Name of Mouse model or mutation:
PIK3R1-FLOX-EM1-B6
PIK3R1-FLOX-EM2-B6

Description:
Floxed allele made by CRISPR/Cas9 gene editing.

Type of mutation:
Floxed exon: ENSMUSE00000290469

Delivery method:
Pronuclear injection into 1-cell stage embryo

Genetic Background:
C57BL/6J

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
gctattataaaattctggtc	AGG
ctattataaaattctggtca	GGG
atggaacgttaatttccgac	TGG
gaaattaacgttccatcaac	TGG

lsDNA donor sequence (5'-3'):

LOCUS Pik3r1 755 bp DNA linear 23-JUN-2020
FEATURES Location/Qualifiers
misc_feature 144..177
/note="loxP"
misc_feature 136..143
/note="AsiSI (SfaAI)"
PCR_primer 115..135
/note="LoxPF"
misc_feature 1..114
/note="5' Homology Arm"
misc_feature 178..579
/note="Critical region"
misc_feature 580..613

```
                /note="loxP"
     misc_feature    614..621
                /note="MreI"
     PCR_primer      complement(622..641)
                /note="LoxPR"
     misc_feature    642..755
                /note="3' Homology Arm"
     source          1..755
                /dnas_title="Pik3r1 donor sequence"
     exon            295..420
                /note="ENSMUSE00000290469"
     exon            1..11
                /note="ENSMUSE00000569198"
ORIGIN
        1 aataccagca ggtaattcac acagagctcc tcccgctgca gtaacaaggg ggccagagac
       61 tcttcagcat aggaaatgat ctgaaattgg gttttgacaa actttacacc ctgaatccgg
      121 gggtaccgcg tcgagGCGAT CGCATAACTT CGTATAGCAT ACATTATACG AAGTTATcca
      181 gaattttata atagctaaat cagataccat attgactatg ccattaggga ttgttatttg
      241 agtaaatagc ttatcaactg aattcttttt ttccctgctt gtatattctt ccaggatcaa
      301 gttgtcaaag aagataatat tgaagctgta gggaaaaaat tacatgaata taatactcaa
      361 tttcaagaaa aaagtcggga atatgataga ttatatgagg agtacacccg tacttcccag
      421 gtgagtttaa gatattttgg actaacacat actatactca gattcaaagg aagaatcaag
      481 tgtctctaac ccttttcagc ttaacccatg cttataaagt cctacattaa gcttgcttct
      541 aactcgcagt gttactatta tgagcttcca gttgatggaA TAACTTCGTA TAGCATACAT
      601 TATACGAAGT TATCGCCGGC Gggtctgagc tcgccatcag ttaatttccg actggttact
      661 tctgcctagg ctagacattg ataataactg ctaatgtcag tttgtccaag ctctagaatg
      721 gaaagttcag acccatccct cctgctgtga ggaga
//
```

Microinjection mixes:

Microinjection buffer (MIB; 10 mM Tris–HCl, 0.1 mM EDTA, 100 mM NaCl, pH7.5) was prepared and filtered through a 2 nm filter and autoclaved. Cas9 mRNA, sgRNAs and ssODNs were diluted and mixed in MIB to the working concentrations of 100 ng/µl, 50 ng/µl each and 50 ng/µl, respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Sequence details

WT

ACCTTTTTGGTACGAGACGCATCTACTAAAATGCACGGCGATTACACTCTTACACTAAGGTGAGCCA
GGAAGTCAGCTGTAATTGCGATGTCTCAGTTGTCATGAAAACATTTCTTCATTAGATTGCCTATTTTTC
CTCCTTAAATTAGCATATAAAGTAATGGGTTTCACTATAGGATTTTTTTTCAAACATCTTCGATTCGTC
CCTTTCTCCTACTGCCTTCTCCTCCCCATCCTGAGAGCCCTCCTTCTGCATCAGAAAATGTTAATAATC
ATTATTTTTGTATTGTATTTACAGGAAAGGAGGAAATAACAAATTAATCAAAATCTTTCACCGTGATG
GAAAATATGGCTTCTCTGATCCATTAACCTTCAACTCTGTGGTTGAGTTAATAAACCACTACCGGAAT
GAGTCTTTAGCTCAGTACAACCCCAAGCTGGATGTGAAGTTGCTCTACCCAGTGTCCAAATACCAGC
AGGTAATTCACACAGAGCTCCTCCCGCTGCAGTAACAAGGGGGCCAGAGACTCTTCAGCATAGGAA
ATGATCTGAAATTGGGTTTTGACAAACTTTACACCCTGACCAGAATTTTATAATAGCTAAATCAGATA
CCATATTGACTATGCCATTAGGGATTGTTATTTGAGTAAATAGCTTATCAACTGAATTCTTTTTTTCCC
TGCTTGTATATTCTTCCAG**GATCAAGTTGTCAAAGAAGATAATATTGAAGCTGTAGGGAAAAAATT**
ACATGAATATAATACTCAATTTCAAGAAAAAAGTCGGGAATATGATAGATTATATGAGGAGTACA
CCCGTACTTCCCAGGTGAGTTTAAGATATTTTGGACTAACACATACTATACTCAGATTCAAAGGAAG
AATCAAGTGTCTCTAACCCTTTTCAGCTTAACCCATGCTTATAAAGTCCTACATTAAGCTTGCTTCTAA
CTCGCAGTGTTACTATTATGAGCTTCCAGTTGATGGAACGTTAATTTCCGACTGGTTACTTCTGCCTA
GGCTAGACATTGATAATAACTGCTAATGTCAGTTTGTCCAAGCTCTAGAATGGAAAGTTCAGACCCA
TCCCTCCTGCTGTGAGGAGAAATGGCCGTGTTGATTATCAGAGAAAGCAGCATGGGTGTCATAAAA
TAAAATGGCTTTTTCAAAGACTGACCGGCTTGTACTTACATACTTAGGAACACACACACACACATGCA
TGCATGCACTCGTTCATTCACACAACATGAATTTGAAGGAACAAGGAGTGGTATGTGAGATGGTTG
AAAGGGAAGGAGGAAATAATGTAATTATGTTATAATCTCAAAAAAAAAAAGAAAACATAATTTTAA
AAAGTGAAAGACAGATAATTCTCTGTCAGGAACATCTGTTTCTTGATCATTTGACGAGAGTTGGTGA
ATTCATGACCAGCCCTGCTAACATACACAGCCCTTGCCTATCTCCTGCTCCTGAATCCTGAAGATGCA
CAGTGTAGGCATCTCCCCGCCCCCTTGCTCCTGTTGTCTAAGCCATACCGTACAGCTATTTACGTAGC
ACTGATGTTGTCTCATGCTCAAGCCA

PIK3R1-FLOX-EM1-B6 or PIK3R1-FLOX-EM2-B6

ACCTTTTTGGTACGAGACGCATCTACTAAAATGCACGGCGATTACACTCTTACACTAAGGTGAGCCA
GGAAGTCAGCTGTAATTGCGATGTCTCAGTTGTCATGAAAACATTTCTTCATTAGATTGCCTATTTTTC
CTCCTTAAATTAGCATATAAAGTAATGGGTTTCACTATAGGATTTTTTTTCAAACATCTTCGATTCGTC
CCTTTCTCCTACTGCCTTCTCCTCCCCATCCTGAGAGCCCTCCTTCTGCATCAGAAAATGTTAATAATC
ATTATTTTTGTATTGTATTTACAGGAAAGGAGGAAATAACAAATTAATCAAAATCTTTCACCGTGATG
GAAAATATGGCTTCTCTGATCCATTAACCTTCAACTCTGTGGTTGAGTTAATAAACCACTACCGGAAT
GAGTCTTTAGCTCAGTACAACCCCAAGCTGGATGTGAAGTTGCTCTACCCAGTGTCCAAATACCAGC
AGGTAATTCACACAGAGCTCCTCCCGCTGCAGTAACAAGGGGGCCAGAGACTCTTCAGCATAGGAA
ATGATCTGAAATTGGGTTTTGACAAACTTTACACCCTGA*atccgggggtaccgcgtcgagGCGATCGC*ATA
ACTTCGTATAGCATACATTATACGAAGTTATCCAGAATTTTATAATAGCTAAATCAGATACCATATTG
ACTATGCCATTAGGGATTGTTATTTGAGTAAATAGCTTATCAACTGAATTCTTTTTTTCCCTGCTTGTA
TATTCTTCCAG**GATCAAGTTGTCAAAGAAGATAATATTGAAGCTGTAGGGAAAAAATTACATGAA**

TATAATACTCAATTTCAAGAAAAAAGTCGGGAATATGATAGATTATATGAGGAGTACACCCGTAC
TTCCCAGGTGAGTTTAAGATATTTTGGACTAACACATACTATACTCAGATTCAAAGGAAGAATCAAG
TGTCTCTAACCCTTTTCAGCTTAACCCATGCTTATAAAGTCCTACATTAAGCTTGCTTCTAACTCGCAG
TGTTACTATTATGAGCTTCCAGTTGATGGAATAACTTCGTATAGCATACATTATACGAAGTTAT*CGCC*
*GGCGgtctgagctcgccatcagt*TAATTTCCGACTGGTTACTTCTGCCTAGGCTAGACATTGATAATAACT
GCTAATGTCAGTTTGTCCAAGCTCTAGAATGGAAAGTTCAGACCCATCCCTCCTGCTGTGAGGAGAA
ATGGCCGTGTTGATTATCAGAGAAAGCAGCATGGGTGTCATAAAATAAAATGGCTTTTTCAAAGACT
GACCGGCTTGTACTTACATACTTAGGAACACACACACACACATGCATGCATGCACTCGTTCATTCACA
CAACATGAATTTGAAGGAACAAGGAGTGGTATGTGAGATGGTTGAAAGGGAAGGAGGAAATAATG
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CTGTCAGGAACATCTGTTTCTTGATCATTTGACGAGAGTTGGTGAATTCATGACCAGCCCTGCTAACA
TACACAGCCCTTGCCTATCTCCTGCTCCTGAATCCTGAAGATGCACAGTGTAGGCATCTCCCCGCCCC
CTTGCTCCTGTTGTCTAAGCCATACCGTACAGCTATTTACGTAGCACTGATGTTGTCTCATGCTCAAG
CCA

LoxP sites are underlined and genotyping handles (restriction enzyme site plus primer unique to each LoxP site) in italics. Floxed exon highlighted in bold.

Please note, alleles EM1 and EM2 have the same sequence, but were derived from different founder animals.

Nucleotide 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:

LoxPF	AAAGGAGGGAAGGAGGCTGA
LoxPR	AGAGAAGCCTGGAGAGAGGG
Taq Polymerase used	Roche Expand Long Range dNTP pack
Annealing Temperature (°C)	61
Elongation time (min)	3
WT product size (bp)	N/A
Mutant product size (bp)	2256
Notes	PCR used to screen for floxed alleles. 3% DMSO used in reactions.

Geno_PIK3R1_F1 primer (5'-3')	ACCTTTTTGGTACGAGACGCA
Geno_PIK3R1_R1 primer (5'-3')	TGGCTTGAGCATGAGACAACA
Taq Polymerase used	ThermoFisher SuperFi PCR Kit
Annealing Temperature (°C)	61
Elongation time (min)	1
WT product size (bp)	1573
Mutant product size (bp)	1698
Notes	Sequenced with the following primers: Geno_PIK3R1_F1 (ACCTTTTTGGTACGAGACGCA) Geno_PIK3R1_R2 primer (AGATAGGCAAGGGCTGTGTATG) LoxPF (AAAGGAGGGAAGGAGGCTGA) LoxPR (AGAGAAGCCTGGAGAGAGGG)

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 sites on the same chromosome with ≤2 mismatches in seed sequence of guide(s) used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
13:6069825-6069847	G**G**TA**A**TAT**G**AAA**A**TCTGGTC AGG	Intergenic	Pik3r1_OT1G1_F1: TCCTCAAAATCATCAACTGCC Pik3r1_OT1G1_R1: CACTGACTTTCCTTATGCCC Sequenced with: PIk3r1_OT1G1_F3: ACAATCCTTGTACTATGCCAAAAC Pik3r1_OT1G1_R2: TGTGCTTTCTTTGTTCCCCCA
13:26316422-26316444	**T**CTATTA**A**A**C**A**T**TTCTGGTC AGG	Intergenic	Pik3r1_OT2G1_F1: GAGGTTAATGCTTCTCCCTGCT Pik3r1_OT2G1_R1: ACTTTTCACTGCTATGAGGGCA Sequenced with: Pik3r1_OT2G1_seqF: ACTTTCTGGTACCATGAAAT Pik3r1_OT2G1_seqR: TCCAACTCTCAGGATTTTAT
13:51258883-51258905	G**T**TATTA**G**AAAA**C**TCTG**C**TC TGG	Intergenic	Pik3r1_OT3G1_F1: TCCAGGGATAAGCAGGGATCTT Pik3r1_OT3G1_R1: CAGATGCGCTGAGTAGAGCAA Sequenced with: Pik3r1_OT3G1_F2: TGTTCTGATGGGTGCCCAAG Pik3r1_OT3G1_R2: GGGAATGCTGAAATGCCAACC
13:93450521-93450543	G**AA**A**A**TATAAAATTCTGG**G**C TGG	Intronic	Pik3r1_OT4G1_F1: CCCACTGCTATCAGAACTCACT Pik3r1_OT4G1_R1: TTTTCCTTGCAGTCTGCCTAAA
13:9714150-9714172	**GC**ATT**T**T**C**AAATTCTGGTCA AGG	Intronic	Pik3r1_OT1G2_F1: AGAACAGGCCCCTCTATCTGG Pik3r1_OT1G2_R1: ATGTCAGACCCTGTGCATGAG Sequenced with: Pik3r1_OT1G2_R2: AGTCCTCTGAATTCTGTTTCACTA
13:33975887-33975909	C**C**ATTA**A**AAAATT**G**TG**T**TCA GGG	Intronic	Pik3r1_OT2G2_F1: CCATACAGTGGGAAAAAGACAGC Pik3r1_OT2G2_R1: ACAGGGTCCCTTCAGCAAAAT Sequenced with: Pik3r1_OT2G2_F2: GGCTTGCGCTGTAAGATCAAAA Pik3r1_OT2G2_R2:

			TTCCGGTTGTATTTTCTGAGGA
13:34362525-34362547	CTATTCAAAATTTCTAGTCA TGG	Intergenic	Pik3r1_OT3G2_F1: AATAGCTTGCTGCCGTGACTTA Pik3r1_OT3G2_R1: GCCCCTTTGAAGTCTGGAAGG Sequenced with: Pik3r1_OT3G2_F2: TATACTGCAGAGAGTATAAACCTGG Pik3r1_OT3G2_R2 GTCTTCTGCACACTCCCACA
13:46914819-46914841	TTTTTAAAAAATTCTGCTCA AGG	Intronic	Pik3r1_OT4G2_F1: GGTAAACGCTGTGGTTCGGAT Pik3r1_OT4G2_R1: GGCTGAAGGGACCATTACTCT
13:51078208-51078230	TTATCATTAAATTCTGCTCA TGG	Intergenic	Pik3r1_OT5G2_F1: TTTGTCATTTGCTGACTCCTTCG Pik3r1_OT5G2_R1: TTACAAGGTTGGGGTCTCCCT
13:54546657-54546679	GCATTAGAAAATTCAGGTCA AGG	Intronic	Pik3r1_OT6G2_F1: CCCCAGCTAACCAGAACTACA Pik3r1_OT6G2_R1: CAGGGACGTGGAGTTGTTCA Sequenced with: Pik3r1_OT6G2_F2: CACCAAGGAAGTCAGGACTGG Pik3r1_OT6G2_R2: GGGAGTTACCCAGAAGCACATT
13:89012517-89012539	CTATTAACAAATTTAGGTCA AGG	Intronic	Pik3r1_OT7G2_F1: TAGCAAAGATGACCCAACAGGT Pik3r1_OT7G2_R1: TGGCTTAAGTGGTTGTCAGCA
13:98904544-98904566	CTACTACAAAGTTCTGGGCA AGG	Intronic	Pik3r1_OT8G2_F1: AATGTAAGATGGTGCTGAACAGGG Pik3r1_OT8G2_R1: TTTGTCTTGGCTGTGTTTACCTG Sequenced with: Pik3r1_OT8G2_F2: ATCCAGACTGAGGAAGACCCG Pik3r1_OT8G2_R3: GAAAATGTTAGCATTTAATT
13:34596127-34596149	TAAATGAACTTTCCATCAAG AGG	Intergenic	Pik3r1_OT1G3_F1: TCAGGGACCAGTTTCTTTCTCT Pik3r1_OT1G3_R1: ACCACTTATGCAGGGCTAGAAT

All amplicons were sent for Sanger sequencing. No evidence of 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	PIK3R1-FLOX-5'
Forward Primer (5'-3')	GACAAACTTTACACCCTGAATCC
Reverse Primer (5'-3')	ACAATCCCTAATGGCATAGTCA
Probe (5'-3')	TCGAGGCGATCGCATAACTTCG
Label	FAM

This ddPCR assay is specific to the 5' LoxP region of the PIK3R1 Flox donor and only floxed 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.

Assay name	PIK3R1-FLOX-3'
Forward Primer (5'-3')	TGAGCTTCCAGTTGATGGAATAA
Reverse Primer (5'-3')	GCAGAAGTAACCAGTCGGAAA
Probe (5'-3')	AAGTTATCGCCGGCGGGTCTGA
Label	FAM

This ddPCR assay is specific to the 3' LoxP region of the PIK3R1 Flox donor and only floxed 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.

Assay name	PIK3R1-FLOX-CR-LOA
Forward Primer (5'-3')	GTACTTCCCAGGTGAGTTTAAG
Reverse Primer (5'-3')	CACTGCGAGTTAGAAGCAA
Probe (5'-3')	TCAAAGGAAGAATCAAGTGTCTCTAACCCT
Label	FAM

This ddPCR assay is universal to Pik3r1 - both WT and floxed 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.

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 random integrations were detected in the animals selected for breeding.

Pik3r1-Flox

Allele Description

This is a CRISPR/Cas9 induced mutation creating a conditional knock-out by floxing critical exon, ENSMUSE00000290469 of *Pik3r1*. The stock was generated at MRC Harwell via pronuclear injection 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 wildtype loss of allele (WT-LOA) 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:

- Universal probe and Universal primer designed 5' of the deleted region.
- Wildtype specific primer situated within the deleted region.
- Mutant specific primer that binds to the inserted LoxP sequence

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

Pik3r1-Flox

Pik3r1-Flox-3'-WT1 assay (FAM labelled)

GGACTAACACATACTATACTCAGATTCAAAGGAAGAATCAAGTGTCTCTAACCCTTTTCAG**CTTAACC CATGCTTATAAAGTCCTACA**TTA**AGCTTGCTTCTAACTCGCAGTGTT**ACTATTATGAGCTTCCAGTTG AT**GGAACGTTAATTTCCGACTGGT**TACTTCTGCCTAGGCTAGACATTGATAATAACTGCTAATGTCA

Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo Pik3r1-Flox	5' label	Sequence 5' → 3'	3' label	Oligo Type
Pik3r1-Flox-WT_F	n/a	**CTTAACCCATGCTTATAAAGTCCTACA**	n/a	WT Forward
Pik3r1-Flox-WT_PROBE	FAM	**AGCTTGCTTCTAACTCGCAGTGTT**	Zen-IBFQ	WT Probe
Pik3r1-Flox-WT_R	n/a	**ACCAGTCGGAAATTAACGTTCC**	n/a	WT Reverse

Pik3r1-Flox -3'-MUT1 assay (FAM labelled)

TGTCTCTAACCCTTTTCAGCTTAACCCATGCTTATAAAGTCCTACATTAAGCTTGCTTCTAACTCGCAG TGTTACTATTA**TGAGCTTCCAGTTGATGGAataa**cttcgtatagcatacattatacg**aagttat*CGCCGGCG*GGT CTGA**GCTCGCCATCAGTTAA**TTTCCGACTGGTTACTTCTGC**CTAGGCTAGACATTGATAATAACTGCT AATGTCAGTTTGTCCAAGCTCTAGAATGGAAAGTTCAGACCCATCCCTCCTGCTGTGAGGAGAAATG

Lower case letters denote the inserted LoxP sequence
Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo Pik3r1-Flox	5' label	Sequence 5' → 3'	3' label	Oligo Type
Pik3r1-Flox-MUT_F	n/a	**TGAGCTTCCAGTTGATGGAATAA**	n/a	Mutant Forward
Pik3r1-Flox-MUT_PROBE	FAM	**AAGTTATCGCCGGCGGGTCTGA**	Zen-IBFQ	Mutant Probe
Pik3r1-Flox-MUT_R	n/a	**GCAGAAGTAACCAGTCGGAAA**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo Pik3r1-Flox	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

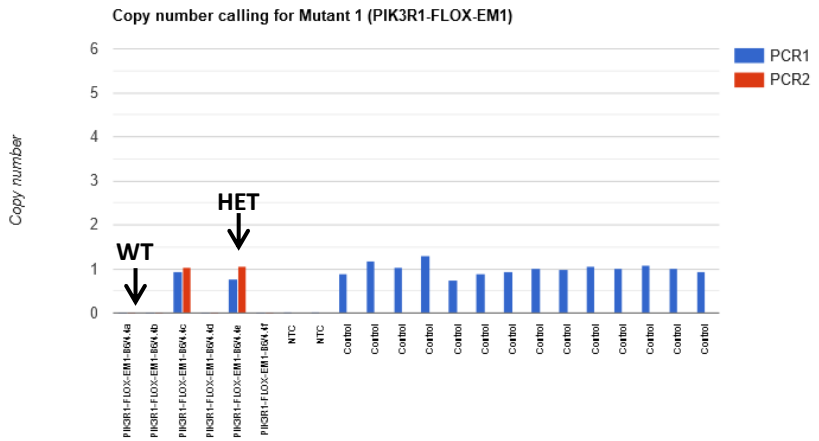
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

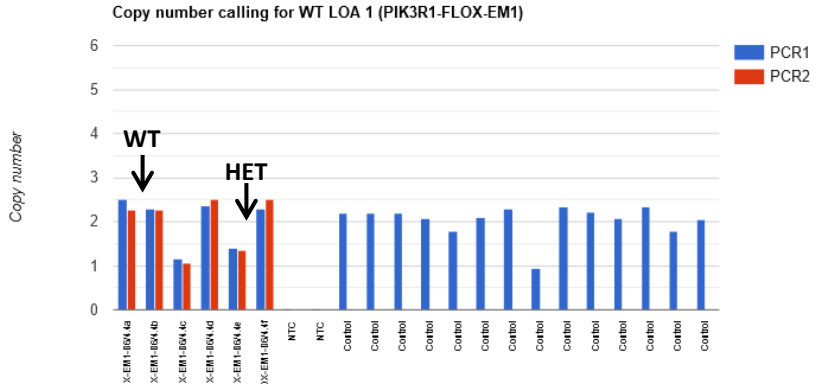
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Pik3r1-Flox'-WT1 and Pik3r1-Flox-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 321184 results)





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Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman