Name of Mouse model or mutation:
Chrne-P141L-EM1-B6J

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

Type of mutation:
SNP: P141L

Delivery method:
Electroporation into 1-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
90:10 Deactivated Cas9: Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
AGGTGCTGCGGTAGATGGCT	GGG

ssODN donor sequence (5'-3'):
ATGTGAAAAGCTTGGTTTCCACAGTATTGATGGGCAGTTTGGAGTGGCCTACGACAGCAATGTTC
TAGTCTATGAGGGAGGCTATGTGAGCTGGTTGCCaCtAGCCATCTACCGCAGCACCTGCGCAGT
GGAGGTCACCTATTTCCCCTTTGACTGGCAGAACTGCTCTCTCATTTTTCGGTAGGAAGAATCAC
TTCAAT

Electroporation mixes:
90:10 Deactivated Cas9: Cas9 protein, sgRNAs and ssODNs were diluted and mixed in Electroporation buffer (EB; Gibco Opti-MEM I Reduced Serum Media – (Thermo Fisher Scientific)) to the working concentrations of 650 ng/µl, 130 ng/µl and 400 ng/µl, respectively. Embryos were electroporated using the following conditions: 30 V, 3 ms pulse length, 100 ms pulse interval, 12 pulses. Electroporated embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Sequence details

Chrne WT

ATAGGCAGAAGCCAGGGAAAGAATGAAGAGCTTAGCCTGTATCACCATCTCTTCGACAATTATGATC
CAGAATGCCGGCCAGTTAGGAGACCTGAGGACACTGTCACCATCACCCTCAAGGTCACCCTAACCAA
CCTCATCTCACTGGTAAGACCTTCCCCCAATTCTAATCCTATACCCTCACTTCTACCCCCTAACTCCTGG
CTTCAGCAGGCTTCTTTCTGTTGTAGAACGAGAAAGAAGAAACTCTGACCACCAGTGTCTGGATTGG
CATTGTGAGTCAGACCTGGGGAGCAGTGGGAGGGTCCCCAGCCAAGCCCCTTCTCTTCTGCCAGGT
GGGAGCCGGAGAGCACACACTCTCTCTCGCCCCCACCTCTTAGGACTGGCACGACTATCGGCTCAAC
TACAGCAAGGACGATTTTGCAGGTGTAGGAATCCTCCGGGTCCCTTCAGAACATGTATGGCTGCCAG
AGATTGTTCTAGAAAACAAGTGAGGATCACCCAAGAGGGCAGGCTGCAAAGGGAACTGGCTTTGG
GGGATTTGGTTGGTGAAGGAGGTTCAGACATGTGAAAAGCTTGGTTTCCACAGTATTGATGGGCAG
TTTGGAGTGGCCTACGACAGCAATGTTCTAGTCTATGAGGGAGGCTATGTGAGCTGGTTGCCCCCA
GCCATCTACCGCAGCACCTGCGCAGTGGAGGTCACCTATTTCCCCTTTGACTGGCAGAACTGCTCTCT
CATTTTTCGGTAGGAAGAATCACTTCAATCATGATATTGAGAAGGGCCTAGAGCAGGGGACAGGGC
CTGCTAAGGAGGGATAGGTGGGGTCTTCTTTGGAAGGTCTTTGGGAAGGAGTTGGAGTGAGGCAC
TGGAGTGAAGGGGTGGGAAGCTGGTGTAGGTTTGCAGTAGGCGTGGGCACAGGTTTCTTGCTGCC
ATCTTGTAGTTTGAGCGCATAGTCGCAGCTATTCCCCAGGAGGAGTCTGAGGCATAAAAATTACTTG
AGTCCAGGAATCCCTTGGATAGCACGGTAAGCCCATCAGCAACCCAAAACAGAACAGAAAAGGAAG
CAGGATTGGGAACGCGCTGGGGAATGTGGGCAAAAGAAGAGAGCCATATTACCCTGCCAGATTGA
AGCTTCCAGGGTGGAACTAAGATGGCTGCCAGCTTCTGGGGTAAAGACTGGCCCTGTTTCACAGCTC
CCAGACCTACAATGCTGAGGAGGTGGAGTTCATCTTTGCCGTGGATGACGACGGCAATACCATCAA
CAAGATTGACATTGACACGGCAGCTTTTACCGGTGAAGGGATATACCCCCTTTCCAAAAATTCTTCTC
CCAGATAAGGTTCAAGGGGGGACGTTCAGGGTAGGGCTTGGCCTGAACCACACAACCGGAAG

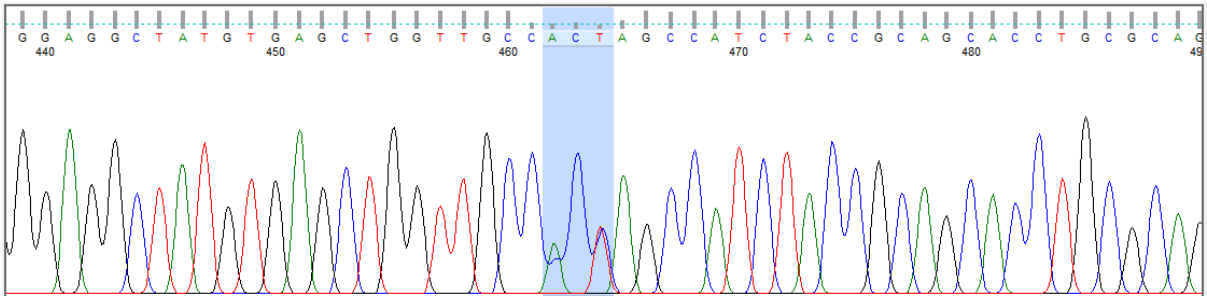
Chrne-P141L-EM1-B6J

ATAGGCAGAAGCCAGGGAAAGAATGAAGAGCTTAGCCTGTATCACCATCTCTTCGACAATTATGATC
CAGAATGCCGGCCAGTTAGGAGACCTGAGGACACTGTCACCATCACCCTCAAGGTCACCCTAACCAA
CCTCATCTCACTGGTAAGACCTTCCCCCAATTCTAATCCTATACCCTCACTTCTACCCCCTAACTCCTGG
CTTCAGCAGGCTTCTTTCTGTTGTAGAACGAGAAAGAAGAAACTCTGACCACCAGTGTCTGGATTGG
CATTGTGAGTCAGACCTGGGGAGCAGTGGGAGGGTCCCCAGCCAAGCCCCTTCTCTTCTGCCAGGT
GGGAGCCGGAGAGCACACACTCTCTCTCGCCCCCACCTCTTAGGACTGGCACGACTATCGGCTCAAC
TACAGCAAGGACGATTTTGCAGGTGTAGGAATCCTCCGGGTCCCTTCAGAACATGTATGGCTGCCAG
AGATTGTTCTAGAAAACAAGTGAGGATCACCCAAGAGGGCAGGCTGCAAAGGGAACTGGCTTTGG
GGGATTTGGTTGGTGAAGGAGGTTCAGACATGTGAAAAGCTTGGTTTCCACAGTATTGATGGGCAG
TTTGGAGTGGCCTACGACAGCAATGTTCTAGTCTATGAGGGAGGCTATGTGAGCTGGTTGCCaCtAG
CCATCTACCGCAGCACCTGCGCAGTGGAGGTCACCTATTTCCCCTTTGACTGGCAGAACTGCTCTCTC
ATTTTTCGGTAGGAAGAATCACTTCAATCATGATATTGAGAAGGGCCTAGAGCAGGGGACAGGGCC
TGCTAAGGAGGGATAGGTGGGGTCTTCTTTGGAAGGTCTTTGGGAAGGAGTTGGAGTGAGGCACT
GGAGTGAAGGGGTGGGAAGCTGGTGTAGGTTTGCAGTAGGCGTGGGCACAGGTTTCTTGCTGCCA
TCTTGTAGTTTGAGCGCATAGTCGCAGCTATTCCCCAGGAGGAGTCTGAGGCATAAAAATTACTTGA

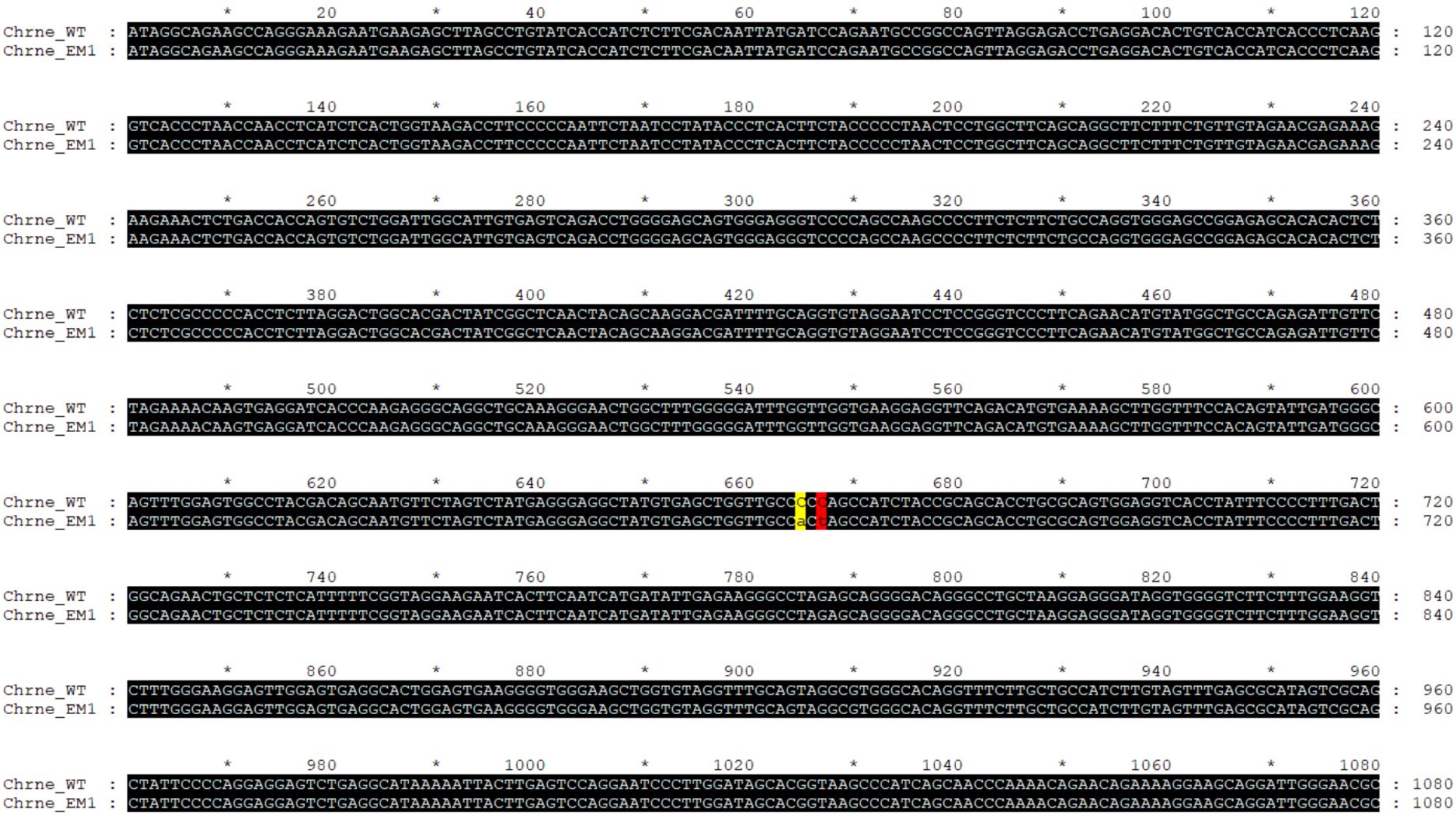
GTCCAGGAATCCCTTGGATAGCACGGTAAGCCCATCAGCAACCCAAAACAGAACAGAAAAGGAAGC
AGGATTGGGAACGCGCTGGGGAATGTGGGCAAAAGAAGAGAGCCATATTACCCTGCCAGATTGAA
GCTTCCAGGGTGGAACTAAGATGGCTGCCAGCTTCTGGGGTAAAGACTGGCCCTGTTTCACAGCTCC
CAGACCTACAATGCTGAGGAGGTGGAGTTCATCTTTGCCGTGGATGACGACGGCAATACCATCAAC
AAGATTGACATTGACACGGCAGCTTTTACCGGTGAAGGGATATACCCCCTTTCCAAAAATTCTTCTCC
CAGATAAGGTTCAAGGGGGGACGTTCAGGGTAGGGCTTGGCCTGAACCACACAACCGGAAG

Red highlight and bold = nominated change. **Yellow highlight and bold = silent mutation** to prevent the reprocessing of the engineered allele.

Heterozygous F1 animal sequence trace:



Nucleotide Alignment:



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                *      1100        *      1120        *      1140        *      1160        *      1180        *      1200
Chrne_WT  : GCTGGGGAATGTGGGCAAAAGAAGAGAGCCATATTACCCTGCCAGATTGAAGCTTCCAGGGTGGAACTAAGATGGCTGCCAGCTTCTGGGGTAAAGACTGGCCCTGTTTCACAGCTCCCA : 1200
Chrne_EM1 : GCTGGGGAATGTGGGCAAAAGAAGAGAGCCATATTACCCTGCCAGATTGAAGCTTCCAGGGTGGAACTAAGATGGCTGCCAGCTTCTGGGGTAAAGACTGGCCCTGTTTCACAGCTCCCA : 1200

                *      1220        *      1240        *      1260        *      1280        *      1300        *      1320
Chrne_WT  : GACCTACAATGCTGAGGAGGTGGAGTTCATCTTTGCCGTGGATGACGACGGCAATACCATCAACAAGATTGACATTGACACGGCAGCTTTTACCGGTGAAGGGATATACCCCCTTTCCAA : 1320
Chrne_EM1 : GACCTACAATGCTGAGGAGGTGGAGTTCATCTTTGCCGTGGATGACGACGGCAATACCATCAACAAGATTGACATTGACACGGCAGCTTTTACCGGTGAAGGGATATACCCCCTTTCCAA : 1320

                *      1340        *      1360        *      1380        *
Chrne_WT  : AAATTCTTCTCCCAGATAAGGTTCAAGGGGGGACGTTCAGGGTAGGGCTTGGCCTGAACCACACAACCGGAAG : 1393
Chrne_EM1 : AAATTCTTCTCCCAGATAAGGTTCAAGGGGGGACGTTCAGGGTAGGGCTTGGCCTGAACCACACAACCGGAAG : 1393
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Predicted Protein Alignment:

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              120         *       140         *       160
Chrne_WT  : IDGQFGVAYDSNVLVYEGGYVSWLPLAIYRSTCAVEVTYFPFDWQNCSLIF : 51
Chrne_EM1 : IDGQFGVAYDSNVLVYEGGYVSWLPLAIYRSTCAVEVTYFPFDWQNCSLIF : 51
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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_Chrne_P141L_F1 (5' to 3')	ATAGGCAGAAGCCAGGGAAAG
Geno_Chrne_P141L_R1 (5' to 3')	CTTCCGGTTGTGTGGTTCAGG
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	1393
Mutant product size (bp)	1393
Notes	Please sequence with the following primers (5' to 3'): Geno_Chrne_P141L_F2: ATACCCTCACTTCTACCCCCT Geno_Chrne_P141L_R2: GCAGCCATCTTAGTTCCACC

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')
11:99082178-99082200	**TT**GT**C**CTG**A**GGTAGATGGCT GGG	Intronic	Chrne_P141L_OT4F1: TCGGTGACTGGAGATTGTGTG Chrne_P141L_OT4R1: TGTGTGTCCCTGTGGCTAAG
11:102659253-102659275	AGGTG**GCTT**GGTAGATGGCT TGG	Intergenic	Chrne_P141L_OT5F1: AGGCATGCTTGGCTCTTATG Chrne_P141L_OT5R1: TTGCTAAACATGGTGCTGGC Please sequence with the following primer: Chrne_P141L_OT5R2: GCTTCGCAATAAGGAACGCA
11:63982834-63982856	AG**C**TGCTG**T**GGTAGA**G**GGCT GGG	Intronic	Chrne_P141L_OT6F1: ACATGAGCTGTGCCTAACCC

			Chrne_P141L_OT6R1: CTTGGCAGGGAGGTGAGAAG

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	Chrne_P141L_MUT1
Forward Primer (5'-3')	GTGAGCTGGTTGCCACT
Reverse Primer (5'-3')	AGCAGTTCTGCCAGTCAAA
Probe (5'-3')	TGCGCAGTGGAGGTCACCTATTTC
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.

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.