Name of Mouse model or mutation:
CRY1-FLOX-EM2-B6

Description:
Floxed allele made by CRISPR/Cas9 gene editing.

Type of mutation:
Floxed allele: ENSMUSE00000100037

Delivery method:
Pronuclear injection into 1-cell stage embryo

Genetic Background:
C57BL/6J

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	**PAM sequence**
GTAAAACATGCTATCAGTAG	**TGG**
TAAAACATGCTATCAGTAGT	**GGG**
GAATGCACATGAGATATTTT	**TGG**
GACAATGTGCATCCTAAAAA	**TGG**

lssDNA donor sequence (5'-3'):

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LOCUS       Flox                1307 bp    DNA     linear      21-JUL-2020
FEATURES             Location/Qualifiers
     misc_feature    180..213
                     /note="loxP"
     misc_feature    1095..1128
                     /note="loxP"
     misc_feature    172..179
                     /note="AsiSI (SfaAI)"
     misc_feature    1129..1136
                     /note="MreI"
     PCR_primer      151..171
                     /note="LoxPF"
     PCR_primer      complement(1137..1156)
                     /note="LoxPR"
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   misc_feature    1157..1306
                   /note="3' HA"
   misc_feature    214..1094
                   /note="Critical region"
   misc_feature    616..800
                   /note="ENSMUSE00000100037"
   misc_feature    1..150
                   /note="5' HA"
   source          1..1307
                   /dnas_title="Flox gBlock template"
ORIGIN
        1 TCCATAGAGG AAATTGGGAC ACAGCAGAAA GGGCAAGAGG AGATAGGGTT TCTCTGTGTA
       61 GCCCTGGCTG TCCTAGAACT CACCCTGTAG ACCAAGCTGA CCTGGCATTT ATAGAGACCC
      121 ACCTGCCTTT GCCTCCGAGT GCTGGGATTG atccgggggt accgcgtcga gGCGATCGCA
      181 TAACTTCGTA TAGCATACAT TATACGAAGT TATATTATTT CTCGTTAGAA TTATCAATCT
      241 ACTTTAGTTT TATTGGTGCC TCTTGAGATT TATTAAATTG GTTCATTTTT TTCACTTGTC
      301 CATTTATATT AATTTTTCTA TACATGATCT ATAGGCTAGT TGAACTACTT TGTTAGTTTT
      361 TAGTCACAAT CCTAAATACT TGCCATCAAT GATTTGATGT TTGCATACTG TATTATTTGA
      421 CACCTAGTAG ACATCTAGGA ATTCAAGAGA ATGGTTTTAC ATTCATTAAA TATTTTTGAA
      481 CACATTTTCT GGTCTTATCA GAAGCTAACA TTCAAAATAT CTTAATTGAT AAATTTTCTG
      541 ATATAAGCAT TTTTGAGCTT ATCAGCCAAG CTATATTAGC ATTTACTAAC TGTATCTTAC
      601 TCATTCTTGT AACAGGATCA TAGAACTCAA TGGCGGACAG CCACCTCTAA CATATAAAAG
      661 GTTTCAGACT CTCGTCAGCA AGATGGAGCC ACTGGAGATG CCAGCAGACA CCATCACATC
      721 AGATGTGATA GGAAAGTGCA TGACCCCTCT GTCTGATGAC CATGATGAGA AATATGGCGT
      781 TCCTTCCCTG GAAGAGCTCG GTGGGTGCAG CCGCTGATAA GCACCCAGAG TGCATGGCAG
      841 CACAAAGTGG ACCTGATGGC TGACTAAGAA AAGTCAGAAA GAACACAGTC AGGATGGGTA
      901 GGGAGATCTG GGGGATCTAG GAAGCTTTAG GGGGACAGGT AAGGGCAGTG TGATCAGAAT
      961 ACGATGTGTG AAATTCCCAA GTAATTTAAA AATATATTTT AAAATACTTC ATAGTCATAA
     1021 ATCATTTGGA CATTTCACTT CTAATGTATT TTTACTGGAA GAAGAGAAGA GTAAAGGGGA
     1081 AAATAAAAGA GAAAATAACT TCGTATAGCA TACATTATAC GAAGTTATCG CCGGCGggtc
     1141 tgagctcgcc atcagtTGCA TCCTAAAAAT GGGAACCATG TGTGGTCCTT GGGGCAGGAG
     1201 TCTGTCATTG GGTGGGGCCT AGGCCAGTGT AGTCCTGTGT AGTCCTCTGT AAGCCAGTGT
     1261 AGGCCTGTAG AGTTGTATTT ATTTGTTTAT TTTATTTTTG CTGAGTG
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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.

Sequence details

WT

TGAGTTTCAGTTTGCTGGTAGATAATTAAGAGACACTTAATAGTGTCATTGTAGCATTATTGAATTCA
ACAAACTTCATTATAGCCCTATATGTAATATCAAAATATTAAACCAAAACAAACTGTACTTACAGCAG
GGAGGATAGCTTTTACTCTGAAACCATGTCTAGACTTAATGCAAACCCTTGGAAGTGCTGCTGGCAT
CTTTCCCTAGAACTATTGTAGGATTGAGGTACCCAGTGGTCAGCAATAACTACATAATGCACTTAATA
TTAATGACTATACTTAAATATAAATGACTTGGAATTTGGGGTGCATGTATGCATAATATTTCTCTCCTT
ATCCATGAGGGCATTCCAAAGCCCTGGTGGCTGTTAAGAACCACTAGTCACGTTGAACCCATGTTCA
CTGTGCTGTCTATCTAAAGAGCACAGCATGGCAAAGCTTGGCTGTAAATTATAAGTTATAGGAGGTT
AACAATGGTATTGAACGTTTAGAACAGTATGCTGTAATAAGTTATGTGAGTCTAGACATACTATTTTT
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TTATCAGAAGCTAACATTCAAAATATCTTAATTGATAAATTTTCTGATATAAGCATTTTTGAGCTTATC
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CCCTTTCCTCCTGCCTGCTTCTTCCTGTTGCTGGAGCCAGGCTGCTGCTTCTGTTCTTCCCACCCGTAT
CAGCTGGCTAACGGGCTGGAGCAGGATTCTGCCTGCAGCCATGCTGCTCTGTCTGAGACTCCAGTG
GGTCCCATGAGACAGTTTCACTTGTCTGTTTTGTTGGTATCACTGGTAATACGTATTGATTGTAGAAA
TTAGCAAGACTCCCATGTATTGATCCTGTTCCGCCACTC

Mutant

TGAGTTTCAGTTTGCTGGTAGATAATTAAGAGACACTTAATAGTGTCATTGTAGCATTATTGAATTCA
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GGAGGATAGCTTTTACTCTGAAACCATGTCTAGACTTAATGCAAACCCTTGGAAGTGCTGCTGGCAT
CTTTCCCTAGAACTATTGTAGGATTGAGGTACCCAGTGGTCAGCAATAACTACATAATGCACTTAATA
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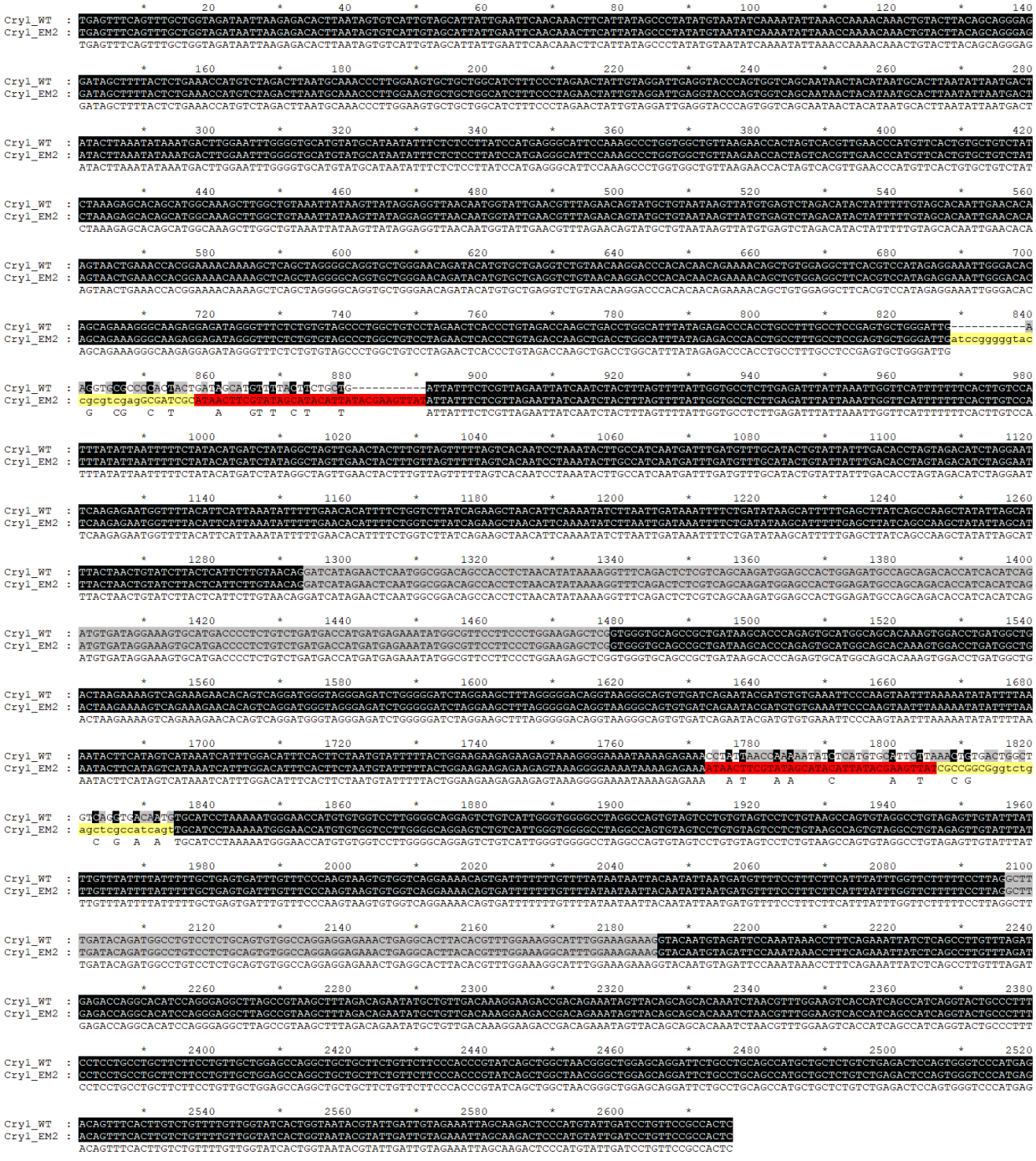
GACTCCAGTGGGTCCCATGAGACAGTTTCACTTGTCTGTTTTGTTGGTATCACTGGTAATACGTATTG
ATTGTAGAAATTAGCAAGACTCCCATGTATTGATCCTGTTCCGCCACTC

LoxP sites are highlighted in red and genotyping handles (restriction enzyme site plus primer unique to each LoxP site) in yellow. Exons are highlighted in grey, with floxed exon in bold also.

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:

Geno_Cry1_Flox_F6 primer (5'-3')	TGAGTTTCAGTTTGCTGGTAGA
Geno_Cry1_Flox_R6 primer (5'-3')	GAGTGGCGGAACAGGATCAA
Taq Polymerase used	ThermoFisher SuperFi II PCR kit
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	2594
Mutant product size (bp)	2616
Notes	Sequenced with the following primers (5'-3'): Geno_Cry1_Flox_F5 primer: ACAAACTGTACTTACAGCAGGGA Geno_Cry1_Flox_R5 primer: GTACCTGATGGCTGATGGTG LoxP_F: ATCCGGGGGTACCGCGTCGAG LoxP_R: ACTGATGGCGAGCTCAGACC

LoxP_F	ATCCGGGGGTACCGCGTCGAG
LoxP_R	ACTGATGGCGAGCTCAGACC
Taq Polymerase used	ThermoFisher SuperFi II PCR kit
Annealing Temperature (°C)	60
Elongation time (min)	1.5
WT product size (bp)	N/A
Mutant product size (bp)	1006
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.

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	CRY1-FLOX-5'-MUT2
Forward Primer (5'-3')	TCCGAGTGCTGGGATTG
Reverse Primer (5'-3')	AATCTCAAGAGGCACCAATAAA
Probe (5'-3')	TCGAGGCGATCGCATAACTTCG
Label	FAM

The ddPCR assay is specific to the Cry1 Flox donor and only floxed 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	CRY1-CR-LOA-WT2
Forward Primer (5'-3')	GGACAGCCACCTCTAACATA
Reverse Primer (5'-3')	GTCATGCACTTTCCTATCACATC
Probe (5'-3')	AGATGCCAGCAGACACCATCACAT
Label	FAM

The ddPCR assay is universal to both WT and floxed alleles which are both 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	CRY1-FLOX-3'-MUT2
Forward Primer (5'-3')	TGGAAGAAGAGAAGAGTAAAGGG
Reverse Primer (5'-3')	TTTAGGATGCAACTGATGGC
Probe (5'-3')	AAGTTATCGCCGGCGGGTCTGA
Label	FAM

The ddPCR assay is specific to the Cry1 Flox donor and only floxed 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 evidence of random integrations were detected in the animals taken forwards to establish the colony.