Name of Mouse model or mutation:
CD1D1-LPETG-EM1-B6

Description:
Double tag insertion (LPETG sortase cassette) made by CRISPR/Cas9 gene editing.

Type of mutation:
Tag 1: Cd1d1 LPETG Sortase
Tag 2: Cd1d2 LPETG Sortase

Delivery method:
Pronuclear injection into 1-cell stage embryo

Genetic Background:
C57BL/6J

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
ACGGGTGCTTGCCTGGCATC	TGG

Cd1d1 lssDNA donor sequence (5'-3'):

```
LOCUS       Cd1d1_Sortase_do        584 bp    DNA     linear      11-NOV-2020
FEATURES             Location/Qualifiers
     misc_feature    158..199
                     /note="LPETG Sortase"
     misc_feature    1..157
                     /note="5' HA"
     misc_feature    200..584
                     /note="3' HA"
     PCR_primer      complement(565..584)
                     /dnas_title="Cd1d1_sortase_RNA_R"
                     /note="Cd1d1_sortase_RNA_R"
     source          1..584
                     /dnas_title="Cd1d1_Sortase_donor template"
ORIGIN
```

1 AGACGTTGAT GCTAAGATAG GGTAACTACC CAGATTCACA CAGTAGCCAG ATCCTGATCA
61 TGAGAAATGG AATGGATATA CTTTAAATTA GAATGGGGAA CTTGAAACAT GTACTGGTTT
121 TCTGTTGAGA GATACATTGT ATTCTTCCAA ACCAGATctg cccgagaccg gcggcggttc
181 tggcggtagc ggcggttccG CCAGGCAAGC ACCCGTGGGC CTGATCGTCT TCATAGTACT
241 GATCATGCTA GTGGTGGTGG GTGCTGTAGT CTACTATATC TGGAGAAGGA
GAAGGTAAGT
301 CTCCCTGTCC ATGTGCTCCT TCCCTCAGCA TCCCTCCTTC ATTCCTTCCT TTTCTCTCCT
361 AATGGCCTCT CTCTTCTTCC AGCGCTTATC AAGACATCCG GTGACTCTTC CTTACACCTG
421 CCTCTCCTGA AATTCAGACT TTCCAGGCTC TAGGACTTCA GTCCTGGTCT GCTCAGGATC
481 TGGGGATGAA GGAGAGGAAT CCTGAAGAAG TGAAGAGCAG CCAGTACGCT
CTTTTCAACA
541 TTAATTATAA GAAATTAATT ATTTGAGTTG TTTCGTCAGT TTCC
//

Cd1d2 lssDNA donor sequence (5’-3’):

LOCUS Cd1d2_Sortase_do 570 bp DNA linear 11-NOV-2020
FEATURES Location/Qualifiers
PCR_primer complement(545..570)
/dnas_title="cd1d2_LPETG_don2_RNAR"
/note=""
misc_feature 159..200
/note="LPETG Sortase"
misc_feature 1..158
/note="5' HA"
misc_feature 201..570
/note="3' HA"
source 1..570
/dnas_title="Cd1d2_Sortase_donor template"
ORIGIN
1 AAAGGACTGG AGGTAGACAG AGTCACACCT GTTTGGATAG CATGTAGCCA GATCCTGATC
61 ATGAGAAATA GAAAGGATAT ACTTTAAATT AGAATGGGGA ACTTGAAACA
GGTACTGGTT
121 TTCTGTTGAG AGATACATTG TATTCTTCCA AACCAGATct gcccgagacc ggcggcggtt
181 ctggcggtag cggcggttcc GCCAGGCAAG CACCCGTGGG CCTGATCGTC TTCATAGTAC
241 TAATCATGCT AGTGGTGGTG GGTGCTGTAG TCTACTATAT CTGGAGAAGG
AGAAGGTAAG
301 TCTCCCTGTC CATGTGCTCC TTCCCTCAGC ATCCCTCCTT CATTCCTTCC TTTTCTCTCC
361 TAATGGTCTC TCTCTTCTTC CAGCGCTTAT CAAGACATCC GGTGACTCTT CCTTACACCT
421 GCCTCTCCTG AAATTCAGAC TTTCCAGGCT CTAGGACTTC AGTCCTGGTC TGCTCAGGAT
481 CTGGGGATGA AAGAGAGGAA TCCTGAAGAA GTGAAGAGCA ACCAGTATGC
TCCTTTAAAT

541 TTAGGAATAT TACTTCCTAA TTAAATTAAG
//

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

Cd1d1 WT

GCAGCCATCAAACATGGAGCGGGAGTTGAACAGATCTAGATTCAGAAATGAAGCCCCTAACTATTA
TAGAAGAGCTAGAAGGAGGGAATTCTAGATAAAAAAGAAACCCCAAAAGACTTGAGATAAGATGT
TCTCTGTATAAGAAGAAAGAAGACCCAGAAACTGACAAGATCCAGAGGTCAGTGGCAAATAAAGAA
GTTTGGGAAGAGCCATTCCCCAGTAGAGAGGACCCAAGGCCCAAGGCATAGCTGCAGTATATTGGG
CGTGTCCTGGGTGAGCACTGGACATTTTGCTGCCCCTACTTCCACTTGTCCCTTAGCTTTGAAGCAAG
TGTTGTTATTTTGAGTTTAAGAATGAAGACGTTGATGCTAAGATAGGGTAACTACCCAGATTCACAC
AGTAGCCAGATCCTGATCATGAGAAATGGAATGGATATACTTTAAATTAGAATGGGGAACTTGAAA
CATGTACTGGTTTTCTGTTGAGAGATACATTGTATTCTTCCAAACCAGATGCCAGGCAAGCACCCGTG
GGCCTGATCGTCTTCATAGTACTGATCATGCTAGTGGTGGTGGGTGCTGTAGTCTACTATATCTGGA
GAAGGAGAAGGTAAGTCTCCCTGTCCATGTGCTCCTTCCCTCAGCATCCCTCCTTCATTCCTTCCTTTT
CTCTCCTAATGGCCTCTCTCTTCTTCCAGCGCTTATCAAGACATCCGGTGACTCTTCCTTACACCTGCC
TCTCCTGAAATTCAGACTTTCCAGGCTCTAGGACTTCAGTCCTGGTCTGCTCAGGATCTGGGGATGA
AGGAGAGGAATCCTGAAGAAGTGAAGAGCAGCCAGTACGCTCTTTTCAACATTAATTATAAGAAAT
TAATTATTTGAGTTGTTTCGTCAGTTTCCATAGTTTAGAACAAACACAACTGCAAATGTGCGTACCTG
CCAGAAACAAGCTGTTGGCAGTGTTTTATGGGATTTGCACTGAACTAGAAAGCATACTTCCTGCCCA
AACAGACGCTCTGAGGTTAGTTGGCAAGTGTAAAGTCCAACACCAACCTGGCTGTACTCTGTATTTT
TCAAGGTGACTAGAAAAATGGATTTGTGTGTGTGTGACATACAGCTTTGGGGTTTGTTGGC

CD1D1-LPETG-EM1-B6 at Cd1d1 locus:

GCAGCCATCAAACATGGAGCGGGAGTTGAACAGATCTAGATTCAGAAATGAAGCCCCTAACTATTA
TAGAAGAGCTAGAAGGAGGGAATTCTAGATAAAAAAGAAACCCCAAAAGACTTGAGATAAGATGT
TCTCTGTATAAGAAGAAAGAAGACCCAGAAACTGACAAGATCCAGAGGTCAGTGGCAAATAAAGAA
GTTTGGGAAGAGCCATTCCCCAGTAGAGAGGACCCAAGGCCCAAGGCATAGCTGCAGTATATTGGG
CGTGTCCTGGGTGAGCACTGGACATTTTGCTGCCCCTACTTCCACTTGTCCCTTAGCTTTGAAGCAAG
TGTTGTTATTTTGAGTTTAAGAATGAAGACGTTGATGCTAAGATAGGGTAACTACCCAGATTCACAC
AGTAGCCAGATCCTGATCATGAGAAATGGAATGGATATACTTTAAATTAGAATGGGGAACTTGAAA
CATGTACTGGTTTTCTGTTGAGAGATACATTGTATTCTTCCAAACCAGATctgcccgagaccggcggcggttct
ggcggtagcggcggttccGCCAGGCAAGCACCCGTGGGCCTGATCGTCTTCATAGTACTGATCATGCTAGT

GGTGGTGGGTGCTGTAGTCTACTATATCTGGAGAAGGAGAAGGTAAGTCTCCCTGTCCATGTGCTCC
TTCCCTCAGCATCCCTCCTTCATTCCTTCCTTTTCTCTCCTAATGGCCTCTCTCTTCTTCCAGCGCTTATC
AAGACATCCGGTGACTCTTCCTTACACCTGCCTCTCCTGAAATTCAGACTTTCCAGGCTCTAGGACTT
CAGTCCTGGTCTGCTCAGGATCTGGGGATGAAGGAGAGGAATCCTGAAGAAGTGAAGAGCAGCCA
GTACGCTCTTTTCAACATTAATTATAAGAAATTAATTATTTGAGTTGTTTCGTCAGTTTCCATAGTTTA
GAACAAACACAACTGCAAATGTGCGTACCTGCCAGAAACAAGCTGTTGGCAGTGTTTTATGGGATTT
GCACTGAACTAGAAAGCATACTTCCTGCCCAAACAGACGCTCTGAGGTTAGTTGGCAAGTGTAAAGT
CCAACACCAACCTGGCTGTACTCTGTATTTTTCAAGGTGACTAGAAAAATGGATTTGTGTGTGTGTG
ACATACAGCTTTGGGGTTTGTTGGC

Cd1d2 WT

CCCAGAAACTGACAAGATCCAGAGGTCAGTGGCAAATAAAGAAGTTTGGGAAGAGCCATTCCCCAG
TAGAGAGGACCCAAGGCCCAAGGCATAGCTGCAGTATATTGGGCGTGTCCTGAGTGAGCACTGGAC
ACTTTGCTGCCCCTACTTCCACTTGTCACTTAGCTTTGAAGCAAGGGTTGTTATTTTGAGTTTAAGAAT
GAGGACGTTGATGCTAAGATAGGGTAACTACCCAGATTCACATAGAAAGGACTGGAGGTAGACAG
AGTCACACCTGTTTGGATAGCATGTAGCCAGATCCTGATCATGAGAAATAGAAAGGATATACTTTAA
ATTAGAATGGGGAACTTGAAACAGGTACTGGTTTTCTGTTGAGAGATACATTGTATTCTTCCAAACC
AGATGCCAGGCAAGCACCCGTGGGCCTGATCGTCTTCATAGTACTAATCATGCTAGTGGTGGTGGG
TGCTGTAGTCTACTATATCTGGAGAAGGAGAAGGTAAGTCTCCCTGTCCATGTGCTCCTTCCCTCAGC
ATCCCTCCTTCATTCCTTCCTTTTCTCTCCTAATGGTCTCTCTCTTCTTCCAGCGCTTATCAAGACATCC
GGTGACTCTTCCTTACACCTGCCTCTCCTGAAATTCAGACTTTCCAGGCTCTAGGACTTCAGTCCTGGT
CTGCTCAGGATCTGGGGATGAAAGAGAGGAATCCTGAAGAAGTGAAGAGCAACCAGTATGCTCCTT
TAAATTTAGGAATATTACTTCCTAATTAAATTAAGATCAATTCAAATTACATGTATGTGCGGAGTGAG
GTGGGGGTGGTGTCTGTGCTCTTGAATACTAGTGATCTTAAAAGTAGGAGAGCCTGCGGTTTCCCTG
CAGCCGGAGTTATAGCCCGAGCAGCCTGATATGGACTATAGGAACCAACTCTAGTCCTCTGCAAGGC
CAGTGTGTGGTCTAAGCCACTGAATCATCTCTCCAGACCCTCTTAAGAAAAGTATTTACTACAGTGAG
AAAAAATAAAAACTATAAGGTATAAGATTTTATTGTTTTATTAAATCTTGGATTCTGAGGCTGGAAAG
CAACTATTTTGGA

CD1D1-LPETG-EM1-B6 at Cd1d2 locus:

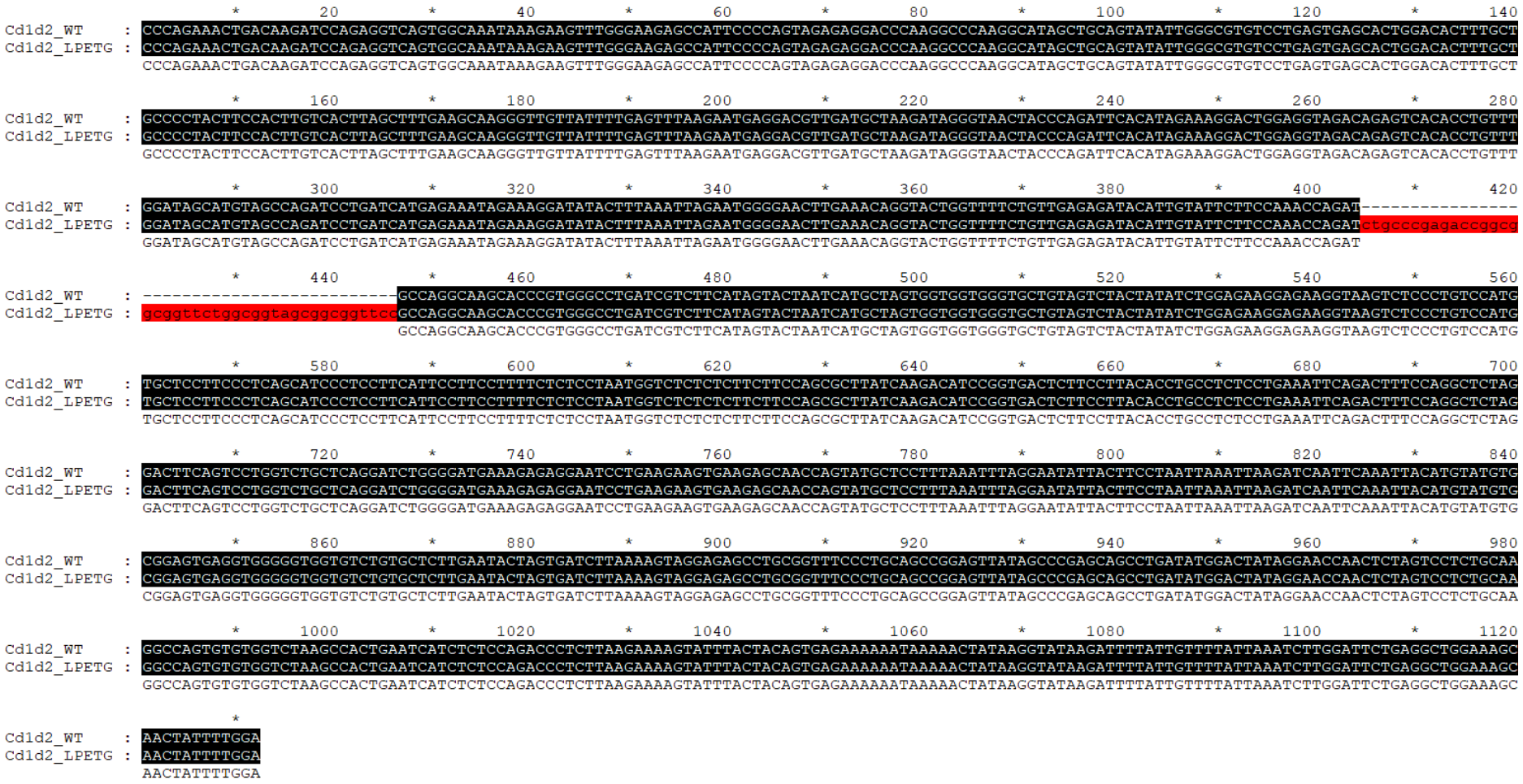
CCCAGAAACTGACAAGATCCAGAGGTCAGTGGCAAATAAAGAAGTTTGGGAAGAGCCATTCCCCAG
TAGAGAGGACCCAAGGCCCAAGGCATAGCTGCAGTATATTGGGCGTGTCCTGAGTGAGCACTGGAC
ACTTTGCTGCCCCTACTTCCACTTGTCACTTAGCTTTGAAGCAAGGGTTGTTATTTTGAGTTTAAGAAT
GAGGACGTTGATGCTAAGATAGGGTAACTACCCAGATTCACATAGAAAGGACTGGAGGTAGACAG
AGTCACACCTGTTTGGATAGCATGTAGCCAGATCCTGATCATGAGAAATAGAAAGGATATACTTTAA
ATTAGAATGGGGAACTTGAAACAGGTACTGGTTTTCTGTTGAGAGATACATTGTATTCTTCCAAACC
AGATctgcccgagaccggcggcggttctggcggtagcggcggttccGCCAGGCAAGCACCCGTGGGCCTGATCGTC
TTCATAGTACTAATCATGCTAGTGGTGGTGGGTGCTGTAGTCTACTATATCTGGAGAAGGAGAAGGT
AAGTCTCCCTGTCCATGTGCTCCTTCCCTCAGCATCCCTCCTTCATTCCTTCCTTTTCTCTCCTAATGGT
CTCTCTCTTCTTCCAGCGCTTATCAAGACATCCGGTGACTCTTCCTTACACCTGCCTCTCCTGAAATTC
AGACTTTCCAGGCTCTAGGACTTCAGTCCTGGTCTGCTCAGGATCTGGGGATGAAAGAGAGGAATC

CTGAAGAAGTGAAGAGCAACCAGTATGCTCCTTTAAATTTAGGAATATTACTTCCTAATTAAATTAAG
ATCAATTCAAATTACATGTATGTGCGGAGTGAGGTGGGGGTGGTGTCTGTGCTCTTGAATACTAGTG
ATCTTAAAAGTAGGAGAGCCTGCGGTTTCCCTGCAGCCGGAGTTATAGCCCGAGCAGCCTGATATG
GACTATAGGAACCAACTCTAGTCCTCTGCAAGGCCAGTGTGTGGTCTAAGCCACTGAATCATCTCTC
CAGACCCTCTTAAGAAAAGTATTTACTACAGTGAGAAAAAATAAAAACTATAAGGTATAAGATTTTA
TTGTTTTATTAAATCTTGGATTCTGAGGCTGGAAAGCAACTATTTTGGA

LPETG Sortase sequence highlighted in red.

Nucleotide Alignment Cd1d1:

Nucleotide Alignment Cd1d2:



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_Cd1d1spec_F3 primer (5'-3')	GCAGCCATCAAACATGGAGC
Geno_Cd1d1spec_R3 primer (5'-3')	GCCAACAAACCCCAAAGCTG
Taq Polymerase used	ThermoFisher SuperFi II PCR kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	1133
Mutant product size (bp)	1175
Notes	PCR to amplify Cd1d1 region specifically. This amplicon sequenced in animals with positive band for LPETG cassette specific PCR (see below). Amplicons sequenced with: Geno_Cd1d1spec_F4:CATGGAGCGGGAGTTGAACA Geno_Cd1d1spec_R4: AGAGTACAGCCAGGTTGGTG

Geno_Cd1d2spec_F1 primer (5'-3')	CCCAGAAACTGACAAGATCCAGA
Geno_Cd1d2spec_R1 primer (5'-3')	TCCAAAATAGTTGCTTTCCAGCC
Taq Polymerase used	ThermoFisher SuperFi II PCR kit
Annealing Temperature (°C)	60
Elongation time (min)	0.75
WT product size (bp)	1090
Mutant product size (bp)	1132
Notes	PCR to amplify Cd1d2 region specifically. This amplicon sequenced in animals with positive band for LPETG cassette specific PCR (see below). Amplicons sequenced with: Geno_Cd1d2spec_F2: CCAGAGGTCAGTGGCAAATAAAG

	Geno_Cd1d2spec_seqR5: CTTAAGAGGGTCTGGAGAGATGATTC

Geno_Cd1d2_LPETG_F9 primer (5'-3')	GGTCTTCCTAGCCAGAGGGA
Geno_Cd1d1&2_LPETG_R8 primer (5'-3')	tctcgggcagATCTGGTTTG
Taq Polymerase used	ThermoFisher SuperFi PCR kit
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	N/A
Mutant product size (bp)	1001
Notes	PCR to screen for presence of LPETG Sortase sequence at either locus.

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')
15:89234145-89234167	AC**C**GGTGCTTGCCTGGCAT**G** TGG	Intergenic	Geno_Cd1d2_OT4_F1 primer: AGTGTGCATCAACAGCCGAAA Geno_Cd1d2_OT4_R1 primer: ACTCCTATTGCAGCCTACCAAG

All amplicons were sent for Sanger sequencing. No off-target activity was detected in those 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	CD1D1-LPETG-UNI1
Forward Primer (5'-3')	TGAAGGAGAGGAATCCTGAA

Reverse Primer (5’-3’)	GAAACTGACGAAACAACTCAAA
Probe (5’-3’)	AAGAGCAGCCAGTACGCTCTTT
Label	FAM

This ddPCR assay is universal to Cd1d1 - both 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	CD1D1-LPETG-MUT2
Forward Primer (5’-3’)	AGCCAGATCCTGATCATGAGAAATGGAAT
Reverse Primer (5’-3’)	TTGCCTGGCGGAACCGC
Probe (5’-3’)	TTCTTCCAAACCAGATCTGCCCGA
Label	FAM

This ddPCR assay is specific to the LPETG insertion in the Cd1d1 gene 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	CD1D2-LPETG-UNI1
Forward Primer (5’-3’)	GAGTCACACCTGTTTGGATAG
Reverse Primer (5’-3’)	ACCAGTACCTGTTTCAAGTTC
Probe (5’-3’)	CATGTAGCCAGATCCTGATCATGAGA
Label	FAM

This ddPCR assay is universal to CD1D2 - both 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	CD1D2-LPETG-MUT2
Forward Primer (5’-3’)	AGCCAGATCCTGATCATGAGAAATAGAAA
Reverse Primer (5’-3’)	TTGCCTGGCGGAACCGC
Probe (5’-3’)	TTCTTCCAAACCAGATCTGCCCGA
Label	FAM

This ddPCR assay is specific to the LPETG insertion in the Cd1d2 gene 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.

CD1D1-LPETG

Allele Description

This is a CRISPR/Cas9 induced insertion of a double LPETG sortase cassette. The stock was generated at MRC Harwell via pronuclear injection of CRISPR/Cas9 reagents into 1-cell stage embryos.

qPCR Copy Counting Genotyping Strategy

The genotyping strategy presented here has been optimized for reagents and conditions used by the Genotyping Core at MRC Harwell. To genotype animals, we recommend researchers validate the assay independently. PCR cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Samples are genotyped using qPCR copy counting with both a wildtype loss of allele (WT-LOA) and a mutant assay against a known reference assay (*Dot1l* on chromosome 10; 2 copies present). Samples for this line are genotyped using the following primers and probe:

- Universal probe and Universal primer designed 5' of the deleted region.
- Wildtype specific primer situated within the deleted region.
- Mutant specific primer that binds to the inserted LoxP sequence

For autosomal genes that have been targeted, the following results would be expected:

Genotype of the Modified allele	WT Assay	Mutant Assay
Wildtype	2	0
Heterozygous	1	1
Homozygous mutant	0	2

CD1D1-LPETG –MUT2 assay (FAM labelled)

AGT**AGCCAGATCCTGATCATGAGAAATGGAAT**GGATATACTTTAAATTAGAATGGGGAACTTGAAA
CATGTACTGGTTTTCTGTTGAGAGATACATTGTA**TTCTTCCAAACCAGATctgcccga**gaccggcggcggttc
tggcggtagcg**gcggttccGCCAGGCAA**GCACCCGTGGGCCTGATCGTCTTCATAGTACTGATCATGCTAG
TGGTGGTGGGTGCTGTAGTCTACTATATCTGGAGAAGGAGAAGGTAAGTCTCCCTGTCCATGTGCTC

Lower case letters denote the inserted sequence
Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo CD1D1-LPETG	5' label	Sequence 5' → 3'	3' label	Oligo Type
CD1D1-LPETG-MUT_F	n/a	**AGCCAGATCCTGATCATGAGAAATGGAAT**	n/a	Mutant Forward
CD1D1-LPETG-MUT_PROBE	FAM	**TTCTTCCAAACCAGATCTGCCCGA**	ZEN-IBFQ	Mutant Probe
CD1D1-LPETG-MUT_R	n/a	**TTGCCTGGCGGAACCGC**	n/a	Mutant Reverse

CD1D2-LPETG –MUT2 assay (FAM labelled)

AGTCACACCTGTTTGGATAGCATGT**AGCCAGATCCTGATCATGAGAAATAGAAA**GGATATACTTTA
AATTAGAATGGGGAACTTGAAACAGGTACTGGTTTTCTGTTGAGAGATACATTGTA**TTCTTCCAAAC**
CAGATctgcccgagaccggcggcggttctggcggtagcg**gcggttccGCCAGGCAA**GCACCCGTGGGCCTGATCG
TCTTCATAGTACTAATCATGCTAGTGGTGGTGGGTGCTGTAGTCTACTATATCTGGAGAAGGAGAAG
GT

Lower case letters denote the inserted sequence
Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo CD1D1-LPETG	5' label	Sequence 5' → 3'	3' label	Oligo Type
CD1D2-LPETG-MUT_F	n/a	**AGCCAGATCCTGATCATGAGAAATAGAAA**	n/a	Mutant Forward
CD1D2-LPETG-MUT_PROBE	FAM	**TTCTTCCAAACCAGATCTGCCCGA**	ZEN-IBFQ	Mutant Probe
CD1D2-LPETG-MUT_R	n/a	**TTGCCTGGCGGAACCGC**	n/a	Mutant Reverse

CD1D1-LPETG

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT CACCCTATGA

Oligo CD1D1-LPETG	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	**GCCCCAGCACGACCATT**	n/a	WT Forward
Dot1l_Probe	VIC	**CCAGCTCTCAAGTCG**	BHQ	WT Probe
Dot1l_Reverse	n/a	**TAGTTGGCATCCTTATGCTTCATC**	n/a	WT Reverse

Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

DNA extraction method

DNA is extracted from ear clips using Applied Biosystems Taqman Sample-to-SNP Kit and qPCR run using 1:10 dilution from the crude preparation.

qPCR master mix 1X

Applied Biosystems GTX Taqman master mix	5 µl
Dot1l_Forward (20 µM)	0.225 µl
Dot1l_Reverse (20 µM)	0.225 µl
Dot1l_Probe (5 µM)	0.2 µl
FAM Assay (probe 5 µM & primers 15 µM each)	0.3 µl
ddH20	1.55 µl
DNA (1:10 dilution of ABI Sample-to-SNP prep)	2.5 µl

Each sample is ran in technical duplicate. Seven WT and/or mutant controls are also included in duplicate along with non-template controls.

qPCR cycling conditions

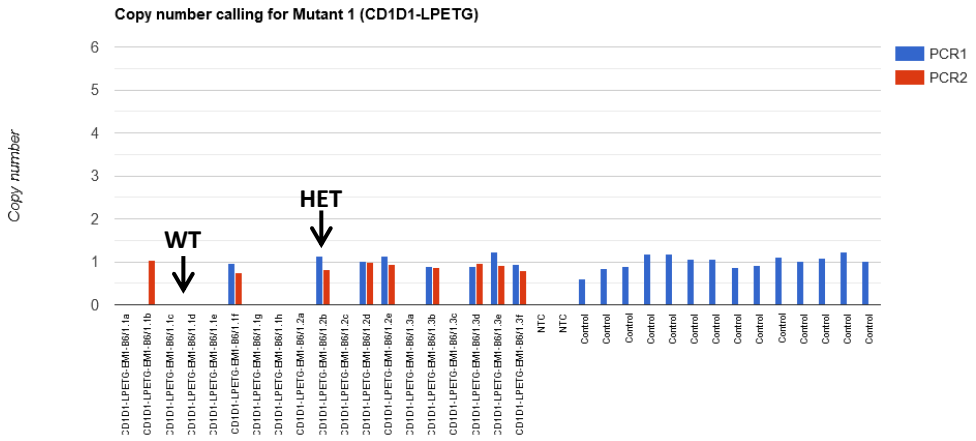
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

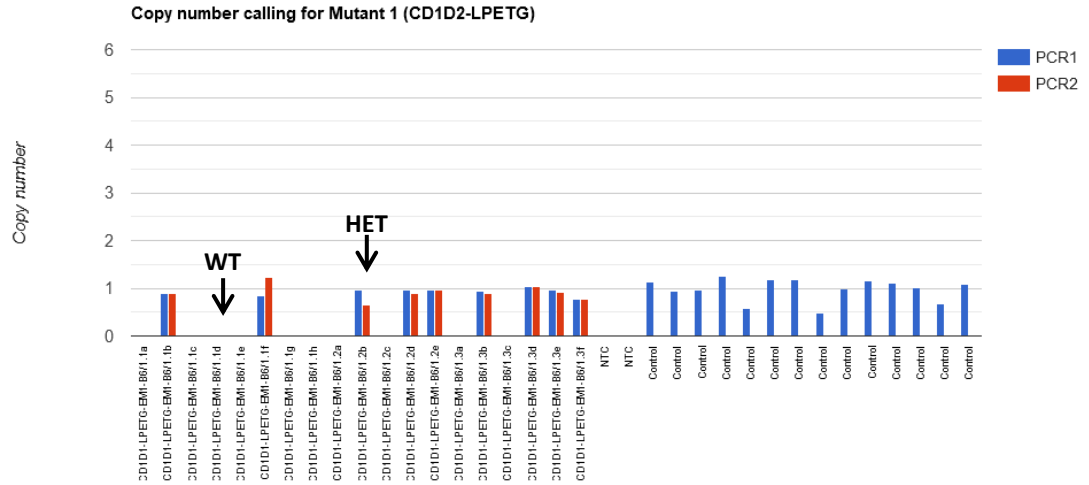
Analysis

The results are analysed using CopyCaller software v2.0 from Applied Biosystems or in-house software that is based on CopyCaller v2.0.

Example of a CD1D1-LPETG-MUT2 assay copy called results, image showing copy number chart for Mutant assay (Task 326129 results)



Example of a CD1D2-LPETG-MUT2 assay copy called results, image showing copy number chart for Mutant assay (Task 326129 results)



Version No.	1
Date:	04/06/2021
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman