Name of Mouse model or mutation:
KCNT1-R409Q-EM1-B6N
KCNT1-R409Q-EM2-B6N

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

Type of mutation:
SNP: R409Q

Delivery method:
Electroporation into 1-cell stage embryo.

Genetic Background:
C57BL/6NTac

Nuclease:
Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
CCTGGAGGTAGATGACCCGC	TGG

ssODN donor sequence (5'-3'):
ATTCAGCCTTGCCAGCAGGACTACTACGTGGTCATCCTGTGTCCCTCTGAAATGGACGTCCAGGT
GCGCAGGGTGCTGCAGATTCCCCTGTGGTC**t**CAGC**a**GGTCATCTACCTCCAGGGCTCTGCCCTC
AAGGACCAGGATCTCATGCGAGCCAAGTGAGTGCTGATCAGGGCAACACAGCCGACCTAGGCC
TGAGGCCA

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

Kcnt1 WT

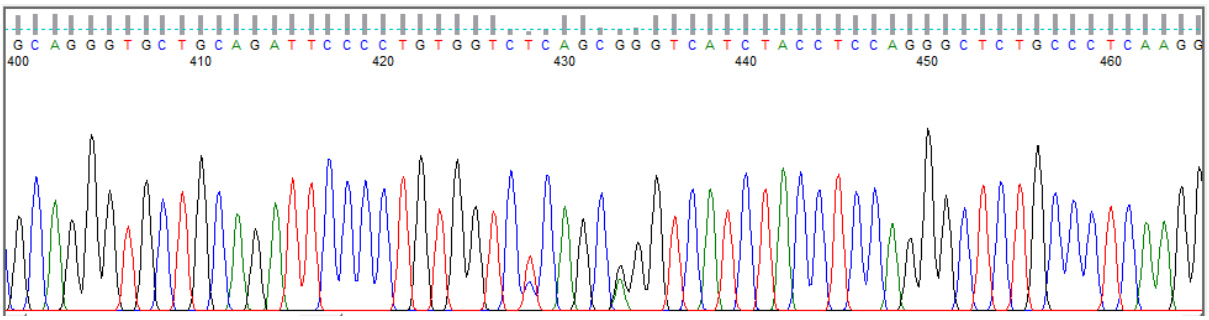
TACCTCTGGATGGAGCGTCAGAAGTCAGGGGGCAACTATAGCCGCCACCGAGCACGGACGGAGAA
GCACGTAGTCCTGTGTGTGAGCTCCCTCAAGATTGATCTCCTCATGGACTTCCTGAATGAGTTCTATG
CCCATCCCCGGCTCCAGGTAAGGCTGGGGGCACCCCTCGGGGGCCTCAGATTTCTTCTAGGCTCCCA
GAACAGTTATCGCCCCTCAACCCCCAACTCCCACTCAAGCGCAGAAATGCCATATGCTTGAGCTAGG
CTCCTGGGTGCTCTAGAGCCTGGGGGTGGGGGGAGCATCTGTGGTGGTCCAATAAGGCCCTGGGA
TGACATCACCCAGGAAACAGAGCAATCTGGGGGTGGGACAGGAGCCTGCTCAGAGACACTGACATT
CAGCCTTGCCAGCAGGACTACTACGTGGTCATCCTGTGTCCCTCTGAAATGGACGTCCAGGTGCGCA
GGGTGCTGCAGATTCCCCTGTGGTCCCAGCGGGTCATCTACCTCCAGGGCTCTGCCCTCAAGGACCA
GGATCTCATGCGAGCCAAGTGAGTGCTGATCAGGGCAACACAGCCGACCTAGGCCTGAGGCCACCA
GCCGTCCTCAGGCAGAGGGAAGGAGGGTCATGACAGTCTTAACTGCCTAGGGCAGACAGGGGTCC
CAGGAGGCAGGGCCAGAGTGGGTGAGTGGAACCCTAGAACCTATCCTCACGTGCTAGTCACTGCTG
GACAGCCTGACGAACACTCCTGGCCGAGCTGTGGTCTCTGCTGTGGACCTTGCCTGTGGGCAAAATT
TTCTCGCTCCTGCTCTGAGTCTGGGGATTTTCTCTGTTAGATTTTAGGGAGCTTAAAATAGACCAGGG
GTGGAAAAAACCTGCTGCGAAGAGTCAGAGACAAAGACACCAGGCTGAGGGTGGCTCTTGATTTAA
AGACAGAGTTGACAGAGTGACTTGAGGTGACAAGAGTGTGCTGTTAACATGCAACTCGAACGTGC

KCNT1-R409Q-EM1-B6N and KCNT1-R409Q-EM2-B6N

TACCTCTGGATGGAGCGTCAGAAGTCAGGGGGCAACTATAGCCGCCACCGAGCACGGACGGAGAA
GCACGTAGTCCTGTGTGTGAGCTCCCTCAAGATTGATCTCCTCATGGACTTCCTGAATGAGTTCTATG
CCCATCCCCGGCTCCAGGTAAGGCTGGGGGCACCCCTCGGGGGCCTCAGATTTCTTCTAGGCTCCCA
GAACAGTTATCGCCCCTCAACCCCCAACTCCCACTCAAGCGCAGAAATGCCATATGCTTGAGCTAGG
CTCCTGGGTGCTCTAGAGCCTGGGGGTGGGGGGAGCATCTGTGGTGGTCCAATAAGGCCCTGGGA
TGACATCACCCAGGAAACAGAGCAATCTGGGGGTGGGACAGGAGCCTGCTCAGAGACACTGACATT
CAGCCTTGCCAGCAGGACTACTACGTGGTCATCCTGTGTCCCTCTGAAATGGACGTCCAGGTGCGCA
GGGTGCTGCAGATTCCCCTGTGGTC**T**CAGC**A**GGTCATCTACCTCCAGGGCTCTGCCCTCAAGGACCA
GGATCTCATGCGAGCCAAGTGAGTGCTGATCAGGGCAACACAGCCGACCTAGGCCTGAGGCCACCA
GCCGTCCTCAGGCAGAGGGAAGGAGGGTCATGACAGTCTTAACTGCCTAGGGCAGACAGGGGTCC
CAGGAGGCAGGGCCAGAGTGGGTGAGTGGAACCCTAGAACCTATCCTCACGTGCTAGTCACTGCTG
GACAGCCTGACGAACACTCCTGGCCGAGCTGTGGTCTCTGCTGTGGACCTTGCCTGTGGGCAAAATT
TTCTCGCTCCTGCTCTGAGTCTGGGGATTTTCTCTGTTAGATTTTAGGGAGCTTAAAATAGACCAGGG
GTGGAAAAAACCTGCTGCGAAGAGTCAGAGACAAAGACACCAGGCTGAGGGTGGCTCTTGATTTAA
AGACAGAGTTGACAGAGTGACTTGAGGTGACAAGAGTGTGCTGTTAACATGCAACTCGAACGTGC

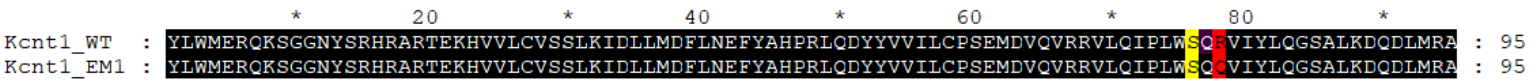
Nucleotide change highlighted in **red and underlined = nominated change**, silent change highlighted in red only.

Heterozygous F1 animal sequence trace:



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_Kcnt1_R409Q_F1 (5'-3')	TACCTCTGGATGGAGCGTCA
Geno_Kcnt1_R409Q_R1 (5'-3')	GCACGTTCGAGTTGCATGTT
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	995
Mutant product size (bp)	995
Notes	Sequence with the following primers Geno_Kcnt1_R409Q_F2 (5'-3'): AACTATAGCCGCCACCGAG Geno_Kcnt1_R409Q_R2 (5'-3'): TAACAGCACACTCTTGTCACCT

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	KCNT1-R409Q-UNI1
Forward Primer (5'-3')	CTTGCCAGCAGGACTACTAC
Reverse Primer (5'-3')	GGAATCTGCAGCACCCT
Probe (5'-3')	TTTCAGAGGGACACAGGATGACCAC
Label	FAM

This ddPCR assay is universal; both the 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	KCNT1-R409Q-MUT1
Forward Primer (5'-3')	CCTTGCCAGCAGGACTAC
Reverse Primer (5'-3')	CCCTGGAGGTAGATGACCT
Probe (5'-3')	TACGTGGTCATCCTGTGTCCCTCT
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.

KCNT1-R409Q

Allele Description

This is a CRISPR/Cas9 induced mutation creating a point mutation; R409Q in exon ENSMUSE00001303880 of *KCNT1*. 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

KCNT1-R409Q

KCNT1-R409Q-WT1 assay (FAM labelled)

TGACATCACCCAGGAAACAGAGCAATCTGGGGGTGGGACAGGAGCCTGCTCAGAGACACTGACATT
CAGCCTTGCCAGCAGGACTACTACGTGGTC**ATCCTGTGTCCCTCTGAAATG**GACGTCCAGGTGCGCA
GGGTGCTGCAGATTCCCC**TGTGGTCcCAGCgGGTCATCTA**CCTCCAGGGCTCTGCC**CTCAAGGACCA**
GGATCTCATGCGAGCCAAGTGAGTGCTGATCAGGGCAACACAGCCGACCTAGGCCTGAGGCCACCA
GCCGTCCTCAGGCAGAGGGAAGGAGGGTCATGACAGTCTTAACTGCCTAGGGCAGACAGGGGTCC

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 KCNT1-R409Q	5' label	Sequence 5' → 3'	3' label	Oligo Type
KCNT1-R409Q- UNI_F	n/a	**ATCCTGTGTCCCTCTGAAATG**	n/a	Universal Forward
KCNT1-R409Q-WT_PROBE	FAM	**TGTGGTCCCAGCGGGTCATCTA**	ZEN/IBFQ	Wild type Probe
KCNT1-R409Q- UNI_R	n/a	**CATGAGATCCTGGTCCTTGAG**	n/a	Universal Reverse

KCNT1-R409Q-MUT1 assay (FAM labelled)

TGACATCACCCAGGAAACAGAGCAATCTGGGGGTGGGACAGGAGCCTGCTCAGAGACACTGACATT
CAGCCTTGCCAGCAGGACTACTACGTGGTC**ATCCTGTGTCCCTCTGAAATG**GACGTCCAGGTGCGCA
GGGTGCTGCAGATTCCCC**TGTGGTCtCAGCaGGTCATCTACCT**CCAGGGCTCTGCC**CTCAAGGACCA**
GGATCTCATGCGAGCCAAGTGAGTGCTGATCAGGGCAACACAGCCGACCTAGGCCTGAGGCCACCA
GCCGTCCTCAGGCAGAGGGAAGGAGGGTCATGACAGTCTTAACTGCCTAGGGCAGACAGGGGTCC

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 KCNT1-R409Q	5' label	Sequence 5' → 3'	3' label	Oligo Type
KCNT1-R409Q- UNI_F	n/a	**ATCCTGTGTCCCTCTGAAATG**	n/a	Universal Forward
KCNT1-R409Q-MUT_PROBE	FAM	**TGTGGTCCCAGCGGGTCATCTA**	BHQ	Mutant Probe
KCNT1-R409Q-UNI_R	n/a	**CATGAGATCCTGGTCCTTGAG**	n/a	Universal Reverse

KCNT1-R409Q

Dot1l internal control (VIC labelled)

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

Oligo KCNT1-R409Q	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

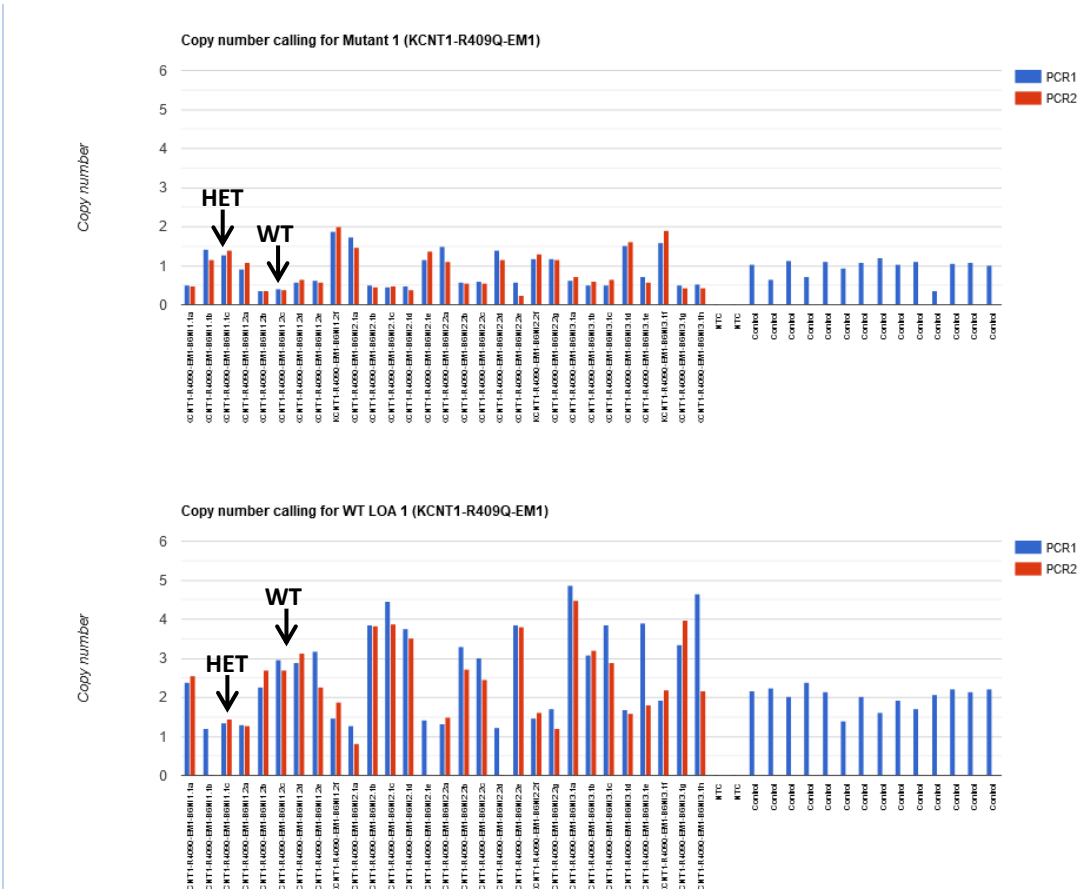
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

We are currently in the process of optimizing our qPCR assays for KCNT1-R409Q as we observe a weak copy number in WT samples when tested with the mutant qPCR assay. We have attached an alternative protocol that can also be used for genotyping.

KCNT1-R409Q-WT1 and KCNT1-R409Q -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 346263 results)



Alternative protocol

Kcnt1 WT

TGACATCACCCAGGAAACAGAGCAATCTGGGGGTGGGACAGGAGCCTGCTCAGAGACACTGACATT
CAGCCTTGCCAGCAGGACTACTACGTGGTCATCCTGTGTCCCTCTGAAATGGACGTCCAGGTGCGCA
GGGTGCTGCAGATTCCCCTGTGGTCCCAGCGGGTCATCTACCTCCAGGGCTCTGCCCTCAAGGACCA
GGATCTCATGCGAGCCAAGTGAGTGCTGATCAGGGCAACACAGCCGACCTAGGCCTGAGGCCACCA
GCCGTCCTCAGGCAGAGGGAAGGAGGGTCATGACAGTCTTAACTGCCTAGGGCAGACAGGGGTCC

KCNT1-R409Q-EM1-B6N and KCNT1-R409Q-EM2-B6N

TGACATCACCCAGGAAACAGAGCAATCTGGGGGTGGGACAGGAGCCTGCTCAGAGACACTGACATT
CAGCCTTGCCAGCAGGACTACTACGTGGTCATCCTGTGTCCCTCTGAAATGGACGTCCAGGTGCGCA
GGGTGCTGCAGATTCCCCTGTGGTC**T**CAGC**A**GGTCATCTACCTCCAGGGCTCTGCCCTCAAGGACCA
GGATCTCATGCGAGCCAAGTGAGTGCTGATCAGGGCAACACAGCCGACCTAGGCCTGAGGCCACCA
GCCGTCCTCAGGCAGAGGGAAGGAGGGTCATGACAGTCTTAACTGCCTAGGGCAGACAGGGGTCC

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KCNT1-R409Q

Version No.	1
Date:	13/08/2021
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman