Name of Mouse model or mutation:
KCNC1-E380C-EM2-B6

Description:
Point mutation model made using CRISPR/Cas9.

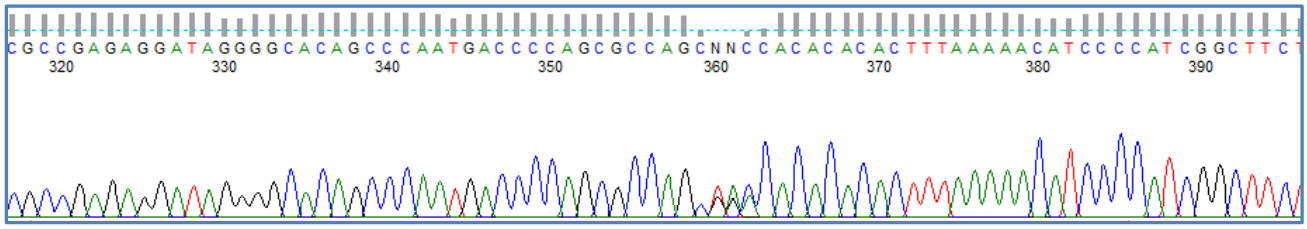
Type of mutation:
SNP: E380C

Sequence details
Kcnc1 WT
CCTCATGCGTGTCGTCTTCTGCCCCAACAAGGTGGAATTCATCAAGAACTCCCTCAATATCATTGACT
TTGTGGCCATTCTCCCCTTCTACCTGGAGGTGGGCCTAAGCGGCCTGTCCTCAAAAGCCGCCAAGGA
CGTTCTGGGCTTCCTGCGCGTCGTCCGCTTCGTGCGCATCCTGCGCATCTTCAAGCTGACCCGCCACT
TCGTGGGCCTGAGGGTCCTGGGCCACACGCTCCGTGCCAGCACCAACGAGTTCCTGCTGCTTATCAT
CTTCCTGGCCCTGGGAGTGCTCATCTTTGCCACCATGATCTACTACGCCGAGAGGATAGGGGCACAG
CCCAATGACCCCAGCGCCAGCGAACACACACACTTTAAAAACATCCCCATCGGCTTCTGGTGGGCTG
TGGTCACCATGACGACACTGGGCTATGGAGACATGTATCCCCAGACGTGGTCTGGAATGCTGGTGG
GAGCCTTGTGTGCTCTGGCTGGTGTGCTGACCATTGCCATGCCGGTGCCTGTCATCGTGAACAATTT
TGGGATGTACTACTCTTTAGCCATGGCTAAGCAGAAACTACCAAAGAAAAAAAAGAAGCATATTCCG
CGGCCACCACAGCTGGGATCTCCCAATTATTGTAAATCTGTCGTAAACTCTCCACACCACAGTACTCA
GAGTGACACATGCCCGCTGGCCCAGGAAGAAATTTTAGAAATTAACAGAGCAGGTAGGAAACCTCT
CAGAGGCATGTCGATCTGACCTTTCACCTCCGCCCCCTGTAGCAATGATTCCAGATCCAGTCAGACTG
CTTCCTTAGTTCCACGGGCG

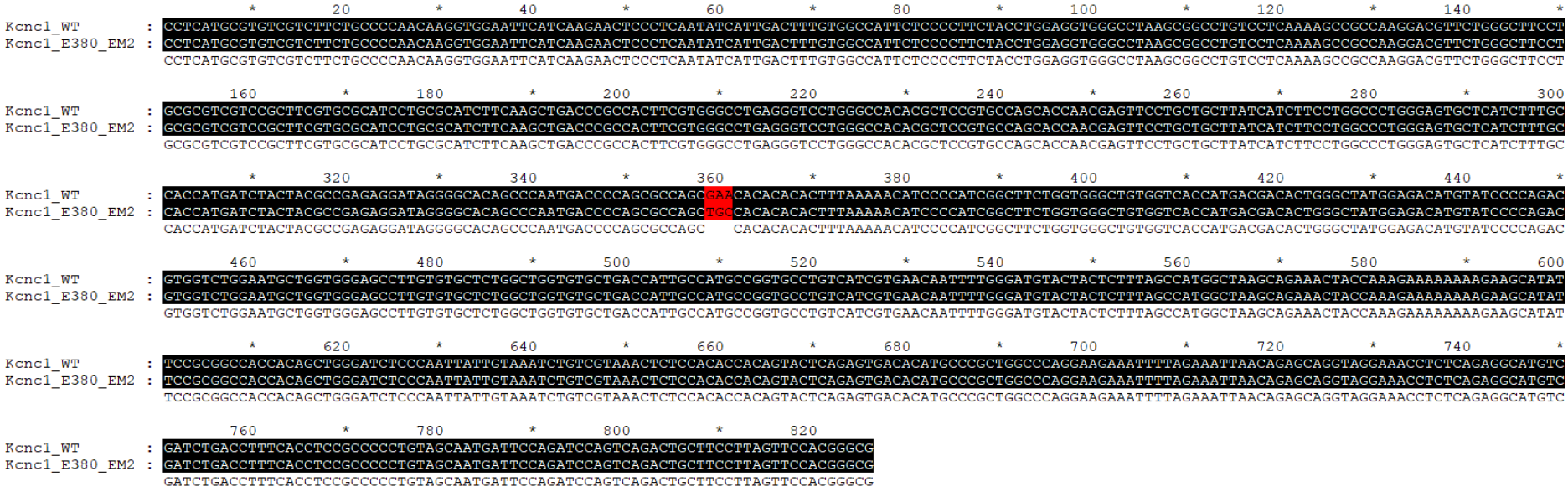
KCNC1-E380C-EM2-B6
CCTCATGCGTGTCGTCTTCTGCCCCAACAAGGTGGAATTCATCAAGAACTCCCTCAATATCATTGACT
TTGTGGCCATTCTCCCCTTCTACCTGGAGGTGGGCCTAAGCGGCCTGTCCTCAAAAGCCGCCAAGGA
CGTTCTGGGCTTCCTGCGCGTCGTCCGCTTCGTGCGCATCCTGCGCATCTTCAAGCTGACCCGCCACT
TCGTGGGCCTGAGGGTCCTGGGCCACACGCTCCGTGCCAGCACCAACGAGTTCCTGCTGCTTATCAT
CTTCCTGGCCCTGGGAGTGCTCATCTTTGCCACCATGATCTACTACGCCGAGAGGATAGGGGCACAG
CCCAATGACCCCAGCGCCAGC**TGC**CACACACACTTTAAAAACATCCCCATCGGCTTCTGGTGGGCTG
TGGTCACCATGACGACACTGGGCTATGGAGACATGTATCCCCAGACGTGGTCTGGAATGCTGGTGG
GAGCCTTGTGTGCTCTGGCTGGTGTGCTGACCATTGCCATGCCGGTGCCTGTCATCGTGAACAATTT
TGGGATGTACTACTCTTTAGCCATGGCTAAGCAGAAACTACCAAAGAAAAAAAAGAAGCATATTCCG
CGGCCACCACAGCTGGGATCTCCCAATTATTGTAAATCTGTCGTAAACTCTCCACACCACAGTACTCA
GAGTGACACATGCCCGCTGGCCCAGGAAGAAATTTTAGAAATTAACAGAGCAGGTAGGAAACCTCT

CAGAGGCATGTCGATCTGACCTTTCACCTCCGCCCCCTGTAGCAATGATTCCAGATCCAGTCAGACTG
CTTCCTTAGTTCCACGGGCG

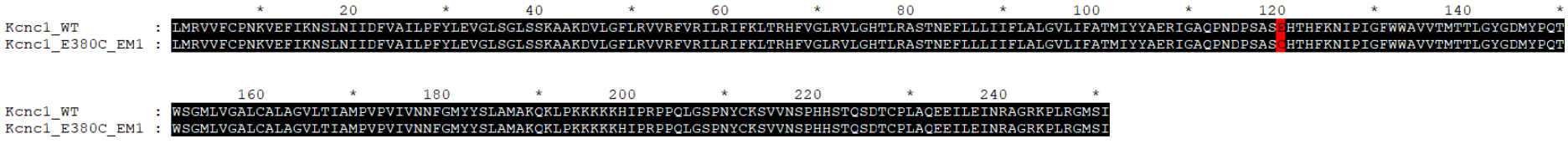
KCNC1-E380C-EM2-B6 Heterozygous F1 animal sequence trace:



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_Kcnc1_E380C_F1 (5'-3')	CCTCATGCGTGTCGTCTTCT
Geno_ Kcnc1_E380C_R1 (5'-3')	CGCCCGTGGAACTAAGGAAG
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	64
Elongation time (min)	0.5
WT product size (bp)	826
Mutant product size (bp)	826
Notes	

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 2 were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
5:125898958-125898980	GTGTGTGTT**G**G**A**TGGCGCTGAGG	Intergenic	Geno_Kcnc1_E380C_OT1_F1: GCCAGCATTCACCATGCTTC Geno_Kcnc1_E380C_OT1_R1: GCCACATTCTGTGGGAAAGC
6:117990521-117990543	GTGTGTG**A**TCGCTGG**A**GCTGTGG	Intergenic	Geno_Kcnc1_E380C_OT2_F1: CACCCAAGGAAACAAGCGTC Geno_Kcnc1_E380C_OT2_R1: GCTCACCTGTGTATCGGTCAA

All amplicons were sent for Sanger sequencing.

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	KCNC1-E380C-MUT1
Forward Primer (5’-3’)	CCTGGGAGTGCTCATCTTTGC
Reverse Primer (5’-3’)	GGATGTTTTTAAAGTGTGTGTGGCA
Probe (5’-3’)	CCATGATCTACTACGCCGAGAGGATAGG
Label	FAM-BHQ1

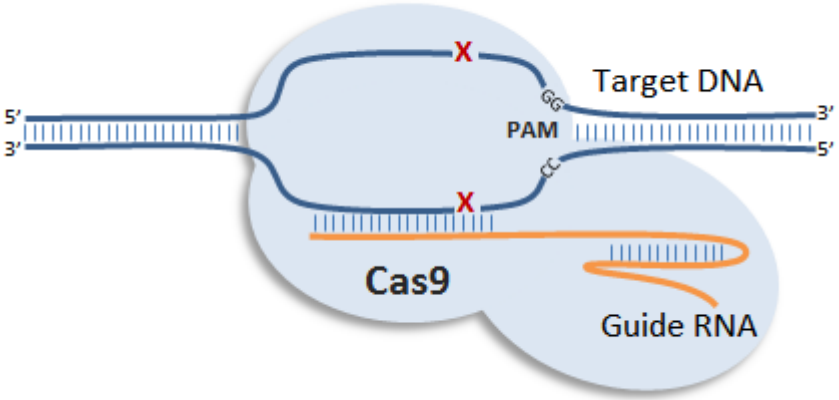
The ddPCR assay is specific to the E380C mutation in the KCNC1 gene and only mutant E380C alleles are expected to be recognised by this assay. WT controls are expected to call at 0 copies and a single integration for a correct mutation is expected to call at 1 copy for F1 (HET) animals.

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.

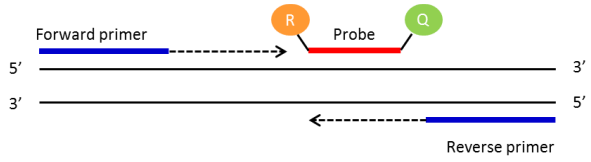
Kcnc1-E380C-EM2 Genotyping Strategy

Animals have been engineered using the CRISPR/Cas9 technology.

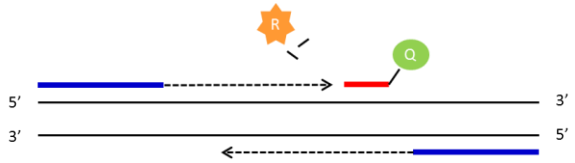


qPCR genotyping strategy

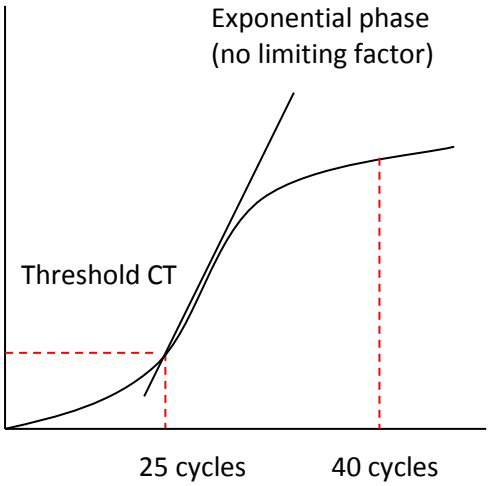
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that is complimentary to part of the DNA sequence between the forward and reverse primers. At the 5' end of the probe there is a fluorescent reporter (R) and at the 3' end a quencher (Q). Whilst they are in close contact with each other there is no fluorescent signal.



As the forward primer is extended the reporter is cleaved from the probe resulting in a fluorescent signal being detected. Once the primer extends enough to release the quencher this signal is blocked. By using probes with different fluorescent signals multiple PCR assays can be multiplexed and run together.



PCR reaction plot



The number of cycles the PCR takes to reach a set threshold is known as the CT value. This is inversely correlated to the amount of template DNA in the sample.

e.g. CT 25 = 2 x template DNA
CT 26 = 1 x template DNA
CT above 30 = no template represented in the sample

CT value can be used to determine how many copies of a particular allele samples have.

All our qPCR are run in duplicate. A FAM labelled genotyping assay is run in multiplex with a VIC labelled internal control Dot1l.

References:

Billard A., Laval V., Fillinger S., Leroux P., Lachaise H., Beffa R., et al. (2012).
The allele-specific probe and primer amplification assay, a new real-time PCR method for fine quantification of single-nucleotide polymorphisms in pooled DNA.
Appl. Environ. Microbiol. 78 1063–1068. 10.1128/AEM.06957-11

Kcnc1-E380C-EM2 Genotyping Strategy

Samples are genotyped with both WT and Mutant assays. These are FAM labelled assays that are designed to detect the critical exon that has been modified. If the animal contains the modified allele the copy number of the WT assay should drop by 1 and the mutant assay should raise by 1. For autosomal genes that have been targeted this means the following

WT= 2 copies of the WT assay and 0 copies of the Mutant assay
HET = 1 copy of the WT assay and 1 copy of the Mutant assay
HOM = 0 copies of the WT assay and 2 copies of the Mutant assay

Kcnc1-E380C-EM2 CRISPR/Cas9 mutant in which SNPs are as highlighted

WT CAATGACCCCAGCGCCAGCGAACACACACACTTTAAAAACATCCC
Mutant CAATGACCCCAGCGCCAGCTGCCACACACACTTTAAAAACATCCC

Kcnc1-E380C-WT1 assay (FAM labelled probe)

TCGTGGGCCTGAGGGTCCTGGGCCACACGCTCCGTGCCAGCACCAACGAGTTCCTGCTGCTTATCAT
CTTCCTGGCCCTGGGAGTGCTCATCTTTGCCACCATGATCTACTACGCCGAGAGGATAGGGGCACAG
CCCAATGACCCCAGCGCCAGCGAACACACACACTTTAAAAACATCCCCATCGGCTTCTGGTGGGCTG
TGGTCACCATGACGACACTGGGCTATGGAGACATGTATCCCCAGACGTGGTCTGGAATGCTGGTGG

Kcnc1-E380C-WT1 primers and probe

Primer 1 = GGGCACAGCCCAATGACC
Primer 2 = ATGGGGATGTTTTTAAAGTGTGTGTGT
Probe = CAGCGCCAGCGAACA

Allele specific primer and probes

KCNC1-E380C-EM2-MUT1 assay (FAM labelled probe)

TCGTGGGCCTGAGGGTCCTGGGCCACACGCTCCGTGCCAGCACCAACGAGTTCCTGCTGCTTATCAT
CTTCCTGGCCCTGGGAGTGCTCATCTTTGCCACCATGATCTACTACGCCGAGAGGATAGGGGCACAG
CCCAATGACCCCAGCGCCAGCTGCCACACACACTTTAAAAACATCCCCATCGGCTTCTGGTGGGCTG
TGGTCACCATGACGACACTGGGCTATGGAGACATGTATCCCCAGACGTGGTCTGGAATGCTGGTGG

KCNC1-E380C-EM2-MUT1 primers and probe

Primer 1 = ATAGGGGCACAGCCCAATGAC
Primer 2 = ATGGGGATGTTTTTAAAGTGTGTGTGG
Probe = AGCGCCAGCTGCCAC

Dot1l internal control (VIC labelled)

CCCCTCTAGTCGTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGA
ATGGTCGTGCTGGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCC

Primer 1 = GCCCCAGCACGACCATT
Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

qPCR master mix

ABI GTX Taqman master mix	5µl
Primers Dot1L_2F (20µM)	0.225µl
Primers Dot1L_R (20µM)	0.225µl
Probe DotL_2M (5µM)	0.2µl
FAM Assay (probe 5µM & primers 15µM each)	0.3µl
ddH20	1.55µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

Kcnc1-E380C-EM2 copy called result, image showing both replicates and controls (261006)