MRC Harwell

GEMM
GENOME EDITING MICE FOR MEDICINE

Name of Mouse model or mutation:
Kcnj11-E23K-EM1-B6N

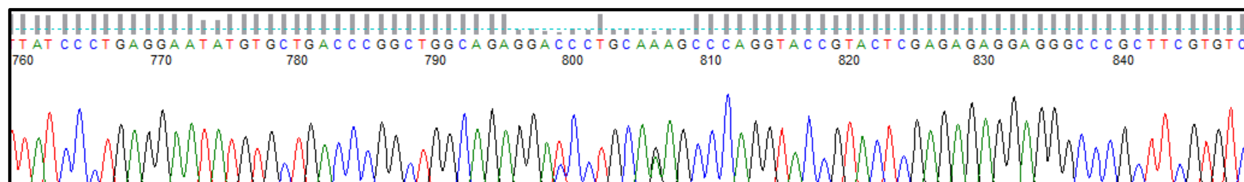
Description:
Point mutation model made using CRISPR/Cas9.

Type of mutation:
SNP: E23K

Sequence details
WT:
CCTAGGCCAAGCCAGTGTAGTGCCTCCCCCCATGGGGGAAACCCCTTCCCGGGGCCAACGGAGCCA
TGCTGTCCCGAAAGGGCATTATCCCTGAGGAATATGTGCTGACCCGGCTGGCAGAGGACCCTGCAG
AGCCCAGGTACCGTACTCGAGAGAGGAGGGCCCGCTTCGTGTCCAAGAAAGGCAACTGCAACGTCG
CCCACAAGAACATTCGAGAGCAGGGCCGCTTCCTGCAGGATGTGTTCACCACGCTGGTGGACCTCA
AATGGCCACACACTCTGCTCATTTTCACCATGTCCTTCCTGTGCAGCTGGCTGCTCTTTGCCATGGTCT
GGTGGCTCATCGCCTTCGCCCACGGTGACCTGGCCCCCGGAGAGGGCACCAATGTGCCCTGCGTCA
CAAGCATCCACTCCTTTTCATCTGCCTTCCTTTTCTCCATCGAGGTCCAGGTGACCATTGG

Kcnj11-E23K-EM1-B6N Mutant:
CCTAGGCCAAGCCAGTGTAGTGCCTCCCCCCATGGGGGAAACCCCTTCCCGGGGCCAACGGAGCCA
TGCTGTCCCGAAAGGGCATTATCCCTGAGGAATATGTGCTGACCCGGCTGGCAGAGGA**T**CCTGCA**A**
AGCCCAGGTACCGTACTCGAGAGAGGAGGGCCCGCTTCGTGTCCAAGAAAGGCAACTGCAACGTCG
CCCACAAGAACATTCGAGAGCAGGGCCGCTTCCTGCAGGATGTGTTCACCACGCTGGTGGACCTCA
AATGGCCACACACTCTGCTCATTTTCACCATGTCCTTCCTGTGCAGCTGGCTGCTCTTTGCCATGGTCT
GGTGGCTCATCGCCTTCGCCCACGGTGACCTGGCCCCCGGAGAGGGCACCAATGTGCCCTGCGTCA
CAAGCATCCACTCCTTTTCATCTGCCTTCCTTTTCTCCATCGAGGTCCAGGTGACCATTGG

Kcnj11-E23K-EM1-B6N Heterozygous F1 animal sequence trace:



Nucleotide Alignment:

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                       *        20         *        40         *        60         *        80         *       100         *       120         *       140
Kcnj11_WT   : CCTAGGCCAAGCCAGTGTAGTGCCTCCCCCCATGGGGAAACCCCTTCCCGGGGCCAACGGAGCCATGCTGTCCCGAAAGGGCATTATCCCTGAGGAATATGTGCTGACCCGGCTGGCAGAGGACCCTGCAGAGCCCAGG : 140
Kcnj11_E23  : CCTAGGCCAAGCCAGTGTAGTGCCTCCCCCCATGGGGAAACCCCTTCCCGGGGCCAACGGAGCCATGCTGTCCCGAAAGGGCATTATCCCTGAGGAATATGTGCTGACCCGGCTGGCAGAGGATCCTGCAAAGCCCAGG : 140
              CCTAGGCCAAGCCAGTGTAGTGCCTCCCCCCATGGGGAAACCCCTTCCCGGGGCCAACGGAGCCATGCTGTCCCGAAAGGGCATTATCCCTGAGGAATATGTGCTGACCCGGCTGGCAGAGGA CCTGCA AGCCCAGG

                       *       160         *       180         *       200         *       220         *       240         *       260         *       280
Kcnj11_WT   : TACCGTACTCGAGAGAGGAGGGCCCGCTTCGTGTCCAAGAAAGGCAACTGCAACGTCGCCCACAAGAACATTCGAGAGCAGGGCCGCTTCCTGCAGGATGTGTTCACCACGCTGGTGGACCTCAAATGGCCACACACTCT : 280
Kcnj11_E23  : TACCGTACTCGAGAGAGGAGGGCCCGCTTCGTGTCCAAGAAAGGCAACTGCAACGTCGCCCACAAGAACATTCGAGAGCAGGGCCGCTTCCTGCAGGATGTGTTCACCACGCTGGTGGACCTCAAATGGCCACACACTCT : 280
              TACCGTACTCGAGAGAGGAGGGCCCGCTTCGTGTCCAAGAAAGGCAACTGCAACGTCGCCCACAAGAACATTCGAGAGCAGGGCCGCTTCCTGCAGGATGTGTTCACCACGCTGGTGGACCTCAAATGGCCACACACTCT
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Predicted Protein Alignment:

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                       *        20         *        40         *        60         *        80         *       100         *       120         *
Kcnj11_WT   : MLSRKGIIPEEYVLTRLAEDPAEPRYRTRERRARFVSKKGNCNVAHKNIREQGRFLQDVFTTLVDLKWPHTLLIFTMSFLCSWLLFAMVWWLIAFAHGDLAPGEGTNVPCVTSIHSFSSAFLFSIEVQVTI : 131
Kcnj11_E23  : MLSRKGIIPEEYVLTRLAEDPAKPRYRTRERRARFVSKKGNCNVAHKNIREQGRFLQDVFTTLVDLKWPHTLLIFTMSFLCSWLLFAMVWWLIAFAHGDLAPGEGTNVPCVTSIHSFSSAFLFSIEVQVTI : 131
              MLSRKGIIPEEYVLTRLAEDPA PRYRTRERRARFVSKKGNCNVAHKNIREQGRFLQDVFTTLVDLKWPHTLLIFTMSFLCSWLLFAMVWWLIAFAHGDLAPGEGTNVPCVTSIHSFSSAFLFSIEVQVTI
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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_Kcnj11 _F1	CCTAGGCCAAGCCAGTGTAG
Geno_Kcnj11 _R1	CCAATGGTCACCTGGACCTC
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	62
Elongation time (min)	1
WT product size (bp)	460
Mutant product size (bp)	460

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 Y chromosome.

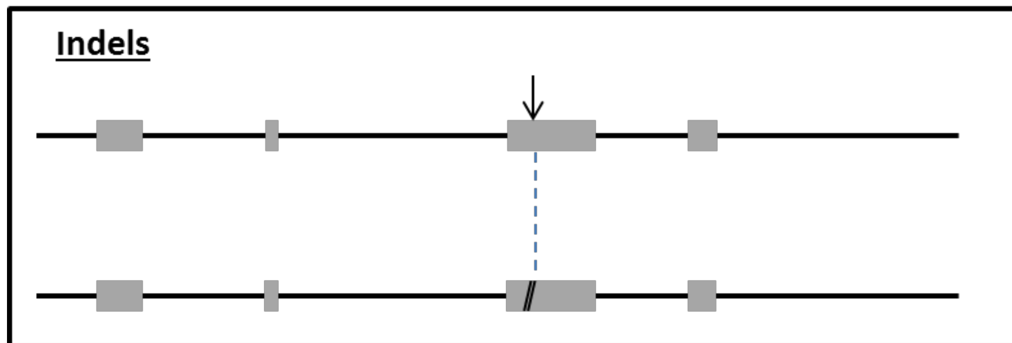
Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assays were used to detect the donor sequence:

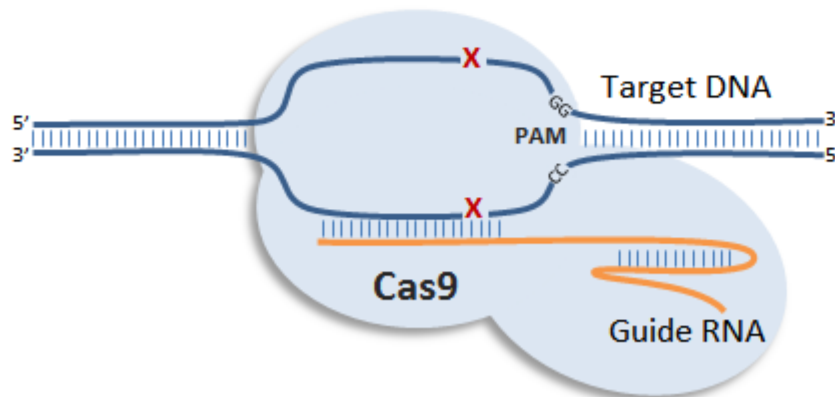
Assay name	**Kcnj11-E23K-donor-WT1**
Forward Primer	GCTGTCCCGAAAGGGCATTATC
Reverse Primer	GGCCCTCCTCTCTCGAGTA
Probe	CTGAGGAATATGTGCTGACCCGGCT
Label	FAM-BHQ1
Notes	The ddPCR assay is universal and will recognise both WT and donor sequences. Therefore WT controls are expected to call at 2 copies and a single integration for a correct mutation is also expected to call at 2 copies for F1 (HET) animals.

Assay name	**Kcnj11-E23K-Legitdonor-MUT1**
Forward Primer	CTGGCAGAGGATCCTGCAA
Reverse Primer	GTTGCCTTTCTTGGACACGAA
Probe	CCAGGTACCGTACTCGAGAGAGGA
Label	FAM-BHQ1
Notes	The ddPCR assay is specific to the E23K mutation of Kcnj11 and the WT gene is not 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.

Kcnj11-E23K Genotyping Strategy

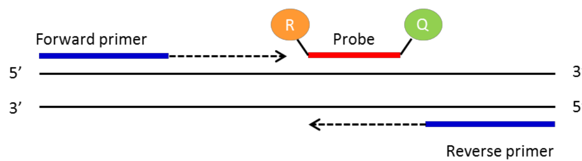
Animals have been engineered using the CRISPR/Cas9 technology. Most of the knockout alleles generated through this method will be obtained by deletion of a critical exon or by introduction of an indel (insertion/deletion) within the coding sequence of a critical exon (see picture below).



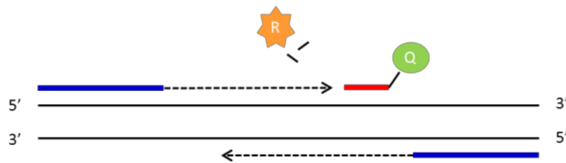


qPCR genotyping strategy

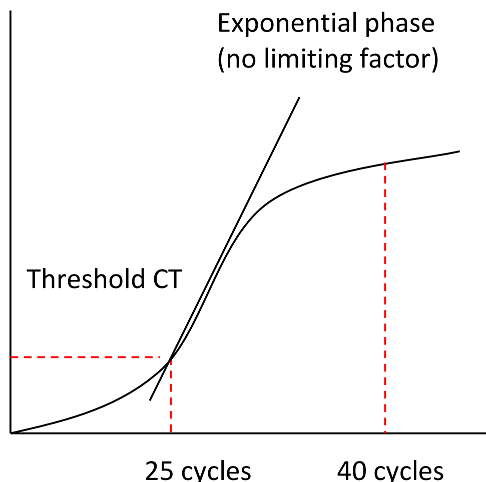
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that 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.

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Kcnj11-E23K Genotyping Strategy

Samples are genotyped with a Mutant assay. This is a FAM labelled assay that is designed to detect the critical exon that has been targeted. If the animal contains the modified allele the copy number of this assay should raise by 1. For autosomal genes that have been targeted this means the following

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

Kcnj11-E23K-Legitdonor-MUT1 assay (FAM labelled probe)

Fragment Sequence
The following sequence is the mutant sequence obtained by CRISPR/Cas9 editing from Kcnj11 gene.
The two SNPs are highlighted in bold underlined letters
WT **C**CCTGCA**G**
Mutant **T**CCTGCA**A**

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CCTAGGCCAAGCCAGTGTAGTGCCTCCCCCCATGGGGAAACCCCTTCCCGGGGCCAACGGAGCCATGCTGTCCC
GAAAGGGCATTATCCCTGAGGAATATGTGCTGACCCGGCTGGCAGAGGATCCTGCAAAGCCCAGGTACCGTACT
CGAGAGAGGAGGGCCCGCTTCGTGTCCAAGAAAGGCAACTGCAACGTCGCCCACAAGAACATTCGAGAGCAGGGC
CGCTTCCTGCAGGATGTGTTCACCACGCTGGTGGACCTCAAATGGCCACACACTCTGCTCATTTTCACCATGTCC
TTCCTGTGCAGCTGGCTGCTCTTTGCCATGGTCTGGTGGCTCATCGCCTTCGCCCACGGTGACCTGGCCCCCGGA
GAGGGCACCAATGTGCCCTGCGTCACAAGCATCCACTCCTTTTCATCTGCCTTCCTTTTCTCCATCGAGGTCCAG
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Kcnj11-E23K-Legitdonor-MUT1 primers and probe

Primer 1 = CTGGCAGAGGATCCTGCAA
Primer 2 = GTTGCCTTTCTTGGACACGAA
Probe = CCAGGTACCGTACTCGAGAGAGGA

Dot1l internal control (VIC labelled)

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TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCT
GGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG
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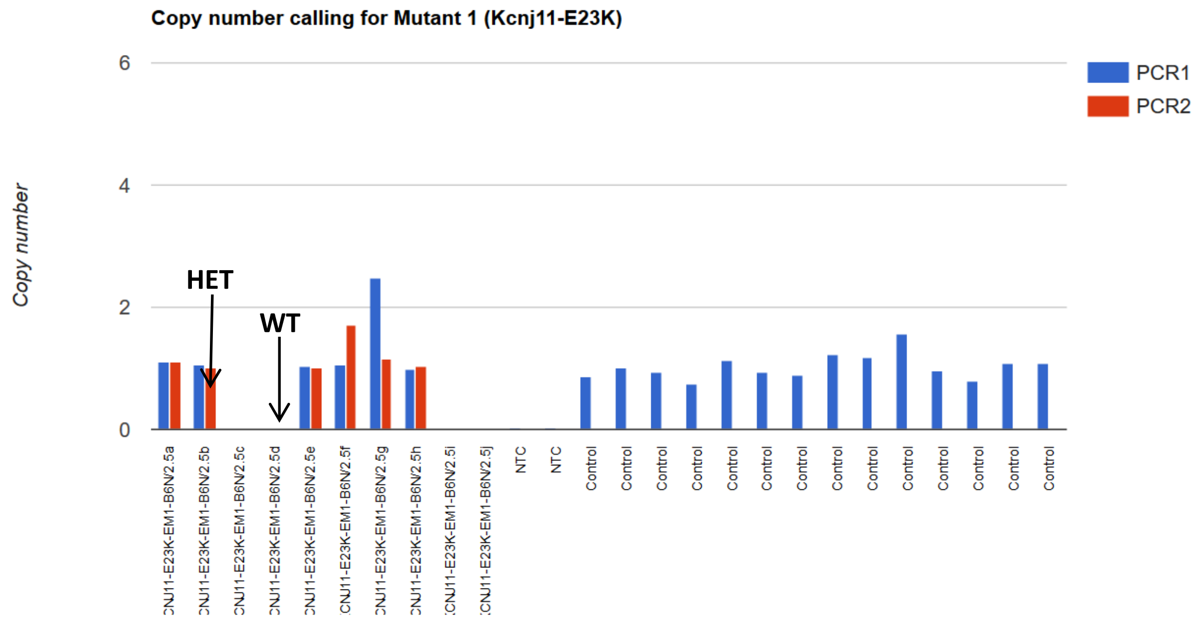
Primer 1 = GCCCCAGCACGACCATT
Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

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

Kcnj11-E23K LOA copy called result, image showing both replicates and controls



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Date: 23.01.2018

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