Name of Mouse model or mutation:
STAT2-FLOX-EM1-B6N

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

Type of mutation:
Flox: LoxP sites flanking exons ENSMUSE00000969764 and ENSMUSE00001035174.

Delivery method:
Pronuclear injection into 1-cell stage embryo.

Genetic Background:
C57BL/6NTac

Nuclease:
Cas9 mRNA

sgRNAs:

Protospacer sequence	PAM sequence
ACCCTCTGCGGTAAAATTTC	AGG
AATTTCAGGCCCTGATTTTA	AGG
AATAGTTAGCAAATGACGGG	GGG
ATAGTTAGCAAATGACGGGG	GGG

lssDNA long donor sequence (5'-3'):

```
LOCUS       Tm1c                1252 bp    DNA     linear      17-SEP-2020
FEATURES             Location/Qualifiers
     misc_feature    229..262
                     /note="loxP"
     misc_feature    991..1024
                     /note="loxP"
     misc_feature    221..228
                     /note="AsiSI (SfaAI)"
     misc_feature    1025..1032
                     /note="MreI"
     PCR_primer      200..220
                     /note="LoxPF"
     PCR_primer      complement(1033..1052)
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                /note="LoxPR"
     misc_feature    1..96
                /note="Stat2-201 transcript Exon 4: ENSMUSE00001063374"
     misc_feature    1..199
                /note="5'HA "
     misc_feature    562..651
                /note="Stat2- 201 transcript Exon 5: ENSMUSE00000969764"
     misc_feature    263..990
                /note="Critical Region"
     misc_feature    740..815
                /note="Stat2-201 transcript Exon 6: ENSMUSE00001035174"
     misc_feature    1053..1252
                /note="3'HA "
     misc_feature    1242..1252
                /note="Stat2-201 transcript Exon 7: ENSMUSE00001039638"
     source          1..1252
                /dnas_title="Tm1c gBlock Stat2 Flox2"
ORIGIN
        1 ACCTTTCCCA ATGGCCCTAC CCAGTTGGCT GAGATGATCT TTAATCTTCT TCTGGAAGAG
       61 CAAAGGATTC TGAATCAGGC TCAAAGAGCT CAAGAGGTAA GGTTAGTGTG ATGTTGACAT
      121 TCAAAAGCCC CTGGGACAGC AAGGTGCCTG GAGGAGAGTC AGGGAGCTTG TCTCAGACCA
      181 GGATCTCCTC CACCCTCTGa tccgggggta ccgcgtcgag GCGATCGCAT AACTTCGTAT
      241 AGCATACATT ATACGAAGTT ATTTAAGGAT GAAAACTCTA GAGTTCATGT GGGAGTGAGG
      301 GGATGTAGCC CACACCGTTA ATCCTAGCAT TCTGGGCTGC AGAGGCAGGT GGATCTCTGT
      361 GAGTTTGAGC CCTGCTTGGT CTATGTAGTG AGTTCCAGAA CATCCAGAAC ATACATAGAG
      421 AAACCCTGTC TTGAAAACAA ACAAACAAAC AAATAAACAA AACCACACAA TAACAAAAAA
      481 ACAAACAAAA AAACCCAAAA CCTGACAAAC AAAAAAACAA GTCAGAGTTC AAACACACTC
      541 GGAACCTCTT ACCTCATTCA GGTGCAGCCC CCACCAGCCC CCGAAGCAGT TGTGGAGAGC
      601 CAGCAGCTTG AGATTGAAAA TCGAATCCAG GGTTTACATG TGGACATTGA GGTTGGCGGG
      661 TGTGGCAAGT GCTGGGTGGG GAGGACTCGG TTTTCCTGCC CTCAGAAGCA GCCTCACTGG
      721 GGTCCCATCT CCTCTACAGT TCTTGGTGAG ATCCATCAGG CAGCTGAAGG ACGAACAGGA
      781 TGTCTTCAGC TTCAGATACA CAGTTTTCAG TCTGAGTAGG TCCTCTCAAT GTGGGAGGAG
      841 GACAGGGGCC ATTTTTGGGG TCTAGGGGAA GGACGGACAA CAGAAGCTAA ATGATCCAGA
      901 GAGCAGTGGT CACATATGGC CGGTGGTAGT ACAGCATCAG AGGTGTCGGG TGTGTTGTGG
      961 AGGGTGGACC TGAAGAATAG TTAGCAAATG ATAACTTCGT ATAGCATACA TTATACGAAG
     1021 TTATCGCCGG CGggtctgag ctcgccatca gtCTGTGTAG AAAACTGCCT TAGCAGATTG
     1081 AGTGGGCTTC TGGTCTGTCC AAAATTTGCC TGTGGGATTC CATGTACCTG TACTTTATCT
     1141 GGTTCTCCCT TCCTCCCCAT CCAACATTTT CGGTTTCTGA GACCCTGAGG CAGAAAAGAG
     1201 CAGAAGGACC CTGTGGTCCC AGTTTTGGTC TTGCCTTTCA GAGAAGACGT CG
//
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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

Stat2 WT

CTTGCGAAAATTCAGCCGGGATATTCAGGTACTTGGAAGAGCCGGGAATAGTGGCATGCAGCTGGC
CTTTAAGTATAGGGTAAGAGCTGGGGTACAGAGTCTGGACGTGGAGTGTCCCTAGCAGAGTAAAG
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ATTGGCAGGTCACTAAGGTAGTTAAATGGGAGCTTGCCTCTGTTTTTCCTACTTAGACCTTTCCCAAT
GGCCCTACCCAGTTGGCTGAGATGATCTTTAATCTTCTTCTGGAAGAGCAAAGGATTCTGAATCAGG
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GGCCCTGATTTTAAGGATGAAAACTCTAGAGTTCATGTGGGAGTGAGGGGATGTAGCCCACACCGT
TAATCCTAGCATTCTGGGCTGCAGAGGCAGGTGGATCTCTGTGAGTTTGAGCCCTGCTTGGTCTATG
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TCAGGAGCTCTCTTTCCTAGGAGGTGCTGGACATCTCCAAAGGACTGGTTGGCCGATTAACCACCCT
GGTCGACCTATTGCTGCCCAAGCT

STAT2-FLOX-EM1-B6N

CTTGCGAAAATTCAGCCGGGATATTCAGGTACTTGGAAGAGCCGGGAATAGTGGCATGCAGCTGGC
CTTTAAGTATAGGGTAAGAGCTGGGGTACAGAGTCTGGACGTGGAGTGTCCCTAGCAGAGTAAAG
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GGCCCTACCCAGTTGGCTGAGATGATCTTTAATCTTCTTCTGGAAGAGCAAAGGATTCTGAATCAGG
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GCCTGGAGGAGAGTCAGGGAGCTTGTCTCAGACCAGGATCTCCTCCACCCTCTGatccgggggtaccgcg
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TGAAAATCGAATCCAGGGTTTACATGTGGACATTGAGGTTGGCGGGTGTGGCAAGTGCTGGGTGG
GGAGGACTCGGTTTTCCTGCCCTCAGAAGCAGCCTCACTGGGGTCCCATCTCCTCTACAG**TTCTTGGT**
GAGATCCATCAGGCAGCTGAAGGACGAACAGGATGTCTTCAGCTTCAGATACACAGTTTTCAGTCT
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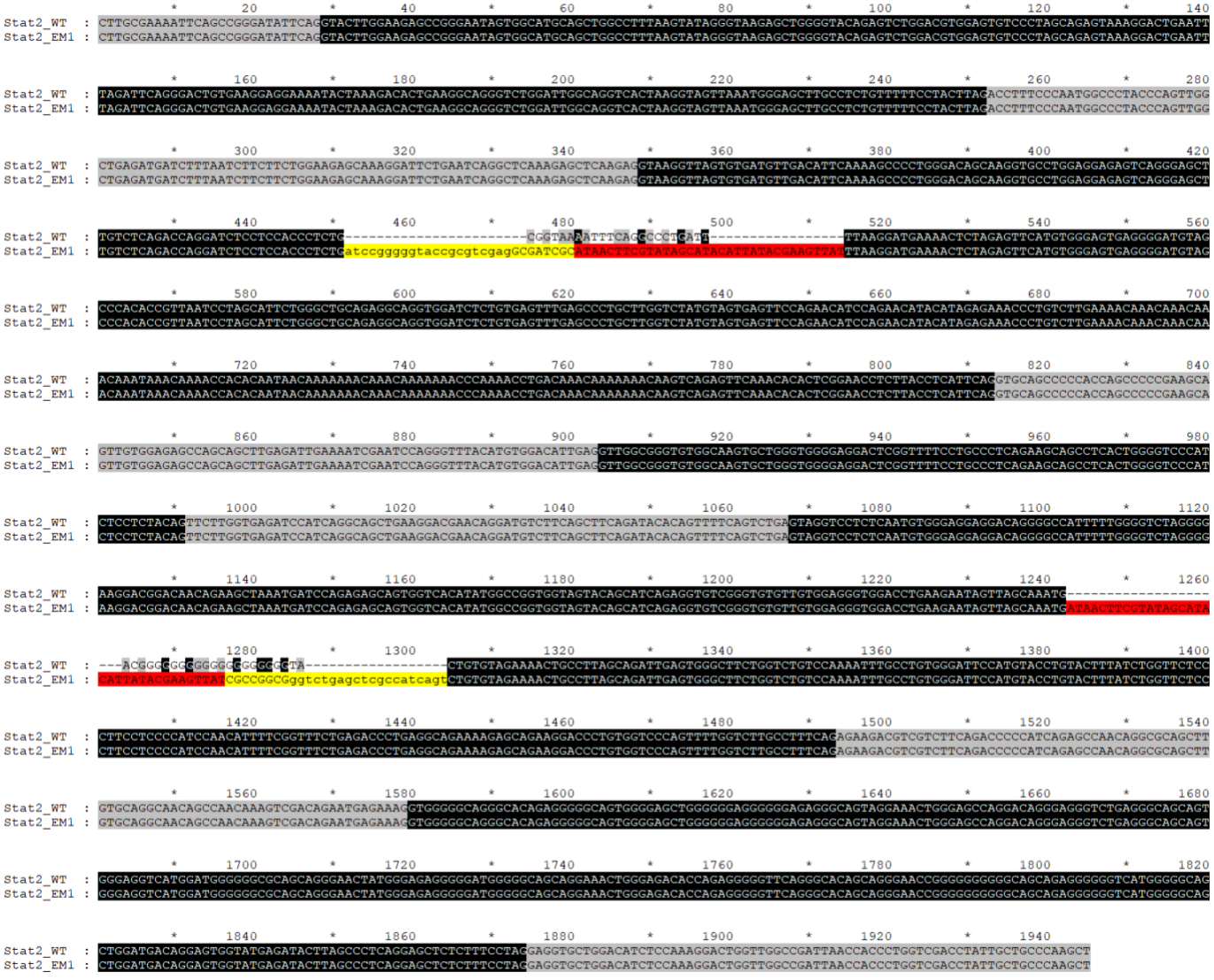
CTCAGGAGCTCTCTTTCCTAGGAGGTGCTGGACATCTCCAAAGGACTGGTTGGCCGATTAACCACCC
TGGTCGACCTATTGCTGCCCAAGCT

LoxP sites are highlighted in red and genotyping handles (restriction enzyme site plus primer unique to each LoxP site) in yellow. Exons are highlighted in grey, with floxed exons in bold also.

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:

LoxP_F	ATCCGGGGGTACCGCGTCGAG
LoxP_R	ACTGATGGCGAGCTCAGACC
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	60
Elongation time (min)	1.5
WT product size (bp)	N/A
Mutant product size (bp)	853
Notes	Screening PCR to identify floxed allele.

Geno_Stat2_F1 primer (5'-3')	CTTGCGAAAATTCAGCCGGG
Geno_Stat2_R1 primer (5'-3')	AGCTTGGGCAGCAATAGGTC
Taq Polymerase used	ThermoFisher SuperFi PCR kit
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	1866
Mutant product size (bp)	1945
Notes	Sequenced using the following primers (5'-3'): Geno_Stat2_F2 primer: TTGGAAGAGCCGGGAATAGTG Geno_Stat2_R1 primer: TTAATCGGCCAACCAGTCCT LoxPF: ATCCGGGGGTACCGCGTCGAG LoxPR: ACTGATGGCGAGCTCAGACC

Geno_Stat2_F1 primer (5'-3')	CTTGCGAAAATTCAGCCGGG
Geno_Stat2_R4 primer (5'-3')	CCGCCAACCTCAATGTCCA
Taq Polymerase used	ThermoFisher SuperFi II PCR kit
Annealing Temperature (°C)	65

Elongation time (min)	0.75
WT product size (bp)	871
Mutant product size (bp)	911
Notes	PCR caried out to better assess the 5' end of the allele. Sequenced using the amplification primers and the following primer: LoxPF_R: ctcgacgcggtacccccggat

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 in the seed sequence for guide(s) used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
10:116181714-116181736	**C**CCCTCT**A**CGGTAAAATTT**A** AGG	Intronic	Stat2_OT1_F1: TTTTGGGACCAAGGCCAACT Stat2_OT1_R1: ATCGAAGTTGCCCACACACT Sequenced also with Stat2_OT1_F2: GCATGGCTGGGAACTCTGTA

All amplicons were sent for Sanger sequencing. No evidence of any 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	STAT2-FLOX-5'-MUT1
Forward Primer (5'-3')	GATCTCCTCCACCCTCTGAT
Reverse Primer (5'-3')	CCTCACTCCCACATGAACTCTA
Probe (5'-3')	TCGAGGCGATCGCATAACTTCG
Label	FAM

This ddPCR assay is specific to the Stat2 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	STAT2-CR-LOA-WT1
Forward Primer (5'-3')	ATGTGGACATTGAGGTTGG
Reverse Primer (5'-3')	AGAACTGTAGAGGAGATGGG
Probe (5'-3')	TTTCCTGCCCTCAGAAGCAGC
Label	FAM

This ddPCR assay is universal to Stat2 - 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.

Assay name	STAT2-FLOX-3'-MUT1
Forward Primer (5'-3')	GGAGGGTGGACCTGAAGAATA
Reverse Primer (5'-3')	GAAGCCCACTCAATCTGCTAAG
Probe (5'-3')	AAGTTATCGCCGGCGGGTCTGA
Label	FAM

This ddPCR assay is specific to the Stat2 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.

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 random donor integrations were detected in the animals selected for breeding.

STAT2-Flox

Allele Description

This is a CRISPR/Cas9 induced mutation creating a conditional knock-out by floxing critical exon, ENSMUSE00000969764 and ENSMUSE00001035174 of *Stat2*. 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

STAT2-Flox

Stat2-Flox-3'-WT1 assay (FAM labelled)

ATTTTTGGGGTCTAGGGGAAGGACGGACAACAGAAGCTAAATGATCCAGAGAGCAGTGGTCACAT
ATGGCCGGTG**GTAGTACAGCATCAGAGGTGTC**GGGTGTGTTGTGGAGGGTGG**ACCTGAAGAATA**
GTTAGCAAATGACGGGGGGGGGGGGGGGGGGGGTACTGTGTAGAAAACTGC**CTTAGCAGATTGAG**
TGGGCTTCTGGTCTGTCCAAAATTTGCCTGTGGGATTCCATGTACCTGTACTTTATCTGGTTCTCCCTT

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

Oligo Stat2	5' label	Sequence 5' → 3'	3' label	Oligo Type
Stat2-WT_F	n/a	**GTAGTACAGCATCAGAGGTGTC**	n/a	WT Forward
Stat2-WT_PROBE	FAM	**ACCTGAAGAATAGTTAGCAAATGACGGGG**	ZEN-IBFQ	WT Probe
Stat2-WT_R	n/a	**AGCCCACTCAATCTGCTAAG**	n/a	WT Reverse

Stat2-Flox-3'-MUT1 assay (FAM labelled)

AACAGAAGCTAAATGATCCAGAGAGCAGTGGTCACATATGGCCGGTGGTAGTACAGCATCAGAGGT
GTCGGGTGTGTTGT**GGAGGGTGGACCTGAAGAATA**GTTAGCAAATGataacttcgtatagcatacattatac
g**aagttatCGCCGGCGGGTCTGA**GCTCGCCATCAGTCTGTGTAGAAAACTGC**CTTAGCAGATTGAGTG**
GGCTTCTGGTCTGTCCAAAATTTGCCTGTGGGATTCCATGTACCTGTACTTTATCTGGTTCTCCCT

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

Oligo Stat2	5' label	Sequence 5' → 3'	3' label	Oligo Type
Stat2-MUT_F	n/a	**GGAGGGTGGACCTGAAGAATA**	n/a	Mutant Forward
Stat2-MUT_PROBE	FAM	**AAGTTATCGCCGGCGGGTCTGA**	ZEN-IBFQ	Mutant Probe
Stat2-MUT_R	n/a	**GAAGCCCACTCAATCTGCTAAG**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**
CCAGCACGACCATTCAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT
CACCCTATGA

Oligo Stat2	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

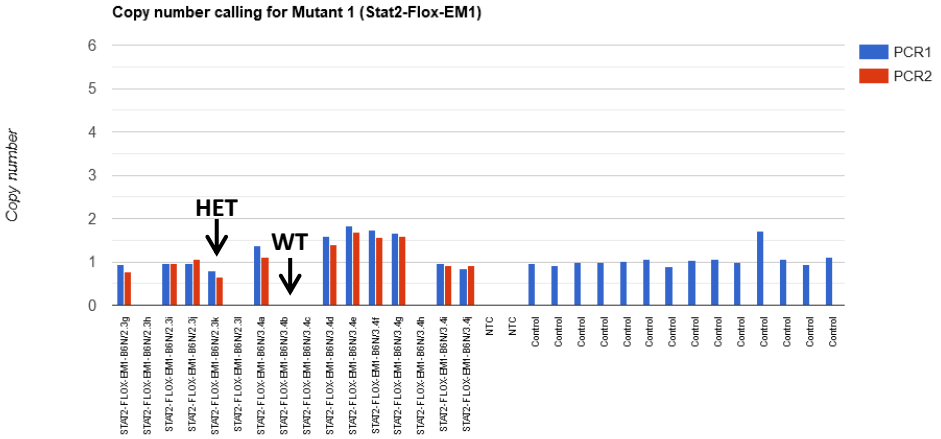
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

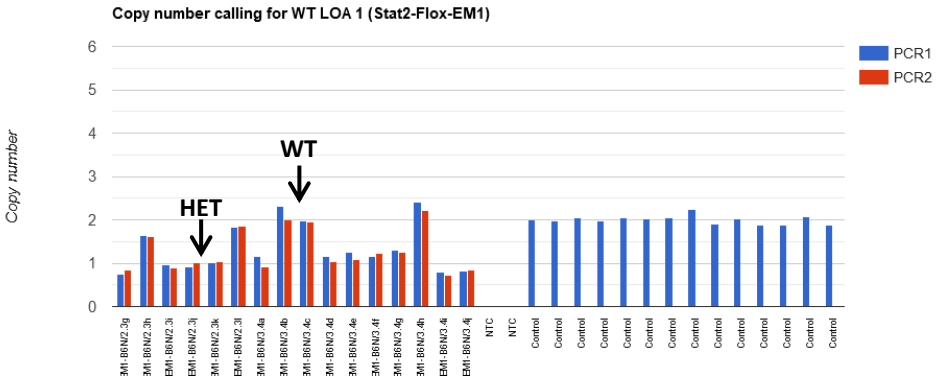
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Stat2'-WT1 and Stat2-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 323514 results)





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