Name of Mouse model or mutation:
FLNC-DEL-EM1-B6J

Description:
Targeted deletion made by CRISPR/Cas9 gene editing.

Type of mutation:
Targeted deletion of exon 40 (ENSMUSE00000564303) of Flnc.

Delivery method:
Cytoplasmic injection into 2-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
CTGGGCTTTCTGTAGGAAAC	TGG
AGCATCACCCGGCAGCAGGA	AGG

ssODN donor sequence (5'-3'):
ATCTGGGCCAAGGTGGTCAGGGATGAGGCCATTTTGACTCACAGAACAGGTGGCCCCCCAGCCTCC
CTGTGAGGACCCCAGTTCCCAGTATTCTCTGCTCACAGAAAGCCCAGAGGGAGGTGGCAGGAGGTG
AGGAGAGAGGCAGGCAGGACAGAGGCCTCAGGGCAGGCTCAAGGCTAAAAAGGGCATCCCATGG
CCGA

Cytoplasmic 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

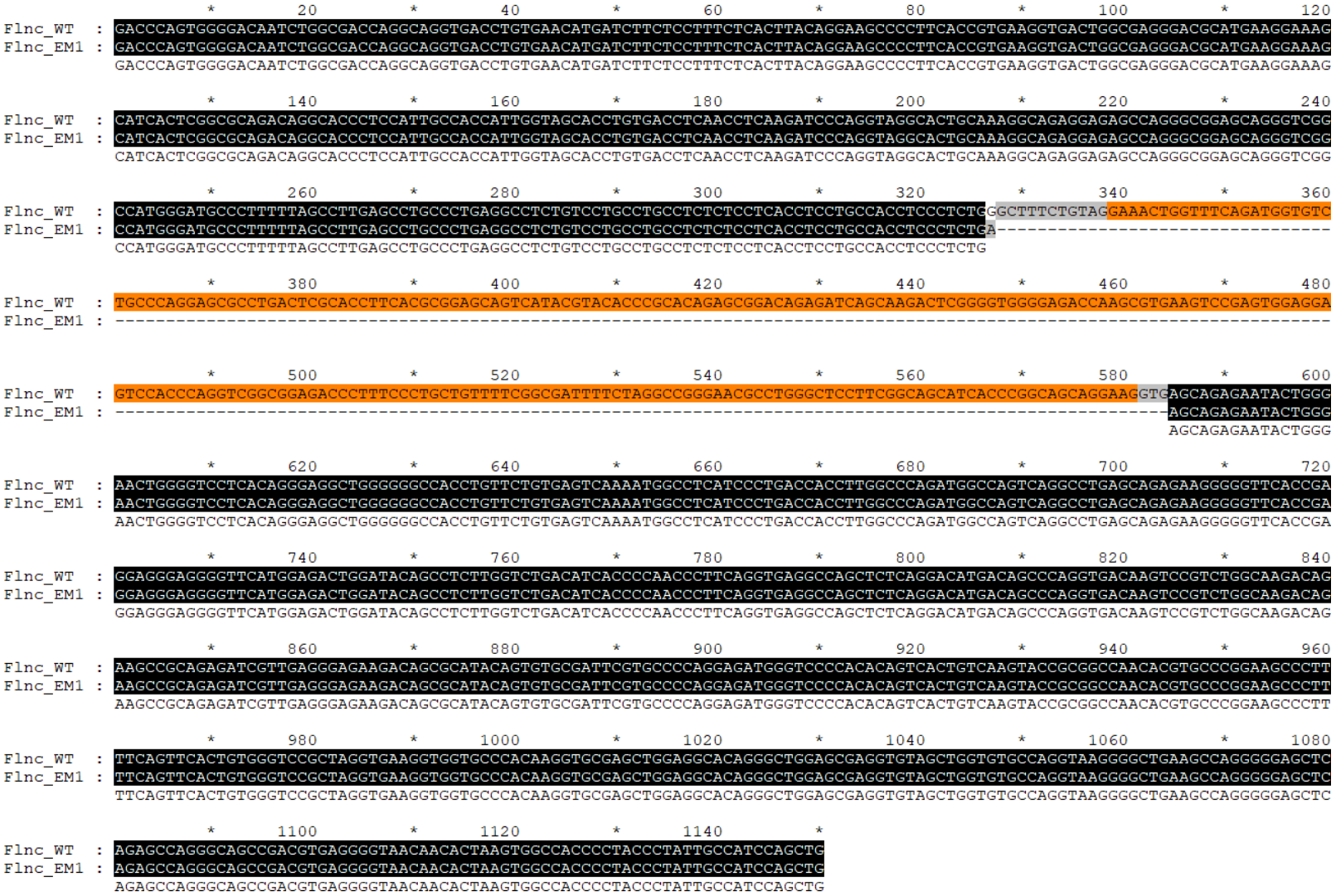
WT

GACCCAGTGGGGACAATCTGGCGACCAGGCAGGTGACCTGTGAACATGATCTTCTCCTTTCTCACTT
ACAGGAAGCCCCTTCACCGTGAAGGTGACTGGCGAGGGACGCATGAAGGAAAGCATCACTCGGCG
CAGACAGGCACCCTCCATTGCCACCATTGGTAGCACCTGTGACCTCAACCTCAAGATCCCAGGTAGG
CACTGCAAAGGCAGAGGAGAGCCAGGGCGGAGCAGGGTCGGCCATGGGATGCCCTTTTTAGCCTT
GAGCCTGCCCTGAGGCCTCTGTCCTGCCTGCCTCTCTCCTCACCTCCTGCCACCTCCCTCTGGGCTTTC
TGTAGGAAACTGGTTTCAGATGGTGTCTGCCCAGGAGCGCCTGACTCGCACCTTCACGCGGAGCAG
TCATACGTACACCCGCACAGAGCGGACAGAGATCAGCAAGACTCGGGGTGGGGAGACCAAGCGTG
AAGTCCGAGTGGAGGAGTCCACCCAGGTCGGCGGAGACCCTTTCCCTGCTGTTTTCGGCGATTTTCT
AGGCCGGGAACGCCTGGGCTCCTTCGGCAGCATCACCCGGCAGCAGGAAGGTGAGCAGAGAATAC
TGGGAACTGGGGTCCTCACAGGGAGGCTGGGGGGCCACCTGTTCTGTGAGTCAAAATGGCCTCATC
CCTGACCACCTTGGCCCAGATGGCCAGTCAGGCCTGAGCAGAGAAGGGGGTTCACCGAGGAGGGA
GGGGTTCATGGAGACTGGATACAGCCTCTTGGTCTGACATCACCCCAACCCTTCAGGTGAGGCCAGC
TCTCAGGACATGACAGCCCAGGTGACAAGTCCGTCTGGCAAGACAGAAGCCGCAGAGATCGTTGAG
GGAGAAGACAGCGCATACAGTGTGCGATTCGTGCCCCAGGAGATGGGTCCCCACACAGTCACTGTC
AAGTACCGCGGCCAACACGTGCCCGGAAGCCCTTTTCAGTTCACTGTGGGTCCGCTAGGTGAAGGT
GGTGCCCACAAGGTGCGAGCTGGAGGCACAGGGCTGGAGCGAGGTGTAGCTGGTGTGCCAGGTA
AGGGGCTGAAGCCAGGGGGAGCTCAGAGCCAGGGCAGCCGACGTGAGGGGTAACAACACTAAGT
GGCCACCCCTACCCTATTGCCATCCAGCTG

FLNC-DEL-EM1-B6J

GACCCAGTGGGGACAATCTGGCGACCAGGCAGGTGACCTGTGAACATGATCTTCTCCTTTCTCACTT
ACAGGAAGCCCCTTCACCGTGAAGGTGACTGGCGAGGGACGCATGAAGGAAAGCATCACTCGGCG
CAGACAGGCACCCTCCATTGCCACCATTGGTAGCACCTGTGACCTCAACCTCAAGATCCCAGGTAGG
CACTGCAAAGGCAGAGGAGAGCCAGGGCGGAGCAGGGTCGGCCATGGGATGCCCTTTTTAGCCTT
GAGCCTGCCCTGAGGCCTCTGTCCTGCCTGCCTCTCTCCTCACCTCCTGCCACCTCCCTCTG**[A]**AGCA
GAGAATACTGGGAACTGGGGTCCTCACAGGGAGGCTGGGGGGCCACCTGTTCTGTGAGTCAAAAT
GGCCTCATCCCTGACCACCTTGGCCCAGATGGCCAGTCAGGCCTGAGCAGAGAAGGGGGTTCACCG
AGGAGGGAGGGGTTCATGGAGACTGGATACAGCCTCTTGGTCTGACATCACCCCAACCCTTCAGGT
GAGGCCAGCTCTCAGGACATGACAGCCCAGGTGACAAGTCCGTCTGGCAAGACAGAAGCCGCAGA
GATCGTTGAGGGAGAAGACAGCGCATACAGTGTGCGATTCGTGCCCCAGGAGATGGGTCCCCACAC
AGTCACTGTCAAGTACCGCGGCCAACACGTGCCCGGAAGCCCTTTTCAGTTCACTGTGGGTCCGCTA
GGTGAAGGTGGTGCCCACAAGGTGCGAGCTGGAGGCACAGGGCTGGAGCGAGGTGTAGCTGGTG
TGCCAGGTAAGGGGCTGAAGCCAGGGGGAGCTCAGAGCCAGGGCAGCCGACGTGAGGGGTAACA
ACACTAAGTGGCCACCCCTACCCTATTGCCATCCAGCTG

Nucleotide Alignment: Exon 40 (ENSMUSE00000564303) highlighted in orange



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_Flnc_Del_F1 (5'-3')	GACCCAGTGGGGACAATCTG
Geno_Flnc_Del_R1 (5'-3')	CAGCTGGATGGCAATAGGGT
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	1150
Mutant product size (bp)	903
Notes	

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')
1:39328940-39328962	CTGGGCT**GC**CTGTAGGAAAC TGG	Intronic	Flnc_DEL_OT1F1 (CAGGGATAGGGAAGGACCAGG) & Flnc_DEL_OT1R1 (ATGGTGCAGTCGCAGGATTA) and sequenced with Flnc_DEL_OT1R2 (ACTGTGCTGGGCACAAAGTC)
5:53958877-53958899	CTGGGC**G**TTCTGTAGGAAA**G** AGG	Intergenic	Flnc_DEL_OT2F1 (GGAGGAAAGAAGAAAGGAAGG) & Flnc_DEL_OT2R1 (CATGACATTTGTGTGGAGAGAG)

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	Flnc-Del-MUT1
Forward Primer (5’-3’)	GAGCAGAGAATACTGGGAACTG
Reverse Primer (5’-3’)	GTCAGGGATGAGGCCATTT
Probe (5’-3’)	TGACTCACAGAACAGGTGGCCC
Label	FAM

This ddPCR assay is universal; both the WT and mutant 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	Flnc-Del-WT1
Forward Primer (5’-3’)	AACTGGTTTCAGATGGTGTCT
Reverse Primer (5’-3’)	CGAGTCTTGCTGATCTCTGTC
Probe (5’-3’)	CTTCACGCGGAGCAGTCATACGT
Label	FAM

This ddPCR assay is specific to the WT allele and only WT alleles are expected to be 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 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.