Name of Mouse model or mutation:
CAMK2A-T286P-EM1-B6
Note this line was re-derived on a 129S9(/SvEV) background due to welfare issues associated with the C57BL/6J background and frozen under CAMK2A-T286P-EM1-129

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

Type of mutation:
SNP: T286P

Delivery method:
Electroporation into 1-cell stage embryo

Genetic Background:
C57BL/6J

Nuclease:
WT Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
CATGCACAGACAGGAGACCG	TGG

ssODN donor sequence (5'-3'):
TGCATGAAACCGATGAAGAGAAGAGCCTGGGGTTACCAGGAATAAGGACAGGTTACCTTCAGTTTC
CTCCTGGCATTGAACTTCTTCAGGCAGTCGACGGGCTCCTGTCTGTGCATGCAGGAGGCCACGGTGG
AGCGGTGCTGGAAAGAGAGGAAGAATTTGTGTGAGGGGAAACACCTGCGGAGCAACGGACCAACC
CA

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

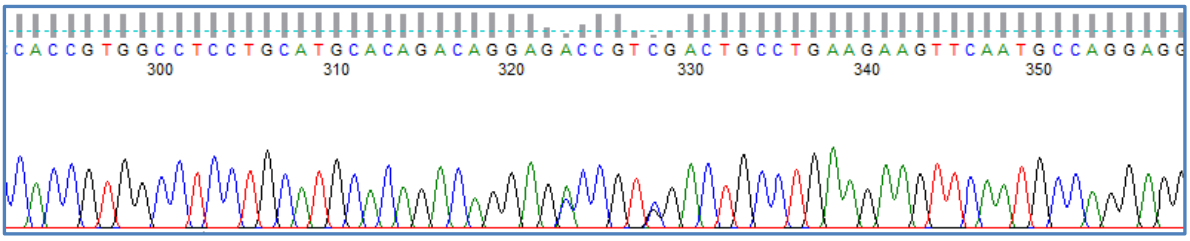
WT

CATGGATCTCGGTGAGCCTTTATCAGCCACACCCACCCAAGAGGCCGTGCTCCTCCAGGTCTTGTTCC
GTGTGGGGTTGAGGGGAATCTCTGGCTCACTGTGGGTCTCAGGGACAACATAGAAATGATGGATAT
TCTGGGTTGGCATTTGTTCGGAGTGTCACCGACATTGACTTGCTAGTGTTCTGATCCTAACCTTGCTA
CACCACTTCAGGGTCATCCTCCTGAAGATATTTTGTCAAGCTTCTGCTCAAATGATATTGGGTTGGTC
CGTTGCTCCGCAGGTGTTTCCCCTCACACAAATTCTTCCTCTCTTTCCAGCACCGCTCCACCGTGGCCT
CCTGCATGCACAGACAGGAGACCGTGGACTGCCTGAAGAAGTTCAATGCCAGGAGGAAACTGAAG
GTAACCTGTCCTTATTCCTGGTAACCCCAGGCTCTTCTCTTCATCGGTTTCATGCATGTTTACTGGACG
TTCTTGCATGCTGGCCACCAAGGGATTTGGGGGAGTGGGTAGCATATCTGACCCCCATCCCCCCATG
TCCACACTCTGGCTCAGAGGGCAATCAGGACTCTGCTGAGGCACGGTGAGTACCGTGTCTGATGGT
GGGAAACATACCAGGGTGATGGCCTTCCCTGGCCTGGAGGATTCTGGGAAGAATCTCAAGTGGCCT
TCCTGGAGACAGTGGAGTTTGAGTTGACTCTTGATAAGCAGGTGTAGAGTGACAATGGGGAGATAT
TCCAGACAGAAAGATGGCCGGTACA

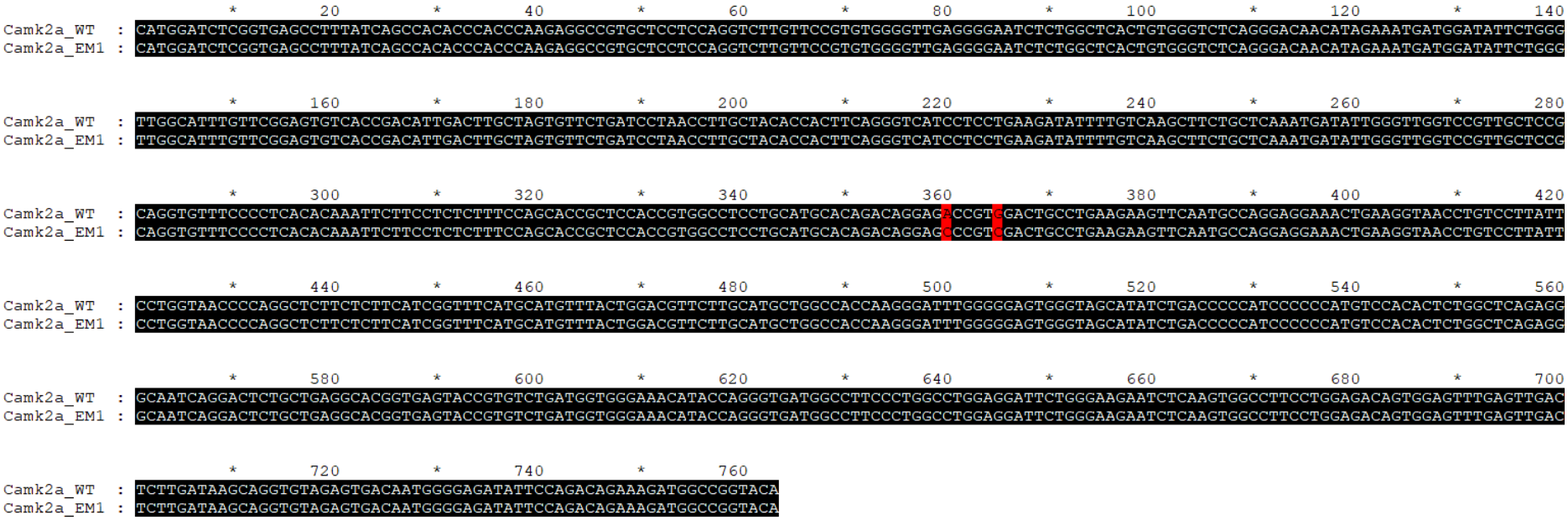
CAMK2A-T286P-EM1-B6

CATGGATCTCGGTGAGCCTTTATCAGCCACACCCACCCAAGAGGCCGTGCTCCTCCAGGTCTTGTTCC
GTGTGGGGTTGAGGGGAATCTCTGGCTCACTGTGGGTCTCAGGGACAACATAGAAATGATGGATAT
TCTGGGTTGGCATTTGTTCGGAGTGTCACCGACATTGACTTGCTAGTGTTCTGATCCTAACCTTGCTA
CACCACTTCAGGGTCATCCTCCTGAAGATATTTTGTCAAGCTTCTGCTCAAATGATATTGGGTTGGTC
CGTTGCTCCGCAGGTGTTTCCCCTCACACAAATTCTTCCTCTCTTTCCAGCACCGCTCCACCGTGGCCT
CCTGCATGCACAGACAGGAG**C**CCGT**C**GACTGCCTGAAGAAGTTCAATGCCAGGAGGAAACTGAAG
GTAACCTGTCCTTATTCCTGGTAACCCCAGGCTCTTCTCTTCATCGGTTTCATGCATGTTTACTGGACG
TTCTTGCATGCTGGCCACCAAGGGATTTGGGGGAGTGGGTAGCATATCTGACCCCCATCCCCCCATG
TCCACACTCTGGCTCAGAGGGCAATCAGGACTCTGCTGAGGCACGGTGAGTACCGTGTCTGATGGT
GGGAAACATACCAGGGTGATGGCCTTCCCTGGCCTGGAGGATTCTGGGAAGAATCTCAAGTGGCCT
TCCTGGAGACAGTGGAGTTTGAGTTGACTCTTGATAAGCAGGTGTAGAGTGACAATGGGGAGATAT
TCCAGACAGAAAGATGGCCGGTACA

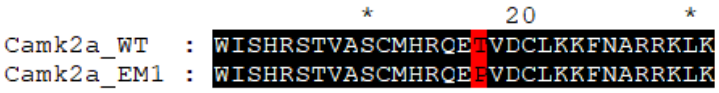
CAMK2A-T286P-EM1-B6 Heterozygous F1 animal sequence trace:



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_Camk2a_(5'-3')	CATGGATCTCGGTGAGCCTTT
Geno_Camk2a_ (5'-3')	TGTACCGGCCATCTTTCTGTC
Taq Polymerase used	ThermoFisher SuperFi II PCR kit
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	763
Mutant product size (bp)	763
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(s) used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
8:71676311-71676333	CATGCAAAGACAGGAGCCCG AGG	Exonic	CAMK2A_G3_OT1_F1: GTGTGGTGTTAAAGGACTTGTGG CAMK2A_G3_OT1_R1: CCATCAACATTGTCTTCCCTGTT
11:5988993-5989015	GATGCACAGACAGGAGACTG TGG	Exonic	CAMK2A_G3_OT2_F1: GCTCACACTACCCACAAATCACA CAMK2A_G3_OT2_R1: GGGTGATCCTGTATATCCTGCTG

All amplicons were sent for Sanger sequencing. No 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	CAMK2A-T286P-UNI1
Forward Primer (5’-3’)	CCTCACACAAATTCTTCCTCTCT
Reverse Primer (5’-3’)	CAGTTTCCTCCTGGCATTGA
Probe (5’-3’)	CCTCCTGCATGCACAGACAGGA
Label	FAM

The ddPCR assay is universal to CAMK2A - both WT and T286P 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	CAMK2A-T286P-MUT1
Forward Primer (5’-3’)	ACAGACAGGAGCCCGTC
Reverse Primer (5’-3’)	GCATGAAACCGATGAAGAGAAGA
Probe (5’-3’)	TCAATGCCAGGAGGAAACTGAAGGT
Label	FAM

The ddPCR assay is specific to the T286P mutation in the CAMK2A gene and only MUT 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 evidence of additional donor integrations was detected in the animals selected to establish the colony.

CAMK2A-T286P

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; T286P in exon ENSMUSE00000572373 of *CAMK2A*. 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

CAMK2A-T286P

CAMK2A-T286P-WT1 assay (FAM labelled)

CGTTGCTCCGCAGGTGTTTCCCCTCACACAAATTCTTCCTCTCTTTCCAGCACCGCTCCACCGTGGCCT
CCTGCAT**GCACAGACAGGAGaCCGTg**G**ACTGCCTGAAGAAGTTCAATGCCAGG**AGGAAACTGAAG
GTAACCTGTCCTTATTCCTGGTAACCCCAGG**CTCTTCTCTTCATCGGTTTCATGC**ATGTTTACTGGACG

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 CAMK2A-T286P	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
CAMK2A-T286P-WT_F	n/a	**GCACAGACAGGAGACCGTG**	n/a	Wild type Forward
CAMK2A-T286P-WT_PROBE	FAM	**ACTGCCTGAAGAAGTTCAATGCCAGG**	ZEN/IBFQ	Wild type Probe
CAMK2A-T286P-WT_R	n/a	**GCATGAAACCGATGAAGAGAAGAG**	n/a	Wild type Reverse

CAMK2A-T286P-MUT1 assay (FAM labelled)

CGTTGCTCCGCAGGTGTTTCCCCTCACACAAATTCTTCCTCTCTTTCCAGCACCGCTCCACCGTGGCCT
CCTGCATGC**ACAGACAGGAGcCCGTc**GACTGCCTGAAGAAGT**TCAATGCCAGGAGGAAACTGAAG**
GTAACCTGTCCTTATTCCTGGTAACCCCAGG**CTCTTCTCTTCATCGGTTTCATGC**ATGTTTACTGGACG

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 CAMK2A-T286P	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
CAMK2A-T286P-MUT_F	n/a	**ACAGACAGGAGCCCGTC**	n/a	Mutant Forward
CAMK2A-T286P-MUT_PROBE	FAM	**TCAATGCCAGGAGGAAACTGAAGGT**	BHQ	Mutant Probe
CAMK2A-T286P-MUT_R	n/a	**GCATGAAACCGATGAAGAGAAGA**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo CAMK2A-T286P	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

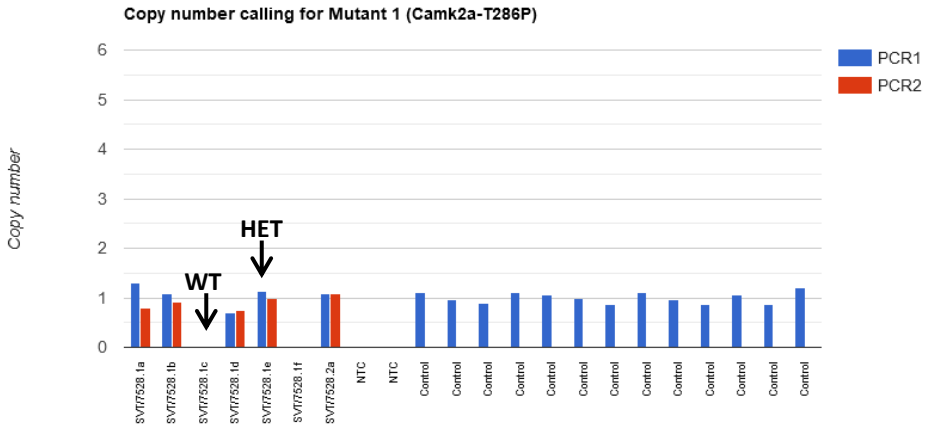
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

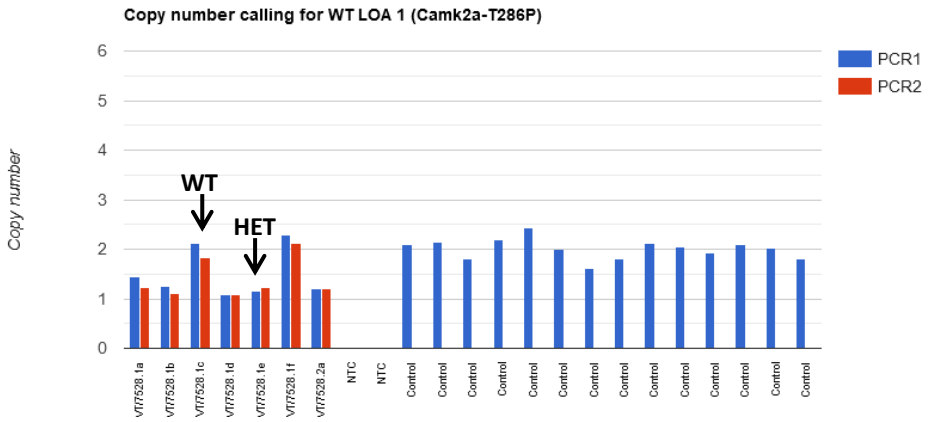
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

CAMK2A-T286P-WT1 and CAMK2A-T286P -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 335750 results)





Version No.	1
Date:	24/08/2021
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman