Name of Mouse model or mutation:
RABEP2-S187AS191A-EM1-B6
RABEP2-S187AS191A-EM2-B6

Description:
Two point mutations introduced by CRISPR/Cas9 gene editing.

Type of mutation:
SNP: S187A S191A

Delivery method:
Electroporation of RNP into 1-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
GTGAGGGGTTCCTCGACAGA	GGG

ssODN donor sequence (5'-3'):
GGACGCTGGCCCTAGCTTCTTCCCTGCAATCCTCCCTAGAGACGACCACGGCAGCCTGCCTCCCTGC
ATGGCTCCACAGAGTTGCTCCCTCTGGCGCGCAACCCCGCACCCCCACTGGAACCCTTGGAGGAGCC
GAGCGGAGACGCCGGCCCAGCCGCCGAGGCCTTTGCCCACAACTGCGATGACAGTGCCTCCATCTC

Electroporation mixes:
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 each 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

WT

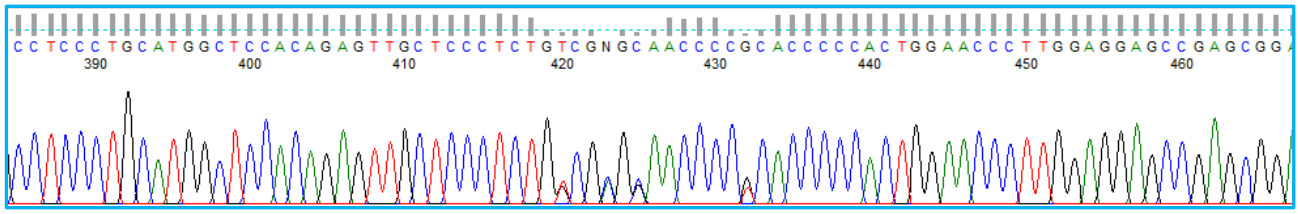
AGCAGCTGAGGTGTAGATGGTATCGGAAAACTGGAACCATTTTCAAACTGTGCCCAGTTAAAATATC
TGAGGTCCTGTGATATAAAAAGGTGCATGTTGAGGCCAGGGGGGACTGGGGTGGATTTGTCCCCCA
AACACTTCCATACATTTATGAAATTCTCAAAGAATAAGTAAAAACGGTTTTAAACGATGGATGTTGC
GGAGGAATTTTGTAAGAATAAGAGGAAAGGAGGAAGCCAGTCATGGCCTTGGCTCCTTTCCCTGGA
AGCACTTAGAGCCCGCGTGTCCTGGAGTCTTGAGTGGGTGGTATAACCATCAGGAAGGATGAGAAC
AGTTAGTGGGCATTGTTCATGATCAAGGGGCTGTCACTTCCCAATGCCCCCGTCGCTCCAGGCTCAT
GAAGACTCGGAAAAGCTTCGAGAGATCGTGTTGCCCATGGAGCAGGAGATTACCGAGCTGAAGGG
GAAGCTGCAGAGGGCGGAAGAGCTGATCCAAGAGATCCAGGTGAGGGCTAGCCGGAGGTGCCGA
GGGAAGGAGGGGTGCCCAGGACGCTGGCCCTAGCTTCTTCCCTGCAATCCTCCCTAGAGACGACCA
CGGCAGCCTGCCTCCCTGCATGGCTCCACAGAGTTGCTCCCTCTGTCGAGGAACCCCTCACCCCCACT
GGAACCCTTGGAGGAGCCGAGCGGAGACGCCGGCCCAGCCGCCGAGGCCTTTGCCCACAACTGCG
ATGACAGTGCCTCCATCTCTTCCTTCTCCCTCGGTGGGGCCGCCGGCAGTGCCTCCCTACGAGGCCCC
CAAGGCCTCAGCCCTGAGCAGGAAGAGACTGCTTCTCTGGTGTCTACTGGCACTCTGGTCCCTGAGG
GCATCTTCCTGCCCCCTCCTGGGTACCAGCTTGTTCCAGATAGCCAGTGGGAGCAACTGCAGGTAGA
GGTGAGTAGACTGGGCTAGGTTCTGTGAACATTCCTGTCTCCCTGGATATATGTGGGCTTGCACTAA
AGAAAGCTACAGTGGAGAGGCCAGTGGGGTGGGGTGGGGGGAGGTCCCCTCCCAGCAAGGGAAC
TTCGGAAGGGCACGTGCACGGGGTGCTGGAGAAGATCAGCAGCTGGGCCGGTGTTATGTTATGGT
GAGAAGTGTCAAGAACAAAACTGAAA

RABEP2-S187AS191A-EM1-B6 and RABEP2-S187AS191A-EM2-B6

AGCAGCTGAGGTGTAGATGGTATCGGAAAACTGGAACCATTTTCAAACTGTGCCCAGTTAAAATATC
TGAGGTCCTGTGATATAAAAAGGTGCATGTTGAGGCCAGGGGGGACTGGGGTGGATTTGTCCCCCA
AACACTTCCATACATTTATGAAATTCTCAAAGAATAAGTAAAAACGGTTTTAAACGATGGATGTTGC
GGAGGAATTTTGTAAGAATAAGAGGAAAGGAGGAAGCCAGTCATGGCCTTGGCTCCTTTCCCTGGA
AGCACTTAGAGCCCGCGTGTCCTGGAGTCTTGAGTGGGTGGTATAACCATCAGGAAGGATGAGAAC
AGTTAGTGGGCATTGTTCATGATCAAGGGGCTGTCACTTCCCAATGCCCCCGTCGCTCCAGGCTCAT
GAAGACTCGGAAAAGCTTCGAGAGATCGTGTTGCCCATGGAGCAGGAGATTACCGAGCTGAAGGG
GAAGCTGCAGAGGGCGGAAGAGCTGATCCAAGAGATCCAGGTGAGGGCTAGCCGGAGGTGCCGA
GGGAAGGAGGGGTGCCCAGGACGCTGGCCCTAGCTTCTTCCCTGCAATCCTCCCTAGAGACGACCA
CGGCAGCCTGCCTCCCTGCATGGCTCCACAGAGTTGCTCCCTCTG**G**CG**C**G**C**AACCCC**G**CACCCCCAC
TGGAACCCTTGGAGGAGCCGAGCGGAGACGCCGGCCCAGCCGCCGAGGCCTTTGCCCACAACTGC
GATGACAGTGCCTCCATCTCTTCCTTCTCCCTCGGTGGGGCCGCCGGCAGTGCCTCCCTACGAGGCC
CCCAAGGCCTCAGCCCTGAGCAGGAAGAGACTGCTTCTCTGGTGTCTACTGGCACTCTGGTCCCTGA
GGGCATCTTCCTGCCCCCTCCTGGGTACCAGCTTGTTCCAGATAGCCAGTGGGAGCAACTGCAGGTA
GAGGTGAGTAGACTGGGCTAGGTTCTGTGAACATTCCTGTCTCCCTGGATATATGTGGGCTTGCACT
AAAGAAAGCTACAGTGGAGAGGCCAGTGGGGTGGGGTGGGGGGAGGTCCCCTCCCAGCAAGGGA
ACTTCGGAAGGGCACGTGCACGGGGTGCTGGAGAAGATCAGCAGCTGGGCCGGTGTTATGTTATG
GTGAGAAGTGTCAAGAACAAAACTGAAA

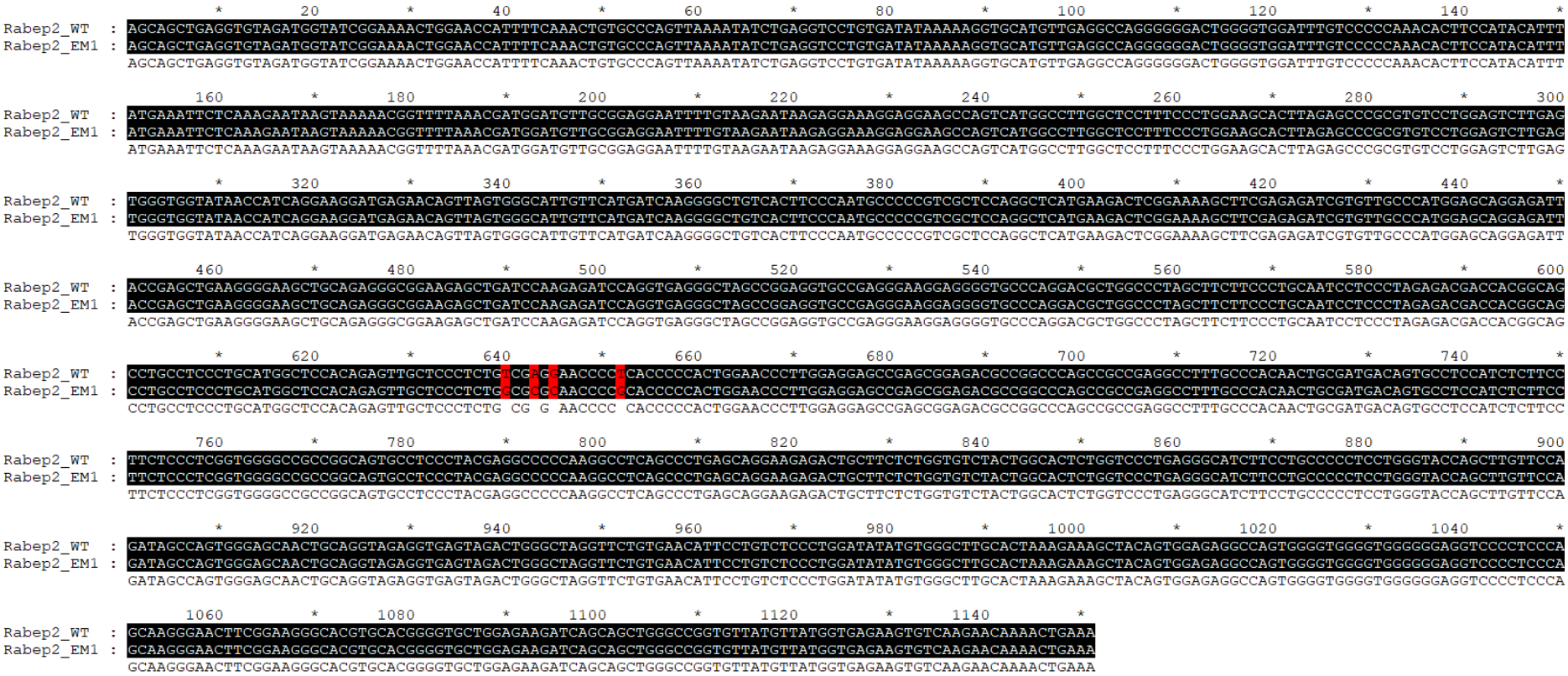
Red and underlined are coding changes, red only are silent changes. Yellow is BssHII site.

RABEP2-S187AS191A-EM1-B6 Heterozygous F1 animal sequence trace:



RABEP2-S187AS191A-EM1-B6 and RABEP2-S187AS191A-EM2-B6 have the same sequence but are derived from different founders.

Nucleotide Alignment:



Predicted Protein 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_RABEP2_F1 primer (5'-3')	AGCAGCTGAGGTGTAGATGGTA
Geno_RABEP2_R1 primer (5'-3')	TTTCAGTTTTGTTCTTGACACTTCT
Taq Polymerase used	ThermoFisher SuperFi
Annealing Temperature (°C)	65
Elongation time (min)	0.75
WT product size (bp)	1151
Mutant product size (bp)	1151
Notes	Sequenced using Geno_RABEP2_F2 primer (5'-3': ACGATGGATGTTGCGGAGGA) and Geno_RABEP2_R3 primer (5'-3': TGCAAGCCCACATATATCCAGG)

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')
17:83157002-83157024	GTGAGGGGATCCTAGACAGAAGG	Intergenic	Geno_RABEP2_OT2_F1: TTGGGTTATTTTCACTCCTGAGAAC Geno_RABEP2_OT2_R1: TCTGTCTTTCCTTTACTGGTGACTT

All amplicons were sent for Sanger sequencing and no evidence of off-target activity was detected.

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	Rabep2-S187AS191A-UNI1
Forward Primer (5’-3’)	CCTAGCTTCTTCCCTGCAATC
Reverse Primer (5’-3’)	TCCTCCAAGGGTTCCAGT
Probe (5’-3’)	ATGGCTCCACAGAGTTGCTCCC
Label	FAM

The ddPCR assay is universal and both WT and MUT Rabep2 alleles are recognised by this assay. 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	Rabep2-S187AS191A-MUT1
Forward Primer (5’-3’)	TGGCCCTAGCTTCTTCCCT
Reverse Primer (5’-3’)	GTGGGGGTGCGGGGTTG
Probe (5’-3’)	AATCCTCCCTAGAGACGACCACGG
Label	FAM

The ddPCR assay is specific to the S187AS191A mutation in the RABEP2 gene and only MUT alleles are expected to be recognised by this assay. 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 random insertions of the donor were detected using these assays.

Rabep2-S187AS191A

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; S187A and S191A in *Rabep2*. The stock was generated at MRC Harwell via microinjection 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 wild type 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:

- Wild type (WT) assay with probe and reverse primer binding to the WT bases mutated in the mutant allele.
- Mutant assay with probe and reverse primer binding to the G601R, F606Y and R609H point mutations.

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

Rabep2-S187AS191A

Rabep2-S187AS191A-WT1 assay (FAM labelled)

CGGCAGCCTGCCTCCCTGCATGGCTCCACAGAGT**TGCTCCCTCTGtCGaGg**AACCCCtCACCCCCACT
GGAACCCTTGGAGGAGCCGAGCGGAGACGCCGGCCCAGCCGCCGAGGC**CTTTGCCCACAACTGCG**
ATGACAGTGCC**TCCATCTCTTCCTTCTCCCTC**GGTGGGGCCGCCGGCAGTGCCTCCCTACGAGGCCC

Lower case letters denote bases changed in the mutant allele.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo Rabep2-S187AS191A	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
Rabep2-S187AS191A-WT_F	n/a	**TGCTCCCTCTGTCGAGG**	n/a	Wild type Forward
Rabep2-S187AS191A-WT_PROBE	FAM	**CTTTGCCCACAACTGCGATGACAG**	ZEN/IBFQ	Wild type Probe
Rabep2-S187AS191A-WT_R	n/a	**GAGGGAGAAGGAAGAGATGGA**	n/a	Wild type Reverse

Rabep2-S187AS191A-MUT1 assay (FAM labelled)

CGGCAGCCTGCCTCCCTGCATGGCTCCACAGA**GTTGCTCCCTCTGgCGc**GcAACCCCgCACCCCCACT
GGAACCCTTGGAGGAGCCGAGCGGAGACGCCGGCCCAGCCGCCGAGGC**CTTTGCCCACAACTGCG**
ATGACAGTGCCTC**CATCTCTTCCTTCTCCCTCGGT**GGGGCCGCCGGCAGTGCCTCCCTACGAGGCCC

Lower case letters denote bases changed in the mutant allele.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo Rabep2-S187AS191A	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
Rabep2-S187AS191A-MUT_F	n/a	**GTTGCTCCCTCTGGCGC**	n/a	Mutant Forward
Rabep2-S187AS191A-MUT_PROBE	FAM	**CTTTGCCCACAACTGCGATGACAG**	ZEN/IBFQ	Mutant Probe
Rabep2-S187AS191A-MUT_R	n/a	**ACCGAGGGAGAAGGAAGAGATG**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo Rabep2-S187AS191A	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

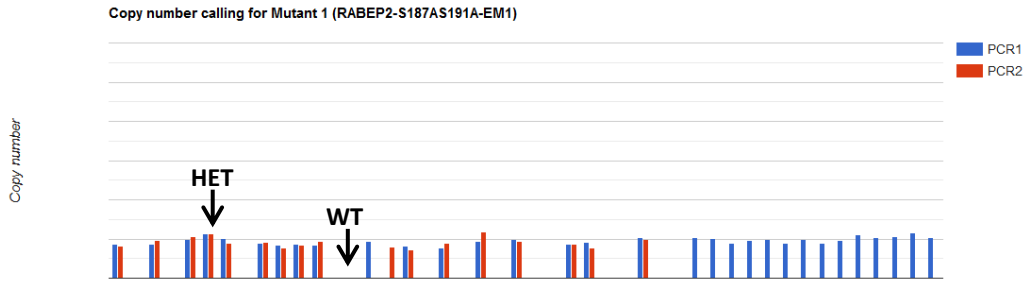
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

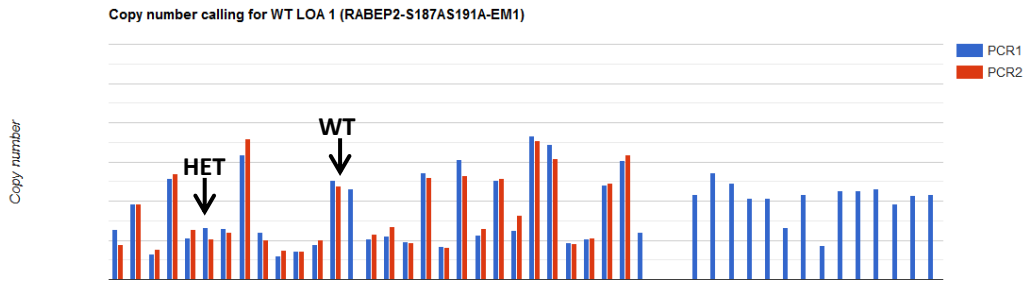
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Rabep2-S187AS191A-WT1 and Rabep2-S187AS191A -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 296542 results)





Version No.	1
Date:	30/07/2020
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman