Name of Mouse model or mutation:
SPTLC1-S331F-EM1-B6N
SPTLC1-S331F-EM2-B6N

Description:
Point mutation model made using CRISPR/Cas9.

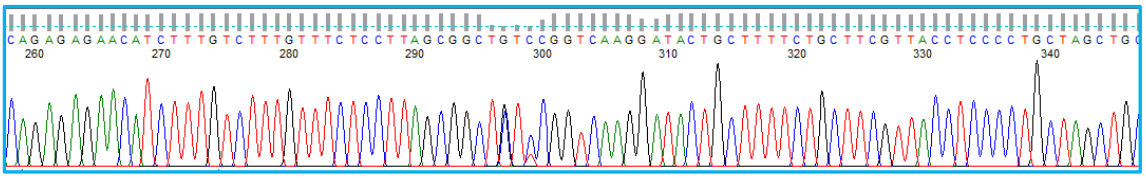
Type of mutation:
SNP: S331F

Sequence details
WT
TCGTGGTTGACCATCAGGTGATGCCTCTTAGAAATGATGCACAGTTGTAGACAGTTGTCCCCACCAC
CCCTTCAGAGCGCAGCTTCCAATATAAGATTTAACTCAGAATTTGTTTCACGGGTTTTATTTAAAAGC
CTGGGGGTGTTATTTTATTTTGTTTTGTTTTATTTTTTGTCATTGTTTTAAAGGTTTTTGTTTTGTTTATT
TATATGAATATACTATAGCTATACATACCAGAAGAAGGTATTAGATCCCATTACAGATGGTTGTGAG
CCCCCTTTTGGTTACTGGGACTTGAACTCAGGACCTCTGGAAGAGCAGTCAGTGCTCTTAACCACTG
AGCCATCTCTCCAGCTCCCTAAAAAAAGCCTGTTTTTAAAGAATAGCTCAAAGTGTTTACAGATACAT
AAAACCGCTATCTCGTGAGAAAGCATCTTGGTTTTGTTACAGCTGCACCCTGTGGTTGAGTCAGTAT
GCCTGCTAGTCAGATGGGTGCCCTACTGTGGGGCCTGGGTGCCTTCCAAAAGTCCCTCTCCACAGAG
AGAACATCTTTGTCTTTGTTTCTCCTTAGCGGCTCTCCGGTCAAGGATACTGCTTTTCTGCTTCGTTAC
CTCCCCTGCTAGCTGCTGCTGCCATTGAGGCCCTCAACATCATGGAAGAGAATCCAGGTAACAGTGC
TAGGAAAGGTGGGGACCTGGGTCAGTGCCAGGCCTCAGTGCTTTCCCAAGCATAATGGCTGCCATG
ACTTGGAGAAGTTAGCTAAGTGCGCTGAGCTTTAGTAGATGTCCTTCCTTTTCTATCTTGTTTTCCCTT
CAGCCTTTGAAGCATCAACTGATAACAATGCTAGCCTGTTCTGTCAGCAAACAACTCTGGGCAGAGG
AAAAAGTTAACTGGGAATTGCAAGTGGCCTTTGACTGTAATAGTGCAGCCTTTAAAACCAAAAAGG
GGCTGGCAAGATGGCTCGGTGGGTAAAGGTGCACACATGCAACCATGGCAGCCTGTGTTCGATCCC
CAGAACCCAGATGCAGGTGCTAGAGAACTAGCTCCATAAAATTGTCCTGTGACCCCCCACACATGCA
CAGTGTTGCATGACTCCAACCCCAAATACACTAATAATGAGTACTGTTAAATGACAGGAAAGGAGAA
GAGGCTTGCTTAGCTGGCACTCCCAAGTTCCCAGCAGAGGTGT

Mutant
TCGTGGTTGACCATCAGGTGATGCCTCTTAGAAATGATGCACAGTTGTAGACAGTTGTCCCCACCAC
CCCTTCAGAGCGCAGCTTCCAATATAAGATTTAACTCAGAATTTGTTTCACGGGTTTTATTTAAAAGC
CTGGGGGTGTTATTTTATTTTGTTTTGTTTTATTTTTTGTCATTGTTTTAAAGGTTTTTGTTTTGTTTATT
TATATGAATATACTATAGCTATACATACCAGAAGAAGGTATTAGATCCCATTACAGATGGTTGTGAG
CCCCCTTTTGGTTACTGGGACTTGAACTCAGGACCTCTGGAAGAGCAGTCAGTGCTCTTAACCACTG
AGCCATCTCTCCAGCTCCCTAAAAAAAGCCTGTTTTTAAAGAATAGCTCAAAGTGTTTACAGATACAT
AAAACCGCTATCTCGTGAGAAAGCATCTTGGTTTTGTTACAGCTGCACCCTGTGGTTGAGTCAGTAT

GCCTGCTAGTCAGATGGGTGCCCTACTGTGGGGCCTGGGTGCCTTCCAAAAGTCCCTCTCCACAGAG
AGAACATCTTTGTCTTTGTTTCTCCTTAGCGGCT**G**T**T**CGGTCAAGGATACTGCTTTTCTGCTTCGTTAC
CTCCCCTGCTAGCTGCTGCTGCCATTGAGGCCCTCAACATCATGGAAGAGAATCCAGGTAACAGTGC
TAGGAAAGGTGGGGACCTGGGTCAGTGCCAGGCCTCAGTGCTTTCCCAAGCATAATGGCTGCCATG
ACTTGGAGAAGTTAGCTAAGTGCGCTGAGCTTTAGTAGATGTCCTTCCTTTTCTATCTTGTTTTCCCTT
CAGCCTTTGAAGCATCAACTGATAACAATGCTAGCCTGTTCTGTCAGCAAACAACTCTGGGCAGAGG
AAAAAGTTAACTGGGAATTGCAAGTGGCCTTTGACTGTAATAGTGCAGCCTTTAAAACCAAAAAGG
GGCTGGCAAGATGGCTCGGTGGGTAAAGGTGCACACATGCAACCATGGCAGCCTGTGTTCGATCCC
CAGAACCCAGATGCAGGTGCTAGAGAACTAGCTCCATAAAATTGTCCTGTGACCCCCCACACATGCA
CAGTGTTGCATGACTCCAACCCCAAATACACTAATAATGAGTACTGTTAAATGACAGGAAAGGAGAA
GAGGCTTGCTTAGCTGGCACTCCCAAGTTCCCAGCAGAGGTGT

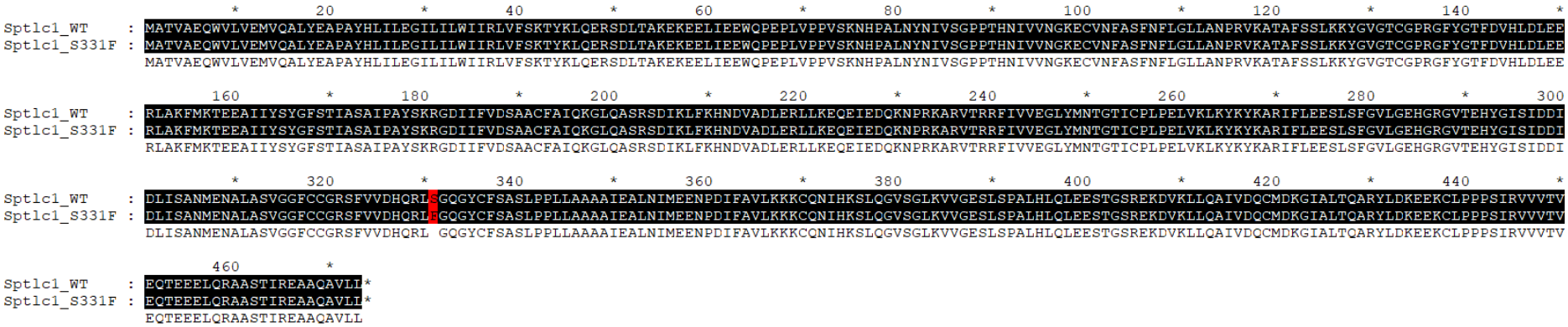
SPTLC1-S331F-EM1-B6N Heterozygous F1 animal sequence trace:



Note: SPTLC1-S331F-EM2-B6N is the same allele sequence, just derived from different founder lines.

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_Sptlc1_F1 primer (5'-3')	TCGTGGTTGACCATCAGGTG
Geno_Sptlc1_R1 primer (5'-3')	ACACCTCTGCTGGGAACTTG
Taq Polymerase used	ThermoFisher SuperFi
Annealing Temperature (°C)	63
Elongation time (min)	0.75
WT product size (bp)	1189
Mutant product size (bp)	1189
Notes	Sequenced with the following primers: Seq_Sptlc1_F1 (5'-3': ATTAGATCCCATTACAGATGGTTG) Seq_Sptlc1_R1 (5'-3': AATTCCCAGTTAACTTTTTCCTCTG)

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	Sptlc1-S331F-UNI1
Forward Primer (5'-3')	CTGCTTTTCTGCTTCGTTACCTC
Reverse Primer (5'-3')	AGCACTGTTACCTGGATTCTCTTC
Probe (5'-3')	CCTGCTAGCTGCTGCTGCCATT
Label	FAM-BHQ1

This ddPCR assay recognises 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.

Assay name	Sptlc1-S331F-MUT1
Forward Primer (5’-3’)	TTGTTTCTCCTTAGCGGCTGTT
Reverse Primer (5’-3’)	ACCTGGATTCTCTTCCATGATGTTG
Probe (5’-3’)	CGGTCAAGGATACTGCTTTTCTGCTTCG
Label	FAM-BHQ1

This ddPCR assay is unique to the mutant allele of the gene as it sits across the targeted region. WT controls are expected to call at 0 copies and 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.

Sptlc1-S331F

Allele Description

This is a CRISPR/Cas9 induced mutation creating a point mutation; S331F. 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

Sptlc1-S331F

Sptlc1-S331F-WT1 assay (FAM labelled)

AAAACCGCTATCTCGTGAGAAAGCATCTTGGTTTTGTTACAGCTG**CACCCTGTGGTTGAGTCAGTAT**
GCCTGCTAGTCAGATGGGTGCCCTACTGTGGGGCCTGGGTGCCTTCCAAAAGTCCCTCTCCACAGA
GAGAACATCTTTGTCTTTGTTTCTCCTTAGCGGC**TcTcCGGTCAAGGATACTGCTTT**TCTGCTTCGTTA

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 Sptlc1-S331F	5' label	Sequence 5' → 3'	3' label	Oligo Type
Sptlc1-S331F-WT_F	n/a	**CACCCTGTGGTTGAGTCAGTATG**	n/a	Wild type Forward
Sptlc1-S331F-WT_PROBE	FAM	**CCTGCTAGTCAGATGGGTGCCC**	BHQ	Wild type Probe
Sptlc1-S331F-WT_R	n/a	**AAAGCAGTATCCTTGACCGGAGA**	n/a	Wild type Reverse

Sptlc1-S331F-MUT1 assay (FAM labelled)

AAAACCGCTATCTCGTGAGAAAGCATCTTGGTTTTGTTACAGCTG**CACCCTGTGGTTGAGTCAGTAT**
GCCTGCTAGTCAGATGGGTGCCCTACTGTGGGGCCTGGGTGCCTTCCAAAAGTCCCTCTCCACAGA
GAGAACATCTTTGTCTTTGTTTCTCCTTAGCGG**CTgTtCGGTCAAGGATACTGCTTTTC**TGCTTCGTTA

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 Sptlc1-S331F	5' label	Sequence 5' → 3'	3' label	Oligo Type
Sptlc1-S331F-MUT_F	n/a	**CACCCTGTGGTTGAGTCAGTATG**	n/a	Mutant Forward
Sptlc1-S331F-MUT_PROBE	FAM	**CCTGCTAGTCAGATGGGTGCCC**	BHQ	Mutant Probe
Sptlc1-S331F-MUT_R	n/a	**GAAAAGCAGTATCCTTGACCGAACAG**	n/a	Mutant Reverse

Sptlc1-S331F

Dot1l internal control (VIC labelled)

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

Oligo Sptlc1-S331F	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

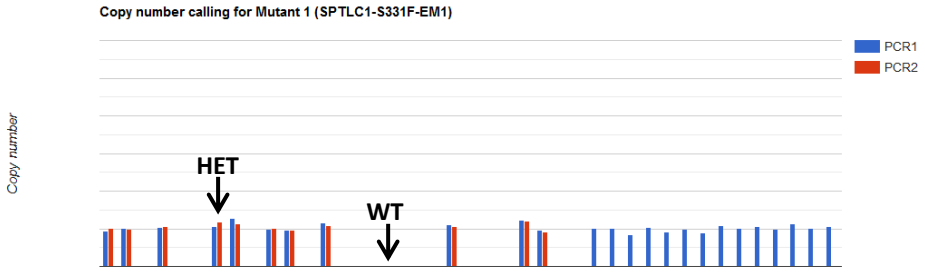
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

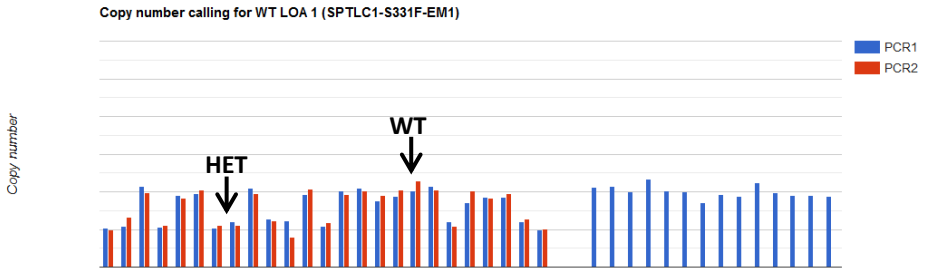
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Sptlc1-S331F-WT1 and Sptlc1-S331F -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 292834 results)





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