Name of Mouse model or mutation:
NOP10-T16M-EM1-B6

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

Type of mutation:
SNP: T16M

Delivery method:
Cytoplasmic injection into 1-cell stage embryo

Genetic Background:
C57BL/6J

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
CGCGATCGCCCTGCTCGTTG	AGG
CCTGCTCGTTGAGGTAATAT	TGG

ssODN donor sequence (5'-3'):
TTGGCGCACGCGATTGAAGCGGCGCACTCGGAGTAAACGCGGCTGGCGGTAGTACGTGTGTCTTTT
TGCTGAGGGCAGTTATGTTTCTCCAATATTAttTaAACGAGCAGGGCGATCGCGTTTATAtGCTGAAG
gtaaggggaggaggaaacagctatatatcaaaaggacgagaaatttagatgggaggaacagttgga

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 ssODNs were diluted and mixed in MIB to the working concentrations of 50 ng/µl, 6.5 ng/µl each and 100 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

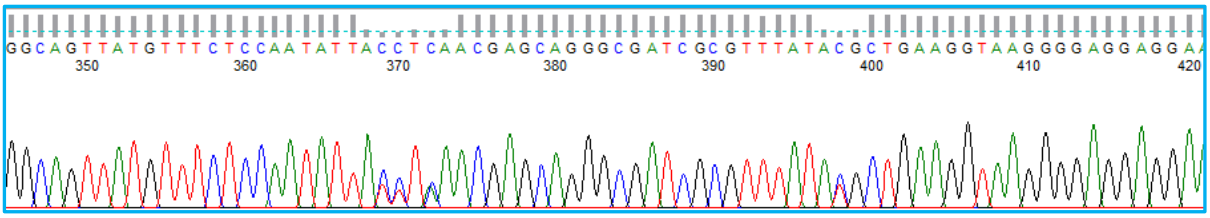
aatccttgagtccttgtttctccaggaattcgaaaagtggtgcctctgaaatgggtgtgcaatttgaaattaaagataggactgatat
caacagtgaatgatgcatcaacattcaacccacatccatccctacttcctaatccctggcaagtggctggcacacagtgggcagttc
catggatcttaataactttgcaaggttgctgctagctggaacccaaggccaagcaagacttgctctactatgccccttctggtcaca
tggatatgactggagaaatggactacatttcccaggatgccttggagagacttccGTCCAATCTGGAAGGTTGTTGGCG
CACGCGATTGAAGCGGCGCACTCGGAGTAAACGCGGCTGGCGGTAGTACGTGTGTCTTTTTGCTGA
GGGCAGTTATGTTTCTCCAATATTACCTCAACGAGCAGGGCGATCGCGTTTATACGCTGAAGgtaagg
ggaggaggaaacagctatatatcaaaaggacgagaaatttagatgggaggaacagttggaaccggcctgcgcagcccactgagt
ggagccatgtcggagccaaaacgtggtagataggcagtggcttctttttctgaccacgcgatctcctggcctcaggtcacccattttct
tgtttctttctatcacaccactcatattgagatatttttttatttttccttgagcagAAATTTGACCCTATGGGACAACAGACT
TGCTCCGCCCATCCTGCTCGGTTCTCCCCAGATGACAAATATTCAAGACACCGAATCACCATCAAGAA
ACGCTT

NOP10-T16M-EM1-B6

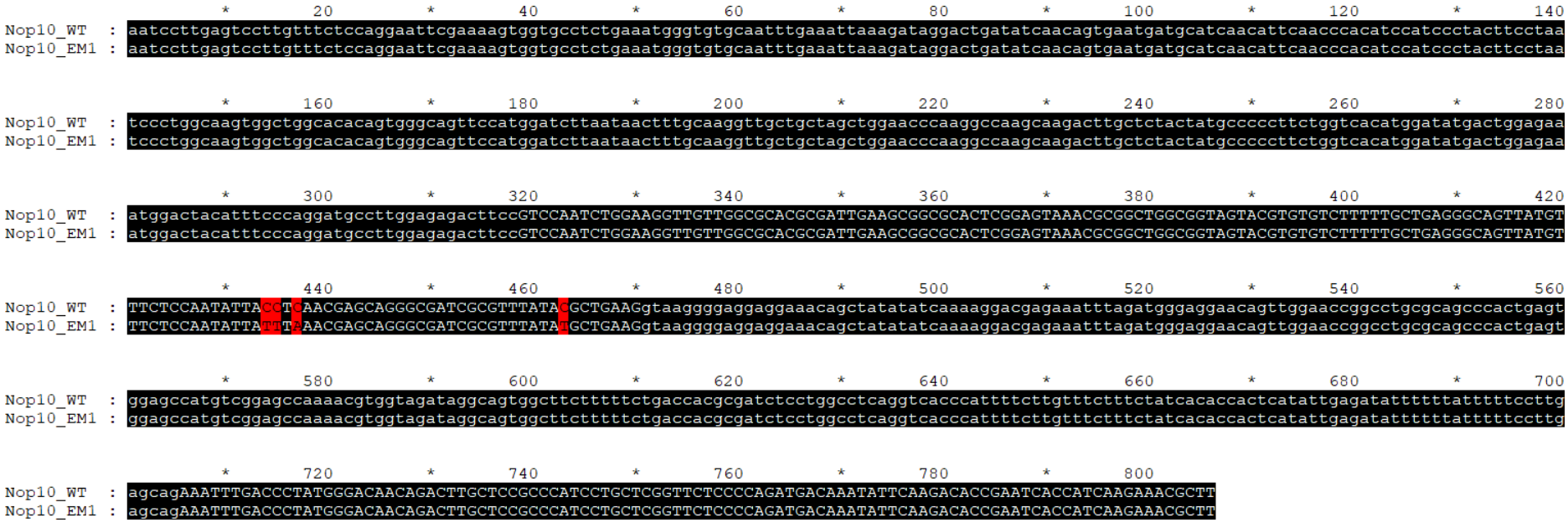
aatccttgagtccttgtttctccaggaattcgaaaagtggtgcctctgaaatgggtgtgcaatttgaaattaaagataggactgatat
caacagtgaatgatgcatcaacattcaacccacatccatccctacttcctaatccctggcaagtggctggcacacagtgggcagttc
catggatcttaataactttgcaaggttgctgctagctggaacccaaggccaagcaagacttgctctactatgccccttctggtcaca
tggatatgactggagaaatggactacatttcccaggatgccttggagagacttccGTCCAATCTGGAAGGTTGTTGGCG
CACGCGATTGAAGCGGCGCACTCGGAGTAAACGCGGCTGGCGGTAGTACGTGTGTCTTTTTGCTGA
GGGCAGTTATGTTTCTCCAATATTATTTAAACGAGCAGGGCGATCGCGTTTATATGCTGAAGgtaagg
ggaggaggaaacagctatatatcaaaaggacgagaaatttagatgggaggaacagttggaaccggcctgcgcagcccactgagt
ggagccatgtcggagccaaaacgtggtagataggcagtggcttctttttctgaccacgcgatctcctggcctcaggtcacccattttct
tgtttctttctatcacaccactcatattgagatatttttttatttttccttgagcagAAATTTGACCCTATGGGACAACAGACT
TGCTCCGCCCATCCTGCTCGGTTCTCCCCAGATGACAAATATTCAAGACACCGAATCACCATCAAGAA
ACGCTT

**Red underlined is T16M mutation, red text are silent mutations and yellow highlight is DraI restriction site.

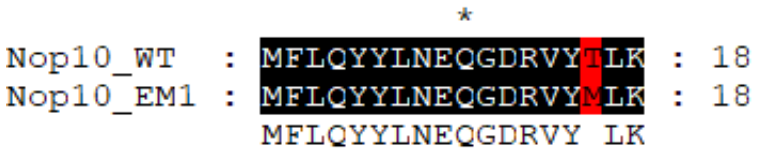
NOP10-T16M-EM1-B6 Heterozygous F1 animal sequence trace:



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_Nop10_T16M_F1 primer (5'-3')	aatccttgagtccttgtttctcc
Geno_Nop10_T16M_R1 primer (5'-3')	AAGCGTTTCTTGATGGTGATTCG
Taq Polymerase used	ThermoFisher Super
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	806
Mutant product size (bp)	806
Notes	Sequenced with Geno_Nop10_T16M_F2 (gaaaagtggtgcctctgaaatg), Geno_Nop10_T16M_R2(CGTTTCTTGATGGTGATTCGGT)

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 in seed for guide(s) used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
2:44090631-44090653	**T**G**G**GA**GG**GCCCTGCTCGTTG TGG	Intronic	Nop10_T16M_OT1_F1: CTCTTTGAAGGGGGCTACCAA Nop10_T16M_OT1_R1: GTTGTGTGCTCCAGGTATGC
2:65948209-65948231	**G**CTG**G**T**T**GTTGAGGTAA**A**AT AGG	Intronic	Nop10_T16M_OT2_F1: TGGCAGGAATGAGATAGAATGC Nop10_T16M_OT2_R1: ACTCAGCTTAATGCAGTACCA

All amplicons were sent for Sanger sequencing and 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	NOP10-T16M-UNI1
Forward Primer (5’-3’)	TGAGGGCAGTTATGTTTCTCCAATA
Reverse Primer (5’-3’)	TCCAACTGTTCCTCCCATCTAAA
Probe (5’-3’)	AACGAGCAGGGCGATCGCGTTTAT
Label	FAM

This ddPCR assay is universal to NOP10 - both WT and MUT alleles are recognised by this assay. Therefore, WT controls are expected to call at 2 copies and a single integration for a correct mutation is expected to call at 2 copies for F1 (HET) animals.

Assay name	NOP10-T16M-MUT1
Forward Primer (5’-3’)	GGCGCACTCGGAGTAAACG
Reverse Primer (5’-3’)	TCCTCCCCTTACCTTCAGCA
Probe (5’-3’)	TCCAATATTATTTAAACGAGCAGGGCGA
Label	FAM

This ddPCR assay is specific to the T16M mutation in the NOP10 gene and only MUT 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.

NOP10-T16M

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; T16M in *Nop10*. 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

NOP10-T16M

NOP10-T16M-WT1 assay (FAM labelled)

TGCTGAG**GGCAGTTATGTTTCTCCAATATTAccTc**AACGAGC**AGGGCGATCGCGTTTATAcGCTGA**A
GGTAAGGGGAGGAGGAAACAGCTATATATCAAAAGGACGAGAAATT**TAGATGGGAGGAACAGTT**
GGAACCGGCCTGCGCAGCCCACTGAGTGGAGCCATGTCGGAGCCAAAACGTGGTAGATAGGCAGT

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 NOP10-T16M	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
NOP10-T16M-WT_F	n/a	**GGCAGTTATGTTTCTCCAATATTACCTC**	n/a	Wild type Forward
NOP10-T16M-WT_PROBE	FAM	**AGGGCGATCGCGTTTATACGCTGA**	BHQ	Wild type Probe
NOP10-T16M-WT_R	n/a	**GTTCCAACTGTTCCTCCCATCTA**	n/a	Wild type Reverse

NOP10-T16M-MUT1 assay (FAM labelled)

TTGGCGCACGCGATTGAAGC**GGCGCACTCGGAGTAAACG**CGGCTGGCGGTAGTACGTGTGTCTTTT
TGCTGAGGGCAGTTATGTTTC**TCCAATATTAttTaAACGAGCAGGGCGA**TCGCGTTTATA**tGCTGAA**
GGTAAGGGGAGGAGGAAACAGCTATATATCAAAAGGACGAGAAATTTAGATGGGAGGAACAGTT

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 NOP10-T16M	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
NOP10-T16M-MUT_F	n/a	**GGCGCACTCGGAGTAAACG**	n/a	Mutant Forward
NOP10-T16M-MUT_PROBE	FAM	**TCCAATATTATTTAAACGAGCAGGGCGA**	BHQ	Mutant Probe
NOP10-T16M-MUT_R	n/a	**TCCTCCCCTTACCTTCAGCA**	n/a	Mutant Reverse

NOP10-T16M

Dot1l internal control (VIC labelled)

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

Oligo NOP10-T16M	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

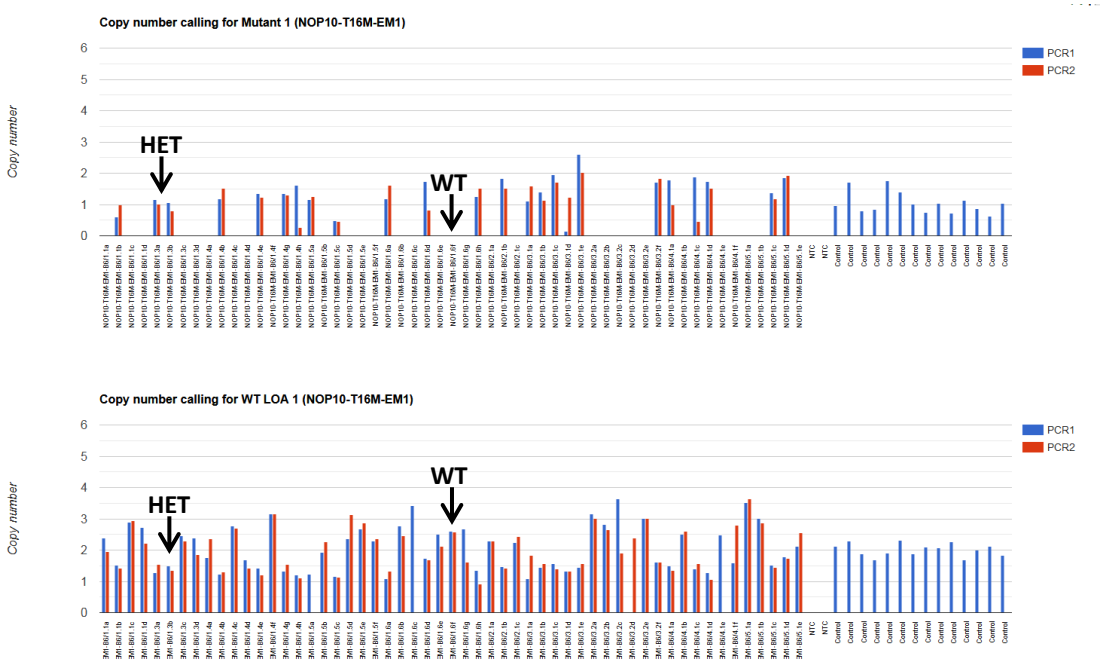
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

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.

NOP10-T16M-WT1 and NOP10-T16M -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 316300 results)



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