-R1211W-EM1-B6N & Gtf3c1-R1211W-EM2-B6N

CTCCAGGAGGGGGTTACCTACAGGAACTGGGTGGCTATAGAGACCCATGGAAGTTCTCAGCTCACC
CCAGTGCTATGCTTACCTTTCAGAACAACACCTCCGAAAATGGGCTCACAGGAAGACTCCAGACCTT
CCTGTCTAAGCGCCCAATGCCCCTTGGGTCCGGAGGTATGTGTCGGACATCCACACAGCGAGCCAGT
ATGAGGCAGTTAAGACCAACACCACCGTGGGTTCTGCTGGCCTGTGTGACCTCTGCAGGGACTGTCC
ACAACAGCTGTGTCCTCTGGTTATTTCTAGGCAGTGGCCGTTTGCCTCTCTGGTCAGAGGGGAGAGC
TGATGCTGAGCTCTGTGCTGACAAGGAAGAACAATTTGAACTGGACAGAGAACCTACCCCTGGCAG
GAACCGGAAAGTGCGTGGTGGGAAGtcaCAGAAGtGGAAGCGGCTGAAGAAGGAGCCTATAAGGA
AGACCAAGCGAAGAAGAAGAGGTCACCTGGGGATGTCGATGACCTGGGTCTTTGCATGGCTGTGG
CACATTGCTGTGGTAGTCCCACAGGGCCTGGGTTCATCCTGCAAAATGTCCGTTTTCCCTGTCTGCAG
CCCTGACTTGGCTGTATCCCTGGCCAACCAAAGCCTGGGCTATTTCCCCAGTTTTCTCTGGCTGTGAA
CTTTGCCAGCCTTTCTCTTTAGCAACTTTCTGATGTAGCAAATTTTGGATTTCATCTCCACTGGAAAAA
ACTCTTCCTAGCAAGATTCTTGTCATCTAAATGAAGTTCCTTTCTTCTCTTGGTTTGTCTTGTTTTGTCT
GCATCTGCTTCAAGCTTAGTGCTGTTGAAGTGAGACTAGGAGGAAGCTGGGAATGGCTTAAATCTG
CACTGTCCCTTCAGAACTAGCATAGCCTTCCTTGGTACTTTCTAGTCTGTGGTTCATCATCTGAACTGC
ATTTCTAATGGAAGTGAGTGATGGCGGGCAGCTCCCTGAGTAAGTAGGCTATATGTTTAATATCAAG
CCTAGATGCAGGCTCTTGAGAAGAAAATGCTGATGCAGGAACCTACTTTATTGGTTTGTGTTGCTGG
CATTCATAATAAACAGCATCCCATCTCTTGATATAGCCCTTAACTTAGCCTGTTACAAATACGTGAAA
GACCAGAATTCCTCAGAGAAAATGGGGCGGCTGGATGTGTATATGT

Nominated change = red highlight and bold. Silent changes to prevent the reprocessing of the engineered allele = yellow highlight.

Please note that the EM1 and EM2 allele are the same and they were from two different founders. These founders were generated from the same microinjection session.

Heterozygous F1 animal sequence trace:



Nucleotide Alignment:

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                  *      1100         *      1120         *      1140         *      1160         *      1180
Gtf3c1_WT   : TAATAAACAGCATCCCATCTCTTGATATAGCCCTTAACTTAGCCTGTTACAAATACGTGAAAGACCAGAATTCCTCAGAGAAAATGGGGCGGCTGGATGTGTATATGT : 1188
Gtf3c1_EM1  : TAATAAACAGCATCCCATCTCTTGATATAGCCCTTAACTTAGCCTGTTACAAATACGTGAAAGACCAGAATTCCTCAGAGAAAATGGGGCGGCTGGATGTGTATATGT : 1188
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Predicted Protein Alignment:

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               *      1180         *      1200         *      1220
Gtf3c1_WT   : SGRLPLWSEGRADAELCADKEEQFELDREPTPGRNRKVRGGKSQKGKRLKKEPIRKTKRRRR : 62
Gtf3c1_EM1  : SGRLPLWSEGRADAELCADKEEQFELDREPTPGRNRKVRGGKSQKGKRLKKEPIRKTKRRRR : 62
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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:

Gtf3c1_R1211W_F1 (5'-3')	CTCCAGGAGGGGGTTACCTA
Gtf3c1_R1211W_R1 (5'-3')	ACATATACACATCCAGCCGCC
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	1188
Mutant product size (bp)	1188
Notes	Sequence using the following primer (5' to 3'): Gtf3c1_R1211W_SeqR: CCTCCTAGTCTCACTTCAACAGC

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')
4:140339191-140339213	GAAGCAGAAGAGGCTGAAGA GGG	Intergenic	Geno_Gtf3c1_OT1F1: ACATATACCTGCACCTGAACCC Geno_Gtf3c1_OT1R1: CCAGAGGGTGAAGTAACCTGTC Sequence with the following primer (5' to 3'): Gtf3c1_OT1_SeqR: TCTCTCTTGAATGCTGAATTTCC
5:28169021-28169043	GAAGCGGAAGAGGCTGGAGA AGG	Intronic	Geno_Gtf3c1_OT2F1: CCCGTCCCCCTTCCTATGTA Geno_Gtf3c1_OT2R1: GGGCAGAGAGTATTTGCCGA Sequence with the following primer (5' to 3'): Geno_Gtf3c1_OT2F2: ACCTTGGGACCACCTCATCA

7:123322771-123322793	GAAGCTGAAGAAGCTGAAGAAGG	Intronic	Geno_Gtf3c1_OT3F1: AAGCTGTCATGTGGTTGCATTC Geno_Gtf3c1_OT3R1: GGGGACAGATACCTAAAGGCTG Sequence with the following primer (5' to 3'): CCTTGTACTAAGTCATTTTCCTCAA

All amplicons were sent for Sanger sequencing.
No off-target activity was detected in the animals selected to establish the colony.

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	Gtf3c1_R1211W_MUT1
Forward Primer (5'-3')	CTCTGTGCTGACAAGGAAGAA
Reverse Primer (5'-3')	CTTCTTCAGCCGCTTCCA
Probe (5'-3')	TGACTTCCCACCACGCACTTTCC
Label	FAM

This ddPCR assay is specific to the donor used to create the engineered mutation and only mutant alleles are expected to be recognised by this assay. Therefore, WT controls are expected to call at 0 copies and a single integration for a correct mutation is expected to call at 1 copy for F1 (HET) animals.

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.

No additional donor integrations were detected in the animals taken forward to establish the colony.