Name of Mouse model or mutation:
SPIB-MCHERRY-EM1-B6J
SPIB-MCHERRY-EM2-B6J

Description:
mCherry knock-in made by CRISPR/Cas9 gene editing.

Type of mutation:
Knock-in: mCherry

Delivery method:
Pronuclear injection into 1-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
GAGTGCTCAGACATGCCGGG	AGG
CATGTCTGAGCACTCCGCTA	AGG

Pronuclear 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 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.

lssDNA donor sequence template (5’-3’, NOTE donor delivered to embryo will be reverse complement (Codner et al., 2018: Figure 1)):

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LOCUS       SpiB                974 bp    DNA     linear      31-MAY-2022
FEATURES             Location/Qualifiers
     misc_feature    101..163
                     /dnas_title="Linker/T2A"
                     /note=""
     misc_feature    164..874
                     /dnas_title="mCherry"
                     /note=""
     misc_feature    1..100
                     /dnas_title="5' HA"
                     /note=""
     misc_feature    1..974
                     /dnas_title="lssDNA donor"
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     exon            1..100
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                     /note=""
     exon            875..877
                     /dnas_title="stop"
                     /note=""
     misc_feature    875..974
                     /dnas_title="3' HA"
                     /note=""
     exon            878..974
                     /dnas_title="UTR"
                     /note=""
     source          1..974
                     /dnas_title="SpiB mCherry long donor sequence"
ORIGIN
        1 AGCGCTGCGC AACTATGCCA AGACAGGCGA AATCCGCAAG GTCAAACGCA AACTCACCTA
       61 CCAGTTTGAC AGCGCGCTGC TGCCAGCCTC CGGCATGTC GGAAGCGGAG AGGGCAGAGG
      121 AAGTCTGCTA ACATGCGGTG ACGTCGAGGA GAATCCTGGA CCTATGGTGA GCAAGGGCGA
      181 GGAGGATAAC ATGGCCATCA TCAAGGAGTT CATGCGCTTC AAGGTGCACA TGGAGGGCTC
      241 CGTGAACGGC CACGAGTTCG AGATCGAGGG CGAGGGCGAG GGCCGCCCCT ACGAGGGCAC
      301 CCAGACCGCC AAGCTGAAGG TGACCAAGGG TGGCCCCCTG CCCTTCGCCT GGGACATCCT
      361 GTCCCCTCAG TTCATGTACG GCTCCAAGGC CTACGTGAAG CACCCCGCCG ACATCCCCGA
      421 CTACTTGAAG CTGTCCTTCC CCGAGGGCTT CAAGTGGGAG CGCGTGATGA ACTTCGAGGA
      481 CGGCGGCGTG GTGACCGTGA CCCAGGACTC CTCCCTGCAG GACGGCGAGT TCATCTACAA
      541 GGTGAAGCTG CGCGGCACCA ACTTCCCCTC AGACGGCCCC GTAATGCAGA AGAAAACCAT
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601 GGGCTGGGAG GCCTCCTCCG AGCGGATGTA CCCCGAGGAC GGCGCCCTGA AGGGCGAGAT
661 CAAGCAGAGG CTGAAGCTGA AGGACGGCGG CCACTACGAC GCTGAGGTCA AGACCACCTA
721 CAAGGCCAAG AAGCCCGTGC AGCTGCCCGG CGCCTACAAC GTCAACATCA AGTTGGACAT
781 CACCTCCCAC AACGAGGACT ACACCATCGT GGAACAGTAC GAACGCGCCG AGGGCCGCCA
841 CTCCACCGGC GGCATGGACG AGCTGTACAA GTAATGAGCA CTCCGCTAAG GACCCCTTTC
901 TGGCCCCTAA GTCCCATGGA GCCCCATATG AGGGCAGTCA GGGTTCTCAG CTCTCCCTAG
961 AGCCTCCCCA GAGT
//

Sequence details

WT

AGAGGATGCAAGAACCAGAGCACCCTAAACACTAATACTTCCCAAGGAAGTTCCCAGATCCATGCTG
CTTGTTGCCTGTGTGACCTTGGTCAAGGGCATCGACCTTTCTGTGCCTGGGTTTCCGTTGTGCACAAA
GACACAGTAACAGCCAAAGGAAACCCAATATATACCTACAATCCTCCTCCCCTTAGGCCTCTGTGTCT
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GGTAAGGTAGGG

SPIB-MCHERRY-EM1-B6J & SPIB-MCHERRY-EM2-B6J

AGAGGATGCAAGAACCAGAGCACCCTAAACACTAATACTTCCCAAGGAAGTTCCCAGATCCATGCTG
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*CGTCGAGGAGAATCCTGGACCT*ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGG
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CAGGCACCCCTTGGGACCCTCTGGGACCTATGTCCGAGATGAATGGCTGGGTAAGGTAGGG

Linker sequence in *italics and underlined*.

mCherry sequence in grey.

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_SpiB_mCherry_F1 (5'-3')	AGAGGATGCAAGAACCAGAGC
Geno_SpiB_mCherry_R1 (5'-3')	CCCTACCTTACCCAGCCATTC
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1.5
WT product size (bp)	1557
Mutant product size (bp)	2331
Notes	External PCR; primers situated external to homology arms of the donor template. Amplicons also sequenced with: Geno_SpiB_mCherry_R4 (GGAGCCGTACATGAACTGAGGG) Geno_SpiB_mCherry_F4 (CTCCGAGCGGATGTACCCC) Geno_SpiCherry_sqF1 (AGAATCCTGGACCTATGGTG) Geno_SpiCherry_sqR1 (GCGTTCGTACTGTTCCACGATG)

Geno_SpiB_mCherry_F3 (5'-3')	GCATGTCGGAAGCGGAGAG
Geno_SpiB_mCherry_R3 (5'-3')	TGGAACCGTAGACGACATCAG
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	-
Mutant product size (bp)	1018
Notes	Donor-specific PCR.

Geno_SpiB_mCherry_F6 (5'-3')	TATACTCCCGCGCTCACTGG
Geno_SpiB_mCherry_R6 (5'-3')	TGGCCTTGTAGGTGGTCTTGA

Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	N/A
Mutant product size (bp)	1185
Notes	PCR specific to correct insertion. Forward primer situated upstream of the donor sequence and reverse primer within the mCherry sequence.

Geno_SpiB_mCherry_F8 (5'-3')	CCTTCGCCTGGGACATCCTG
Geno_SpiB_mCherry_R8 (5'-3')	AGAGGACTGCCACTAGCTTGA
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	-
Mutant product size (bp)	1179
Notes	PCR specific to correct insertion. Forward primer situated within donor sequence and reverse primer downstream of the donor sequence.

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.

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	mCherry_MUT1
Forward Primer (5'-3')	CGCCCTCGATCTCGAACTC

Reverse Primer (5’-3’)	CAAGGGCGAGGAGGATAACATG
Probe (5’-3’)	CCATCATCAAGGAGTTCATGCGCTTCA
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.

Assay name	Spib BP Assay
Forward Primer (5’-3’)	AACTCACCTACCAGTTTGACAG
Reverse Primer (5’-3’)	AGAGCTGAGAACCCTGACT
Probe (5’-3’)	CCTTAGCGGAGTGCTCAGACATGC
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.