Name of Mouse model or mutation:
CAMK2A-T286P-EM2-B6

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

Type of mutation:
SNP: T286P

Delivery method:
Electroporation into 1-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
CATGCACAGACAGGAGACCG	TGG

ssODN donor sequence (5'-3'):
TGCATGAAACCGATGAAGAGAAGAGCCTGGGGTTACCAGGAATAAGGACAGGTTACCTTCAGTTTC
CTCCTGGCATTGAACTTCTTCAGGCAGTC**G**ACGG**G**CTCCTGTCTGTGCATGCAGGAGGCCACGGTGG
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

Camk2a WT

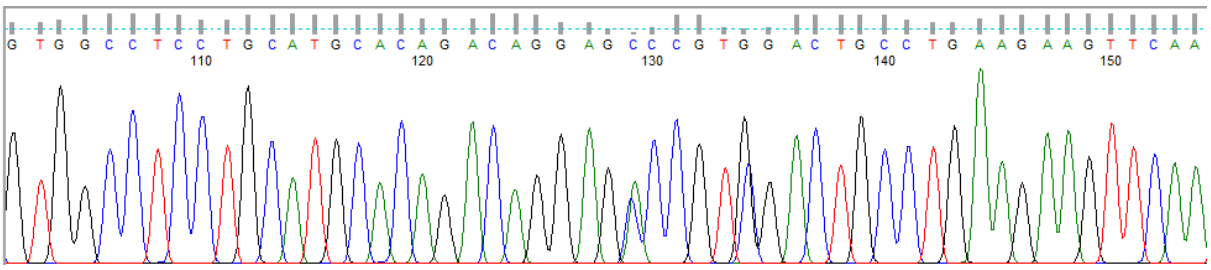
CATGGATCTCGGTGAGCCTTTATCAGCCACACCCACCCAAGAGGCCGTGCTCCTCCAGGTCTTGTTCC
GTGTGGGGTTGAGGGGAATCTCTGGCTCACTGTGGGTCTCAGGGACAACATAGAAATGATGGATAT
TCTGGGTTGGCATTTGTTCGGAGTGTCACCGACATTGACTTGCTAGTGTTCTGATCCTAACCTTGCTA
CACCACTTCAGGGTCATCCTCCTGAAGATATTTTGTCAAGCTTCTGCTCAAATGATATTGGGTTGGTC
CGTTGCTCCGCAGGTGTTTCCCCTCACACAAATTCTTCCTCTCTTTCCAGCACCGCTCCACCGTGGCCT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

CAMK2A-T286P-EM2-B6

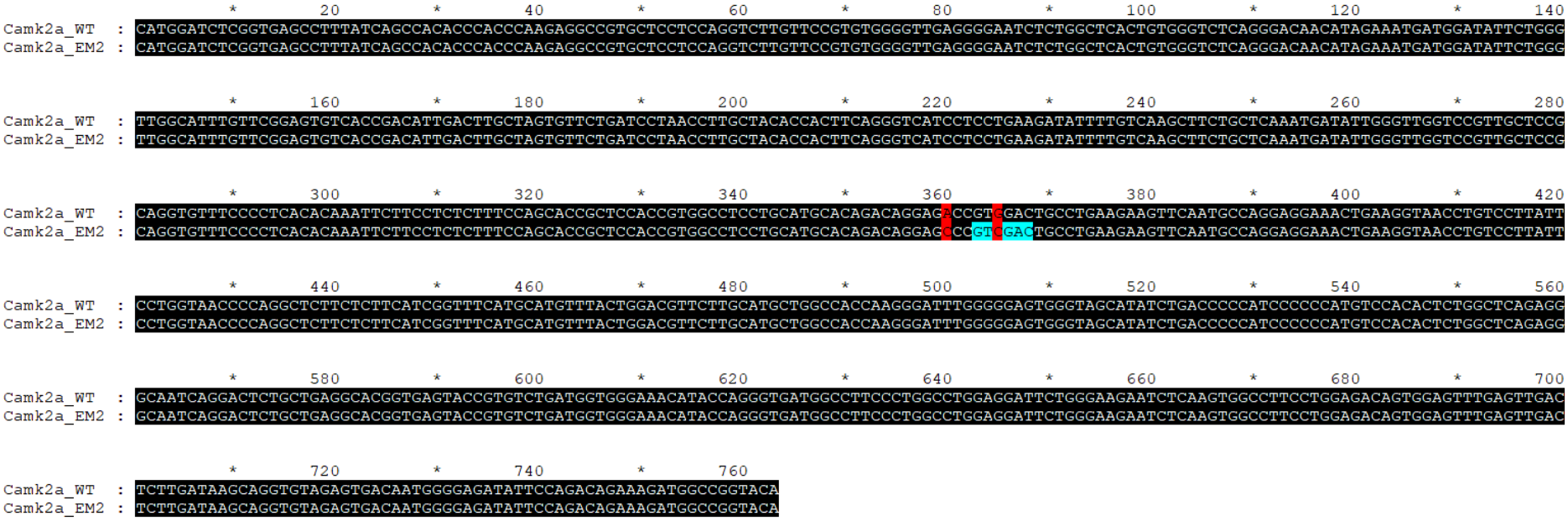
CATGGATCTCGGTGAGCCTTTATCAGCCACACCCACCCAAGAGGCCGTGCTCCTCCAGGTCTTGTTCC
GTGTGGGGTTGAGGGGAATCTCTGGCTCACTGTGGGTCTCAGGGACAACATAGAAATGATGGATAT
TCTGGGTTGGCATTTGTTCGGAGTGTCACCGACATTGACTTGCTAGTGTTCTGATCCTAACCTTGCTA
CACCACTTCAGGGTCATCCTCCTGAAGATATTTTGTCAAGCTTCTGCTCAAATGATATTGGGTTGGTC
CGTTGCTCCGCAGGTGTTTCCCCTCACACAAATTCTTCCTCTCTTTCCAGCACCGCTCCACCGTGGCCT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

Nucleotide changes highlighted in **red, bold and underlined = nominated change**, **silent change** highlighted in red and bold only. Blue highlight is HincII restriction enzyme site.

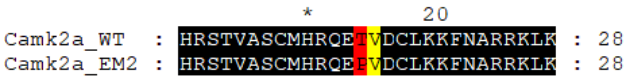
Heterozygous F1 animal sequence trace:



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_F3 (5'-3')	CATGGATCTCGGTGAGCCTTT
Geno_Camk2a_R3 (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	Please sequence with the following primers (5' to 3'): Geno_Camk2a_F4: CTTGCTACACCACTTCAGGGTC Geno_Camk2a_R2: TTCCCACCATCAGACACGGT Geno_Camk2a_R4: AGCAGAGTCCTGATTGCCCT

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.
Off-target activity was detected in the animals selected to establish the colony. We aim to breed out the off-target activity mutations in subsequent generations.

Off-target site 8:71676311-71676333 WT sequence

GTGTGGTGTTAAAGGACTTGTGGATGCTCTGGGTCCCAGGCACCCACCAGGAGGGGCATCTGAGAA
CAGAACTTGGCATGCTTTAGGGAAGCACAGCTGCACAGTTCTCTCCCCCAGGATCACTCTTAGTTCTA
CCCCATGCAGCAGACACTGCCACTGGCAACAGATGGGTAAACGGAGGCACCAGGGTGCATAGCCA
GGGATGGAGGACTGGAGAACGGGCTCCTGCCATCACCATCTGAGGCCTAGGAGTTCTTTTCAGAAT
CCTCCAAGCTGTACCTGTGGGTTCCAGTGATGGAACTCAAGTCGATTTGTGTGGCGATAGCGTATAC
TCCTCAGCCTTCTAGCAAGCCTCATCTCATAGCTTTTGAGCAGAATAAAAGGCGAGGTTTTCAGAGG
AAGTGGGGGCACGCCCAGCAAGCAGGTGGGCTTCCCAGGAGAAGGCCCCTCTTAGTTCATTAGGAC
TGGAGGCCCAGAGGTTTGGTTTTTTTCCGCCCGTAATTTTTTTTTTTAAACATTACGCAGTGTGTCTTT
TAGGAGGTTAAAGGTTGGAGGCGGAGTGGAAACTGAAGCCTGAAATGTGTTCTAGCCGCAGCAAG
TCCCTGCCCGGGCTGGCTCAGGTTGTTCCAAGGACATGCAAAGACAGGAGCCCGAGGCGGGGAGT
GCTCTGTAAGCTCAGGTCCCCAGCAGCACCCCAGCCTTTCTGTCTCTGCACAGCTCCCACCCCAGAAG
GGTGGCCTGCGGCACAGGTTTCTAGTCTCCCCCGGGCTTCCCGCCCCGCCCCGCCCCCAGGGCGCCC
TGACTTTCCGTAAATGACGGCGGCCAGGAAGCTCAGGGGCCGCACAGGAAGGAGCAGGGACTTTC
CTGTCGCAGCCTCGGCTGTCCTTTGCCTCGGCACAGCTGCAGGTAGGAGCGCGGGGCACAGGTTCA
GGGCGCGGGTGTGCGGGCGCGGGAGCGGGAGCGGGGCGCTGCCTAGACACCGCATTGGCGGCTC
AGCAGCGCGCATGCGCGGCGGGCTCCCCAACCCCACCCTCCCTGTTCTAGGAGTGGGGAAGTAAAG
GGCCTGAACTTTCCATTTGGTATAATCTCATACTTCTTTAATATTCGTGTCCGCTTTGTTGTTTTATGTC
TTATATTTTGAGGATGGCTCTCCGTCCGTAGCCCTGCTGGCTTAGAAGTCACTATGTAGCCCCAAACT
CATAGCAATCCTCCTGCAGCCTTCCCAGGAGCAAAAGAACAGGGAAGACAATGTTGATGG

Underlined nucleotides indicate site of 3 nt insertion in the mutant sequence.

Off-target site 8:71676311-71676333 3 nt insertion mutant sequence seen in CAMK2A-T286P-CAS-LINE10-B6J/1.4g, CAMK2A-T286P-CAS-LINE10-B6J/1.1g and CAMK2A-T286P-CAS-LINE10-B6J/1.3b

GTGTGGTGTTAAAGGACTTGTGGATGCTCTGGGTCCCAGGCACCCACCAGGAGGGGCATCTGAGAA
CAGAACTTGGCATGCTTTAGGGAAGCACAGCTGCACAGTTCTCTCCCCCAGGATCACTCTTAGTTCTA
CCCCATGCAGCAGACACTGCCACTGGCAACAGATGGGTAAACGGAGGCACCAGGGTGCATAGCCA
GGGATGGAGGACTGGAGAACGGGCTCCTGCCATCACCATCTGAGGCCTAGGAGTTCTTTTCAGAAT
CCTCCAAGCTGTACCTGTGGGTTCCAGTGATGGAACTCAAGTCGATTTGTGTGGCGATAGCGTATAC
TCCTCAGCCTTCTAGCAAGCCTCATCTCATAGCTTTTGAGCAGAATAAAAGGCGAGGTTTTCAGAGG
AAGTGGGGGCACGCCCAGCAAGCAGGTGGGCTTCCCAGGAGAAGGCCCCTCTTAGTTCATTAGGAC
TGGAGGCCCAGAGGTTTGGTTTTTTTCCGCCCGTAATTTTTTTTTTTTTTAAACATTACGCAGTGTGTC
TTTTAGGAGGTTAAAGGTTGGAGGCGGAGTGGAAACTGAAGCCTGAAATGTGTTCTAGCCGCAGCA
AGTCCCTGCCCGGGCTGGCTCAGGTTGTTCCAAGGACATGCAAAGACAGGAGCCCGAGGCGGGGA
GTGCTCTGTAAGCTCAGGTCCCCAGCAGCACCCCAGCCTTTCTGTCTCTGCACAGCTCCCACCCCAGA
AGGGTGGCCTGCGGCACAGGTTTCTAGTCTCCCCCGGGCTTCCCGCCCCGCCCCGCCCCCAGGGCGC
CCTGACTTTCCGTAAATGACGGCGGCCAGGAAGCTCAGGGGCCGCACAGGAAGGAGCAGGGACTT

TCCTGTCGCAGCCTCGGCTGTCCTTTGCCTCGGCACAGCTGCAGGTAGGAGCGCGGGGCACAGGTT
CAGGGCGCGGGTGTGCGGGCGCGGGAGCGGGAGCGGGGCGCTGCCTAGACACCGCATTGGCGGC
TCAGCAGCGCGCATGCGCGGCGGGCTCCCCAACCCCACCCTCCCTGTTCTAGGAGTGGGGAAGTAA
AGGGCCTGAACTTTCCATTTGGTATAATCTCATACTTCTTTAATATTCGTGTCCGCTTTGTTGTTTTAT
GTCTTATATTTTGAGGATGGCTCTCCGTCCGTAGCCCTGCTGGCTTAGAAGTCACTATGTAGCCCCAA
ACTCATAGCAATCCTCCTGCAGCCTTCCCAGGAGCAAAAGAACAGGGAAGACAATGTTGATGG

Off-target site 11:5988993-5989015 WT sequence

GCTCACACTACCCACAAATCACAGCGTCCAGACTCCTGAGAAGTGGGCGTGGCCAGGAAGGGGTTC
TTGTCCTTCCTAAATGCTGTGGCACCTTAATACAGTTCCTCATGTCGCGGTGATGCTCGGCCACAAAG
TTATCTCGTTACCACTTCATCACTGTAATCTTGCTACTGTGGTGTTATGAATGACATGCGGAACATCT
GATATGTGACCCACTAAAGGGTCTTGACCCACAAGTTGAGAACCATTCCTTTAACAGGCCCAGGGTA
GTCACGGTTGTCCTCAATGCTTTGGGGAAGCTCAATCAAGCAATGGCTCCCGGGTCTACTCCTGTCC
CTGACTGGCCAGCAACTATCAGGATTGGTTCTGTCCACTTGCCTCCCTAACCTGCTGGGCCCACCAG
GACCCTTCTGCTCTGCCCTTTACCAAACCTGCTCTGGCCCAGTACTCCTTTAGCCCTCAGGGTGATGA
AGCGACCAGCTCTGTACCCAGGGCCAAGCTCAACCCTCTGAGCTGTGCCAGCATCTGGAATCCTCTG
GTCTGGACTTGCAAACAGGCATTTGGGGGTTTCTCCTGCTTCCTCTTCTGCAGGTCCTACTGACACCC
CTACTTCCAGCATGGAGCCCACCAGGACGCAGGGTTGGGGACTGAGTCTCGCCTACCTTGAGCTTCC
TCCTTGCATTGAACTTCTTCAGACATTCCACAGTCTCCTGTCTGTGCATCATAGAGGCCACGGTGGAA
CGTTGCTGTTGGGAGAAGGGAAGTCACAGGGCAGTGAGGATCATCAGAGGTGTCAGTCTTGTCCCC
AGAGGCTTAGGAGAGAGGTTCAAGGTGGTGGCACTCTAGGGCCAGCCACAATGACAAAGAGATCG
ATCACTCGTTCCCACCTGCGGTCCCCAACTTATTTCCAAGTTCAAGTCTTGTCCTTCACAGACCAGGG
GAGTGGTCAGACACAAGGTACAGAAGCCCAGGGCCATCTTGGGGGCCCTGGATATACCCACTAGGA
ACTGGCCCCTAGAGTTTGCCAAGAATGACTTACGCAGACCCATGGGTGCTTCAGGGCCTCATGGGCC
GTGATGCGCTTGGCAGGGTTGATGGTCAGCATCTGGTTGATGAGGTTTTTGGCTTCAGGAGTAACG
GTGTCCCACTCAGGGGATGGGAACTACAGAAGGAAGCGGGGCACAGGGCAAGGCTGAAGCCCCTC
CCCTGGGCAGAGGCCATGGAGAGGAGGGGAGACCCAGGGTGCGGCAGTCCCCACCCTCCACGCCG
GCGCCCACTCACATCATACGCCCCAGCCTTGATCTGCTGGTACAGCTTGTGTTGGTCCTCATCCCAGA
AAGGTGGGTAGCCCACCAGCAGGATATACAGGATCACCC

Sequence in red is the 35 nt deleted in the mutant sequence seen in CAMK2A-T286P-CAS-LINE10-B6J/1.4g. Sequence in red and underlined is the 10 nt deleted in the mutant sequence seen in CAMK2A-T286P-CAS-LINE10-B6J/1.4h.

Off-target site 11:5988993-5989015 35 nt deletion mutant sequence seen in CAMK2A-T286P-CAS-LINE10-B6J/1.4g

GCTCACACTACCCACAAATCACAGCGTCCAGACTCCTGAGAAGTGGGCGTGGCCAGGAAGGGGTTC
TTGTCCTTCCTAAATGCTGTGGCACCTTAATACAGTTCCTCATGTCGCGGTGATGCTCGGCCACAAAG
TTATCTCGTTACCACTTCATCACTGTAATCTTGCTACTGTGGTGTTATGAATGACATGCGGAACATCT

GATATGTGACCCACTAAAGGGTCTTGACCCACAAGTTGAGAACCATTCCTTTAACAGGCCCAGGGTA
GTCACGGTTGTCCTCAATGCTTTGGGGAAGCTCAATCAAGCAATGGCTCCCGGGTCTACTCCTGTCC
CTGACTGGCCAGCAACTATCAGGATTGGTTCTGTCCACTTGCCTCCCTAACCTGCTGGGCCCACCAG
GACCCTTCTGCTCTGCCCTTTACCAAACCTGCTCTGGCCCAGTACTCCTTTAGCCCTCAGGGTGATGA
AGCGACCAGCTCTGTACCCAGGGCCAAGCTCAACCCTCTGAGCTGTGCCAGCATCTGGAATCCTCTG
GTCTGGACTTGCAAACAGGCATTTGGGGGTTTCTCCTGCTTCCTCTTCTGCAGGTCCTACTGACACCC
CTACTTCCAGCATGGAGCCCACCAGGACGCAGGGTTGGGGACTGAGTCTCGCCTACCTTGAGCTTCC
TCCT **[35 nt deletion]**
GTCTGTGCATCATAGAGGCCACGGTGGAACGTTGCTGTTGGGAGAAGGGAAGTCACAGGGCAGTG
AGGATCATCAGAGGTGTCAGTCTTGTCCCCAGAGGCTTAGGAGAGAGGTTCAAGGTGGTGGCACTC
TAGGGCCAGCCACAATGACAAAGAGATCGATCACTCGTTCCCACCTGCGGTCCCCAACTTATTTCCA
AGTTCAAGTCTTGTCCTTCACAGACCAGGGGAGTGGTCAGACACAAGGTACAGAAGCCCAGGGCCA
TCTTGGGGGCCCTGGATATACCCACTAGGAACTGGCCCCTAGAGTTTGCCAAGAATGACTTACGCAG
ACCCATGGGTGCTTCAGGGCCTCATGGGCCGTGATGCGCTTGGCAGGGTTGATGGTCAGCATCTGG
TTGATGAGGTTTTTGGCTTCAGGAGTAACGGTGTCCCACTCAGGGGATGGGAACTACAGAAGGAAG
CGGGGCACAGGGCAAGGCTGAAGCCCCTCCCCTGGGCAGAGGCCATGGAGAGGAGGGGAGACCC
AGGGTGCGGCAGTCCCCACCCTCCACGCCGGCGCCCACTCACATCATACGCCCCAGCCTTGATCTGC
TGGTACAGCTTGTGTTGGTCCTCATCCCAGAAAGGTGGGTAGCCCACCAGCAGGATATACAGGATC
ACCC

Off-target site 11:5988993-5989015 10 nt deletion mutant sequence seen in CAMK2A-T286P-CAS-LINE10-B6J/1.4h, CAMK2A-T286P-CAS-LINE10-B6J/1.1f, CAMK2A-T286P-CAS-LINE10-B6J/1.1g, CAMK2A-T286P-CAS-LINE10-B6J/1.2f and CAMK2A-T286P-CAS-LINE10-B6J/1.3b

GCTCACACTACCCACAAATCACAGCGTCCAGACTCCTGAGAAGTGGGCGTGGCCAGGAAGGGGTTC
TTGTCCTTCCTAAATGCTGTGGCACCTTAATACAGTTCCTCATGTCGCGGTGATGCTCGGCCACAAAG
TTATCTCGTTACCACTTCATCACTGTAATCTTGCTACTGTGGTGTTATGAATGACATGCGGAACATCT
GATATGTGACCCACTAAAGGGTCTTGACCCACAAGTTGAGAACCATTCCTTTAACAGGCCCAGGGTA
GTCACGGTTGTCCTCAATGCTTTGGGGAAGCTCAATCAAGCAATGGCTCCCGGGTCTACTCCTGTCC
CTGACTGGCCAGCAACTATCAGGATTGGTTCTGTCCACTTGCCTCCCTAACCTGCTGGGCCCACCAG
GACCCTTCTGCTCTGCCCTTTACCAAACCTGCTCTGGCCCAGTACTCCTTTAGCCCTCAGGGTGATGA
AGCGACCAGCTCTGTACCCAGGGCCAAGCTCAACCCTCTGAGCTGTGCCAGCATCTGGAATCCTCTG
GTCTGGACTTGCAAACAGGCATTTGGGGGTTTCTCCTGCTTCCTCTTCTGCAGGTCCTACTGACACCC
CTACTTCCAGCATGGAGCCCACCAGGACGCAGGGTTGGGGACTGAGTCTCGCCTACCTTGAGCTTCC
TCCTTGCATTGAACTTCTTCAGACAT**[10 nt deletion]**
CCTGTCTGTGCATCATAGAGGCCACGGTGGAACGTTGCTGTTGGGAGAAGGGAAGTCACAGGGCA
GTGAGGATCATCAGAGGTGTCAGTCTTGTCCCCAGAGGCTTAGGAGAGAGGTTCAAGGTGGTGGC
ACTCTAGGGCCAGCCACAATGACAAAGAGATCGATCACTCGTTCCCACCTGCGGTCCCCAACTTATTT
CCAAGTTCAAGTCTTGTCCTTCACAGACCAGGGGAGTGGTCAGACACAAGGTACAGAAGCCCAGGG
CCATCTTGGGGGCCCTGGATATACCCACTAGGAACTGGCCCCTAGAGTTTGCCAAGAATGACTTACG

CAGACCCATGGGTGCTTCAGGGCCTCATGGGCCGTGATGCGCTTGGCAGGGTTGATGGTCAGCATC
TGGTTGATGAGGTTTTTGGCTTCAGGAGTAACGGTGTCCCACTCAGGGGATGGGAACTACAGAAGG
AAGCGGGGCACAGGGCAAGGCTGAAGCCCCTCCCCTGGGCAGAGGCCATGGAGAGGAGGGGAGA
CCCAGGGTGCGGCAGTCCCCACCCTCCACGCCGGCGCCCACTCACATCATACGCCCCAGCCTTGATC
TGCTGGTACAGCTTGTGTTGGTCCTCATCCCAGAAAGGTGGGTAGCCCACCAGCAGGATATACAGG
ATCACCC

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-MUT1
Forward Primer (5'-3')	ACAGACAGGAGcCCGTc
Reverse Primer (5'-3')	GCATGAAACCGATGAAGAGAAGA
Probe (5'-3')	TCAATGCCAGGAGGAAACTGAAGGT
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.