Name of Mouse model or mutation:
PCDH19-DDCRE-EM2-B6J

Description:
Knock-in of ddCre into the 5' UTR of Pcdh19 made by CRISPR/Cas9 gene editing.

Type of mutation:
Knock-in: ddCre

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

Genetic Background:
C57BL/6J

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
TCTCAGCAGAGACTGTCTGG	GGG
GGTCTCAGCAGAGACTGTCT	GGG

Plasmid donor sequence (5'-3'):

LOCUS pUC-GW-Kan.dna 2626 bp ds-DNA circular SYN 08-JUN-2020
SOURCE synthetic DNA construct
ORGANISM synthetic DNA construct
REFERENCE 1 (bases 1 to 2626)
AUTHORS 123
TITLE Direct Submission
JOURNAL Exported Jun 8, 2020 from SnapGene Viewer 4.2.6 to Vector NTI(R) format
http://www.snapgene.com
COMMENT http://www.informaxinc.com/
ORIGDB|GenBank
COMMENT VNTDATE|568684800|
COMMENT VNTDBDATE|915840000|
COMMENT VNTNAME|pUC-GW-Kan|
COMMENT VNTAUTHORNAME|123|
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//

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 plasmid 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

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Mutant PCDH19-DDCRE-EM2-B6J

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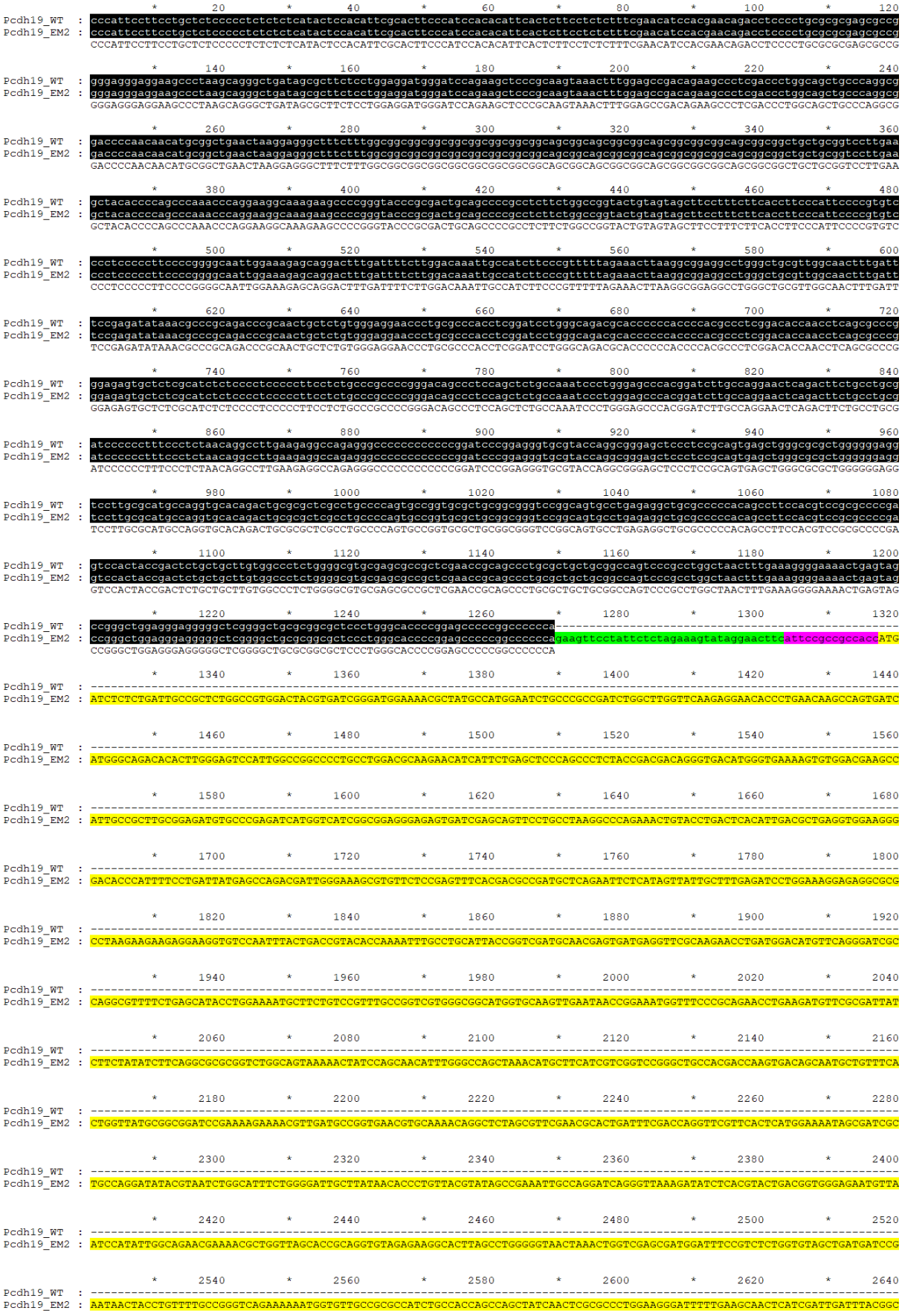
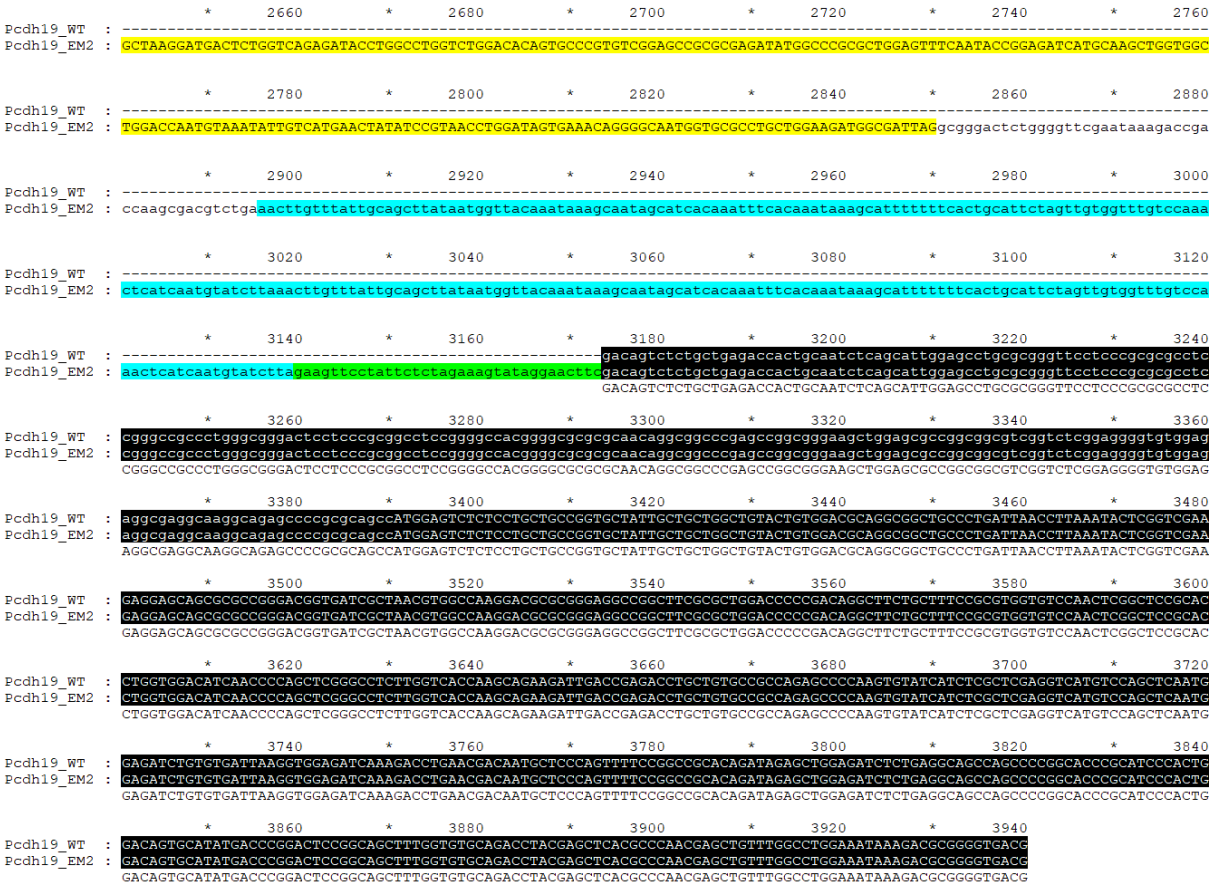
FRT sites = Green highlight
Kozac sequence = Pink highlight
ddCre = Yellow highlight
SV40 sequences = Blue highlight
Lowercase = UTR
Uppercase = CDS

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_Pcdh19_F8 (5'-3')	CCCATTCCTTCCTGCTCTCC
Geno_Pcdh19_R8 (5'-3')	CGTCACCCCGCGTCTTTATT
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	2
WT product size (bp)	2036
Mutant product size (bp)	3940
Notes	PCR external to Knock-in. Primers bind to engineered and WT allele. Sequenced with the following primers (5'-3'): Geno_Pcdh19_F3 (taggaacttcattccgccgc) Geno_Pcdh19_F6 (accaagcgacgtctgaaact)

	Geno_Pcdh19_R4 (cgcCTAATCGCCATCTTCCA) Geno_Pcdh19_R7 (TTCACCCATGTCACCCTGTC)

Geno_Pcdh19_F3 (5’-3’)	taggaacttcattccgccgc
Geno_Pcdh19_R3 (5’-3’)	GCAGGCTCCAATGCTGAGAT
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	-
Mutant product size (bp)	1925
Notes	Knock-in specific PCR. Sequenced with the following primers (5’-3’): Geno_Pcdh19_F4 (GCGCGCCTAAGAAGAAGAGG) Geno_Pcdh19_F5 (CGTACTGACGGTGGGAGAAT) Geno_Pcdh19_R6 (AACATCTTCAGGTTCTGCGGG)

Geno_Pcdh19_F8 (5’-3’)	CCCATTCCTTCCTGCTCTCC
Geno_Pcdh19_R5 (5’-3’)	TCAGCTACACCAGAGACGGA
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1.25
WT product size (bp)	-
Mutant product size (bp)	2513
Notes	Knock-in specific PCR. Sequenced with the following primers (5’-3’): Geno_Pcdh19_F8 (CCCATTCCTTCCTGCTCTCC) Seq_F (ATCCCGGAGGGTGCGTACCA) Seq_R10 (ATCGCAGGCAGAAGTCTGAGTTCC)

Geno_Pcdh19_F6 (5’-3’)	accaagcgacgtctgaaact
Geno_Pcdh19_R8 (5’-3’)	CGTCACCCCGCGTCTTTATT

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)	1061
Notes	Knock-in specific PCR. Sequenced with the following primers (5'-3'): Geno_Pcdh19_R8 (CGTCACCCCGCGTCTTTATT) Geno_Pcdh19_R3 (GCAGGCTCCAATGCTGAGAT)

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 and animals male.

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')
7:131085680-131085702	GGGCTCAGCAGATACTGTCT TGG	Intronic	Pcdh19_OT1_F1 (GTCTTTCCCCATCCTCTTGGT) Pcdh19_OT1_R1 (CTACACTCTATTGCCTTTGGCCT)

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	Pcdh19_ddCre_MUT1
Forward Primer (5'-3')	gcattctagttgtggtttgtcc
Reverse Primer (5'-3')	CAGGCTCCAATGCTGAGAT
Probe (5'-3')	aggaacttcGACAGTCTCTGCTGAGA
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	Pcdh19_ddCre_BP1
Forward Primer (5'-3')	CCGCCTGGCTAACTTTGA
Reverse Primer (5'-3')	GCTGAGATTGCAGTGGTCT
Probe (5'-3')	AAACTGAGTAGCCGGGCTGGAG
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.

Assay name	pUC57-Backbone-Assay2
Forward Primer (5'-3')	GGCGAAACCCGACAGGACTATAA
Reverse Primer (5'-3')	GGGAGAAAGGCGGACAGGTATC
Probe (5'-3')	TCTCCTGTTCCGACCCTGCCGCTTA
Label	FAM

This ddPCR assay is specific to the plasmid backbone of the donor. WT controls and correct F1 (HET) animals should all call at 0 copies.

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 used as the internal calibrator.

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