Name of Mouse model or mutation:

SYT1-F349A-EM1-B6

Description:

Point mutation model made using CRISPR/Cas9.

Type of mutation:

SNP: F349A

Description:

Point mutation model made using CRISPR/Cas9.
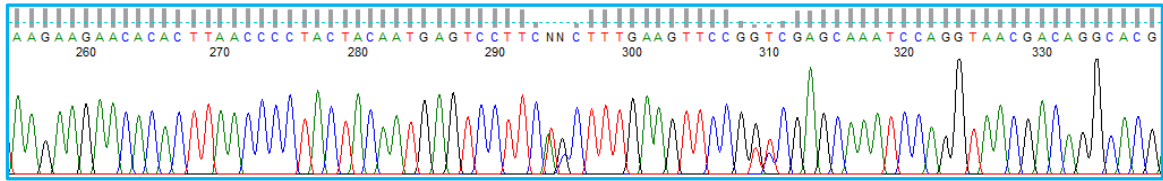

Sequence details

WT
CCTGTTGGGGCTCATTCCTTTTCTGAGCTAATGCATGATCTAAAACATGGCTCTGGAGGACAAGAAG
GTTCAGACAATGAAAGTACCTGCCGCCAAACCTAATGACCTGAGTCTGATCCCGAGGACACACACG
GGAGATGAAGAGGACAGTTCCCACGGGCTGTCCTTTGATCTCCACGCATGCATGCACTGCGGTGTA
CATACCACCGCACACACAGTAAACAACCCGTAATAGAATATTTCAGAGAAGATAAAACACGCGTCTG
AGCTCCTAAAGTCCCTGTGTCTGTCTTTCAGATCCCTATGTAAAGATTCACCTGATGCAGAACGGCAA
GAGACTGAAGAAGAAAAGACAACGATTAAGAAGAACACACTTAACCCCTACTACAATGAGTCCTT
CAGCTTTGAAGTTCCGTTCGAGCAAATCCAGGTAACGACAGGCACGAGTGTGCCCCTCCCCCCCACT
CAGTCTCACGCCTTCAGATCGCATAACTGACAAGCGTAGACCCTGAGAGTCCTCAGTGCCCTGCCTT
CATGAGAACTGGCCCATGCCCCTTTGTGTGATGTCTATGCTCAACTAAGCAGGACATAATAAAACAC
GCAGCACAAGAAGGGCCGATTCATCAGCTGGAACAGCCAGCTGACTTCTGCTGAGCAGAAATAAGG
TAGAAAATAGAAACCAACAGAACCACTTTGCTAGGTCAATAAGTTTGTCTACGGAATCCAAAACCT
CAGAGAGAATGTTCGTTGCCAGGCTACAATAAAAAATCTACTTCTATAGGGCAAAAATAGCCAGGAT
CCCTCCTTCTCACGTCAGCCCATTGTTTACATCACTAGCTCATACACATCCTTACATGCTAGAAAATTA
TATGTATGGATTTATTTTCAACAGCTAAGTAAATCACCAGTATGGTTTTCCTGTCCCTAGTCCCTTGCT
CAGTGGTCTTAACTTGTGAGCACTTTAGATCTTCTGGCATATCCTTGAGACATCAGTTTTCTACCATAA
TTTATTTTGCAATATCTGCTCAGTGAACTTTTTGAGTTCTAAGACCTTATTCCTATGAAAACTAACACC
TCCTGGCATTTTGTTTTTTAATTCCACAGAACAATACAAAGCAAGGTATTGAGATGGAAAAAAAAAA
AAAAAAAAAAACGTGTAAACTTACTCCTTTGGGCTATAATCCTATACATATAAAAATGCATTTCACCT
ATAAATTTCAGAAAGTACAAATATATTGAAAGGGAAGCATATTAAAAACAAAATAAACTTTTCTATTT
GGGGCAGCATTTTAGAGGAGAGAGCCTGAGCCTGACATG

SYT1-F349A-EM1-B6
CCTGTTGGGGCTCATTCCTTTTCTGAGCTAATGCATGATCTAAAACATGGCTCTGGAGGACAAGAAG
GTTCAGACAATGAAAGTACCTGCCGCCAAACCTAATGACCTGAGTCTGATCCCGAGGACACACACG
GGAGATGAAGAGGACAGTTCCCACGGGCTGTCCTTTGATCTCCACGCATGCATGCACTGCGGTGTA
CATACCACCGCACACACAGTAAACAACCCGTAATAGAATATTTCAGAGAAGATAAAACACGCGTCTG
AGCTCCTAAAGTCCCTGTGTCTGTCTTTCAGATCCCTATGTAAAGATTCACCTGATGCAGAACGGCAA
GAGACTGAAGAAGAAAAGACAACGATTAAGAAGAACACACTTAACCCCTACTACAATGAGTCCTT
C**TC**CTTTGAAGTTCCG**GC**CGAGCAAATCCAGGTAACGACAGGCACGAGTGTGCCCCTCCCCCCCACT
CAGTCTCACGCCTTCAGATCGCATAACTGACAAGCGTAGACCCTGAGAGTCCTCAGTGCCCTGCCTT
CATGAGAACTGGCCCATGCCCCTTTGTGTGATGTCTATGCTCAACTAAGCAGGACATAATAAAACAC
GCAGCACAAGAAGGGCCGATTCATCAGCTGGAACAGCCAGCTGACTTCTGCTGAGCAGAAATAAGG
TAGAAAATAGAAACCAACAGAACCACTTTGCTAGGTCAATAAGTTTGTCTACGGAATCCAAAACCT
CAGAGAGAATGTTCGTTGCCAGGCTACAATAAAAAATCTACTTCTATAGGGCAAAAATAGCCAGGAT
CCCTCCTTCTCACGTCAGCCCATTGTTTACATCACTAGCTCATACACATCCTTACATGCTAGAAAATTA
TATGTATGGATTTATTTTCAACAGCTAAGTAAATCACCAGTATGGTTTTCCTGTCCCTAGTCCCTTGCT
CAGTGGTCTTAACTTGTGAGCACTTTAGATCTTCTGGCATATCCTTGAGACATCAGTTTTCTACCATAA
TTTATTTTGCAATATCTGCTCAGTGAACTTTTTGAGTTCTAAGACCTTATTCCTATGAAAACTAACACC
TCCTGGCATTTTGTTTTTTAATTCCACAGAACAATACAAAGCAAGGTATTGAGATGGAAAAAAAAAA
AAAAAAAAAAACGTGTAAACTTACTCCTTTGGGCTATAATCCTATACATATAAAAATGCATTTCACCT

ATAAATTTCAGAAAGTACAAATATATTGAAAGGGAAGCATATTAAAAACAAAATAAACTTTTCTATTT
GGGGCAGCATTTTAGAGGAGAGAGCCTGAGCCTGACATG

SYT1-F349A-EM1-B6 Heterozygous F1 animal sequence trace:

Nucleotide Alignment:

```
                    *        20         *        40         *        60         *        80         *       100         *       120         *       140         *
Syt1_WT    : CCTGTTGGGGCTCATTCCTTTTCTGAGCTAATGCATGATCTAAAACATGGCTCTGGAGGACAAGAAGGTTCAGACAATGAAAGTACCTGCCGCCAAACCTAATGACCTGAGTCTGATCCCGAGGACACACACGGGAGATGAAGAGGACAG
Syt1_F349A : CCTGTTGGGGCTCATTCCTTTTCTGAGCTAATGCATGATCTAAAACATGGCTCTGGAGGACAAGAAGGTTCAGACAATGAAAGTACCTGCCGCCAAACCTAATGACCTGAGTCTGATCCCGAGGACACACACGGGAGATGAAGAGGACAG
             CCTGTTGGGGCTCATTCCTTTTCTGAGCTAATGCATGATCTAAAACATGGCTCTGGAGGACAAGAAGGTTCAGACAATGAAAGTACCTGCCGCCAAACCTAATGACCTGAGTCTGATCCCGAGGACACACACGGGAGATGAAGAGGACAG

                    *       160         *       180         *       200         *       220         *       240         *       260         *       280         *       300
Syt1_WT    : TTCCCACGGGCTGTCCTTTGATCTCCACGCATGCATGCACTGCGGTGTACATACCACCGCACACACAGTAAACAACCCGTAATAGAATATTTCAGAGAAGATAAAACACGCGTCTGAGCTCCTAAAGTCCCTGTGTCTGTCTTTCAGATC
Syt1_F349A : TTCCCACGGGCTGTCCTTTGATCTCCACGCATGCATGCACTGCGGTGTACATACCACCGCACACACAGTAAACAACCCGTAATAGAATATTTCAGAGAAGATAAAACACGCGTCTGAGCTCCTAAAGTCCCTGTGTCTGTCTTTCAGATC
             TTCCCACGGGCTGTCCTTTGATCTCCACGCATGCATGCACTGCGGTGTACATACCACCGCACACACAGTAAACAACCCGTAATAGAATATTTCAGAGAAGATAAAACACGCGTCTGAGCTCCTAAAGTCCCTGTGTCTGTCTTTCAGATC

                    *       320         *       340         *       360         *       380         *       400         *       420         *       440         *
Syt1_WT    : CCTATGTAAAGATTCACCTGATGCAGAACGGCAAGAGACTGAAGAAGAAAAAGACAACGATTAAGAAGAACACACTTAACCCCTACTACAATGAGTCCTTCAGCTTTGAAGTTCCGTTCGAGCAAATCCAGGTAACGACAGGCACGAGTG
Syt1_F349A : CCTATGTAAAGATTCACCTGATGCAGAACGGCAAGAGACTGAAGAAGAAAAAGACAACGATTAAGAAGAACACACTTAACCCCTACTACAATGAGTCCTTCTGCTTTGAAGTTCCGGCCGAGCAAATCCAGGTAACGACAGGCACGAGTG
             CCTATGTAAAGATTCACCTGATGCAGAACGGCAAGAGACTGAAGAAGAAAAAGACAACGATTAAGAAGAACACACTTAACCCCTACTACAATGAGTCCTTC   CTTTGAAGTTCCG   CGAGCAAATCCAGGTAACGACAGGCACGAGTG

                    *       460         *       480         *       500         *       520         *       540         *       560         *       580         *       600
Syt1_WT    : TGCCCTCCCCCCCCACTCAGTCTCACGCCTTCAGATCGCATAACTGACAAGCGTAGACCCTGAGAGTCCTCAGTGCCCTGCCTTCATGAGAACTGGCCCATGCCCCTTTGTGTGATGTCTATGCTCAACTAAGCAGGACATAATAAAACA
Syt1_F349A : TGCCCTCCCCCCCCACTCAGTCTCACGCCTTCAGATCGCATAACTGACAAGCGTAGACCCTGAGAGTCCTCAGTGCCCTGCCTTCATGAGAACTGGCCCATGCCCCTTTGTGTGATGTCTATGCTCAACTAAGCAGGACATAATAAAACA
             TGCCCTCCCCCCCCACTCAGTCTCACGCCTTCAGATCGCATAACTGACAAGCGTAGACCCTGAGAGTCCTCAGTGCCCTGCCTTCATGAGAACTGGCCCATGCCCCTTTGTGTGATGTCTATGCTCAACTAAGCAGGACATAATAAAACA

                    *       620         *       640         *       660         *       680         *       700         *       720         *       740         *
Syt1_WT    : CGCAGCACAAGAAGGGCCGATTCATCAGCTGGAACAGCCAGCTGACTTCTGCTGAGCAGAAATAAGGTAGAAAATAGAAACCAACAGAACCACTTTGCTAGGTCAATAAGTTTGTCTACGGAATCCAAAACCTCAGAGAGAATGTTCGT
Syt1_F349A : CGCAGCACAAGAAGGGCCGATTCATCAGCTGGAACAGCCAGCTGACTTCTGCTGAGCAGAAATAAGGTAGAAAATAGAAACCAACAGAACCACTTTGCTAGGTCAATAAGTTTGTCTACGGAATCCAAAACCTCAGAGAGAATGTTCGT
             CGCAGCACAAGAAGGGCCGATTCATCAGCTGGAACAGCCAGCTGACTTCTGCTGAGCAGAAATAAGGTAGAAAATAGAAACCAACAGAACCACTTTGCTAGGTCAATAAGTTTGTCTACGGAATCCAAAACCTCAGAGAGAATGTTCGT

                    *       760         *       780         *       800         *       820         *       840         *       860         *       880         *       900
Syt1_WT    : TGCCAGGCTACAATAAAAAATCTACTTCTATAGGGCAAAAATAGCCAGGATCCCTCCTTCTCACGTCAGCCCATTGTTTACATCACTAGCTCATACACATCCTTACATGCTAGAAAATTATATGTATGGATTTATTTTCAACAGCTAAGT
Syt1_F349A : TGCCAGGCTACAATAAAAAATCTACTTCTATAGGGCAAAAATAGCCAGGATCCCTCCTTCTCACGTCAGCCCATTGTTTACATCACTAGCTCATACACATCCTTACATGCTAGAAAATTATATGTATGGATTTATTTTCAACAGCTAAGT
             TGCCAGGCTACAATAAAAAATCTACTTCTATAGGGCAAAAATAGCCAGGATCCCTCCTTCTCACGTCAGCCCATTGTTTACATCACTAGCTCATACACATCCTTACATGCTAGAAAATTATATGTATGGATTTATTTTCAACAGCTAAGT

                    *       920         *       940         *       960         *       980         *      1000         *      1020         *      1040         *
Syt1_WT    : AAATCACCAGTATGGTTTTCCTGTCCCTAGTCCCTTGCTCAGTGGTCTTAACTTGTGAGCACTTTAGATCTTCTGGCATATCCTTGAGACATCAGTTTTCTACCATAATTTATTTTGCAATATCTGCTCAGTGAACTTTTTGAGTTCTAA
Syt1_F349A : AAATCACCAGTATGGTTTTCCTGTCCCTAGTCCCTTGCTCAGTGGTCTTAACTTGTGAGCACTTTAGATCTTCTGGCATATCCTTGAGACATCAGTTTTCTACCATAATTTATTTTGCAATATCTGCTCAGTGAACTTTTTGAGTTCTAA
             AAATCACCAGTATGGTTTTCCTGTCCCTAGTCCCTTGCTCAGTGGTCTTAACTTGTGAGCACTTTAGATCTTCTGGCATATCCTTGAGACATCAGTTTTCTACCATAATTTATTTTGCAATATCTGCTCAGTGAACTTTTTGAGTTCTAA

                    *      1060         *      1080         *      1100         *      1120         *      1140         *      1160         *      1180         *      1200
Syt1_WT    : GACCTTATTCCTATGAAAACTAACACCTCCTGGCATTTTGTTTTTTAATTCCACAGAACAATACAAAGCAAGGTATTGAGATGGAAAAAAAAAAAAAAAAAAAACGTGTAAACTTACTCCTTTGGGCTATAATCCTATACATATAAAAA
Syt1_F349A : GACCTTATTCCTATGAAAACTAACACCTCCTGGCATTTTGTTTTTTAATTCCACAGAACAATACAAAGCAAGGTATTGAGATGGAAAAAAAAAAAAAAAAAAAACGTGTAAACTTACTCCTTTGGGCTATAATCCTATACATATAAAAA
             GACCTTATTCCTATGAAAACTAACACCTCCTGGCATTTTGTTTTTTAATTCCACAGAACAATACAAAGCAAGGTATTGAGATGGAAAAAAAAAAAAAAAAAAAACGTGTAAACTTACTCCTTTGGGCTATAATCCTATACATATAAAAA

                    *      1220         *      1240         *      1260         *      1280         *      1300         *
Syt1_WT    : TGCATTTCACCTATAAATTTCAGAAAGTACAAATATATTGAAAGGGAAGCATATTAAAAACAAAATAAACTTTTCTATTTGGGGCAGCATTTTAGAGGAGAGAGCCTGAGCCTGACATG
Syt1_F349A : TGCATTTCACCTATAAATTTCAGAAAGTACAAATATATTGAAAGGGAAGCATATTAAAAACAAAATAAACTTTTCTATTTGGGGCAGCATTTTAGAGGAGAGAGCCTGAGCCTGACATG
             TGCATTTCACCTATAAATTTCAGAAAGTACAAATATATTGAAAGGGAAGCATATTAAAAACAAAATAAACTTTTCTATTTGGGGCAGCATTTTAGAGGAGAGAGCCTGAGCCTGACATG
```

Predicted Protein Alignment:

```
                    *         20         *         40
Syt1_WT    : PYVKIHLMQNGKRLKKKKTTIKKNTLNPYYNESFSFEVPFEQIQ
Syt1_F349A : PYVKIHLMQNGKRLKKKKTTIKKNTLNPYYNESFSFEVPAEQIQ
             PYVKIHLMQNGKRLKKKKTTIKKNTLNPYYNESFSFEVP EQIQ
```

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_Syt1_F349A_F2 primer (5'-3')	CCTGTTGGGGCTCATTCCTT
Geno_Syt1_F349A_R2 primer (5'-3')	CATGTCAGGCTCAGGCTCTC
Taq Polymerase used	ThermoFisher SuperFI
Annealing Temperature (°C)	65
Elongation time (min)	0.75
WT product size (bp)	1319
Mutant product size (bp)	1319
Notes	Sequence from amplicon using primers: Syt1_F349A_F1 (5'-3': TGAAAGTACCTGCCGCCAAA) Syt1_F349A_SeqR (5'-3': TGTAGCCTGGCAACGAACAT)

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.

Copy counting by ddPCR

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	Syt1-F349A-UNI1
Forward Primer (5'-3')	CGAGCAAATCCAGGTAACGA
Reverse Primer (5'-3')	GGGTCTACGCTTGTCAGTTATG
Probe (5'-3')	CCACTCAGTCTCACGCCTTCAGAT
Label	FAM-BHQ1

This ddPCR assay is universal to both the WT allele and the mutant allele of the gene. WT controls are expected to call at 2 copies and 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 animals taken forward to establish the colony showed evidence of random donor integrations.

Syt1-F349A

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; F349A, in *Syt1*. 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

Syt1-F349A

Syt1-F349A-WT1 assay (FAM labelled)

GAGACTGAAGAAGAAAAAGACAACGATTAAGAAGAACACACTTAACCCCTACTACAATGAGTCCT**T
CagCTTTGAAGTTCCGttCG**AGC<mark>**CAAATCCAGGTAACGACAGGCACG**</mark>AGTGTGCCCCTCCCCCCCACTC
AGTCTCACGCCTTCAGATCG**CATAACTGACAAGCGTAGACCC**TGAGAGTCCTCAGTGCCCTGCCTT

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 Syt1-F349A	5' label	Sequence 5' → 3'	3' label	Oligo Type
Syt1-F349A-WT_F	n/a	**TCAGCTTTGAAGTTCCGTTCG**	n/a	Wild type Forward
Syt1-F349A-WT_PROBE	FAM	**CAAATCCAGGTAACGACAGGCACG**	ZEN/IBFQ	Wild type Probe
Syt1-F349A-WT_R	n/a	**GGGTCTACGCTTGTCAGTTATG**	n/a	Wild type Reverse

Syt1-F349A-MUT1 assay (FAM labelled)

GAGACTGAAGAAGAAAAAGACAACGATTAAGAAGAACACACTTAACCCCTACTACAATGAGTCCT**T
CtcCTTTGAAGTTCCGgc**CGAG<mark>**CAAATCCAGGTAACGACAGGCACG**</mark>AGTGTGCCCCTCCCCCCCACT
CAGTCTCACGCCTTCAGATCGC**ATAACTGACAAGCGTAGACCC**TGAGAGTCCTCAGTGCCCTGCCTT

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 Syt1-F349A	5' label	Sequence 5' → 3'	3' label	Oligo Type
Syt1-F349A-MUT_F	n/a	**TCTCCTTTGAAGTTCCGGC**	n/a	Mutant Forward
Syt1-F349A-MUT_PROBE	FAM	**CAAATCCAGGTAACGACAGGCACG**	ZEN/IBFQ	Mutant Probe
Syt1-F349A-MUT_R	n/a	**GGGTCTACGCTTGTCAGTTAT**	n/a	Mutant Reverse

Syt1-F349A

Dot1l internal control (VIC labelled)

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

Oligo Syt1-F349A	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
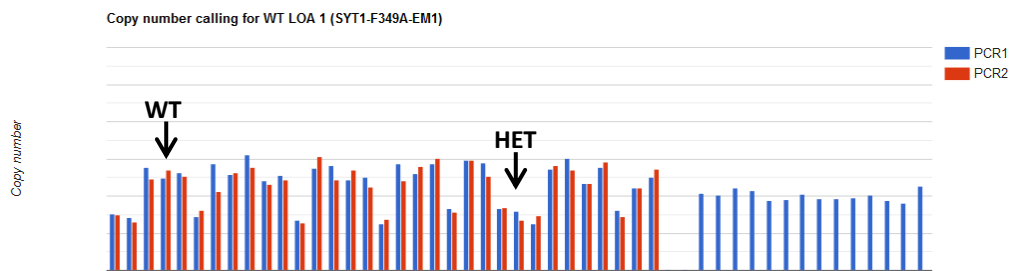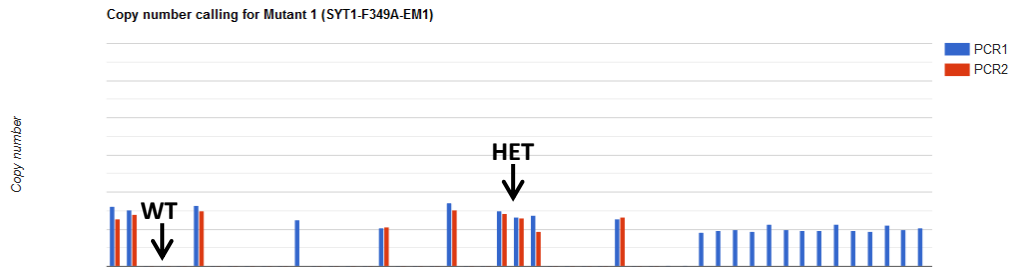
95^oC for 20 sec
Then 40 cycles of;
95^oC for 3 sec
60^oC for 30 sec

Syt1-F349A

Analysis

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

Syt1-F349A-WT1 and Syt1-F349A -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 290875 results)

Version No.   1

Date: 21/07/2020

Created/Updated by: Daniel Ford

Approved by: Rumana Zaman