Name of Mouse model or mutation:
CSF1R-I792T-EM1-B6

Description:
Point mutation model made using CRISPR/Cas9.

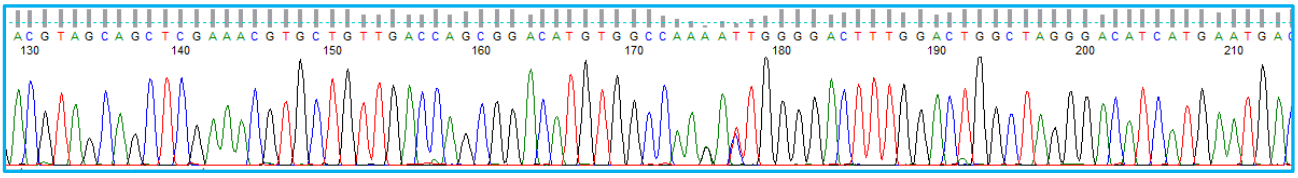
Type of mutation:
SNP: I792T

Sequence details
WT
AGGGGTCTAACGGGTTGTTGTGTATGCAAATGCTTGGGAAAGCACCTGGTATTGTGTACTTGGAGG
TGGCAGCTGTTGGTGTTGTGTATGAGGTGGGCCACAGGCTGCTCACAGCACAGGGACTCATTGCCT
CTGAGGCAGGAGCAGCCCTGTGGCTCCAAGGGCCAGAGGGCCATTGGAAACAGGGCTGTGGGGCT
AGAATTCTAAATGTTAAATTGTCCACATCAAATCGTCTCAGACCTCAGGCCTGCACAGGTTTAGACAG
GAGTAGACAGTAGACTACCAAAACCTGCATCTACTTCAACAGAGACCCAAGACCTCCTGCTCCTCCTC
TGGTCCTCAGGCCTCAGGGAAGGATAAACTGACTAATAATCTCTCTGCGCTTTCTTCAGTGCATCCAC
CGGGACGTAGCAGCTCGAAACGTGCTGTTGACCAGCGGACATGTGGCCAAGATTGGGGACTTTGG
ACTGGCTAGGGACATCATGAATGACTCCAACTATGTTGTCAAGGGCAATGTGAGTGCCGAGAGAGA
GAGAGAGAGAGGGAGGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
GAGAGAGAGAGAGAGAGAGAGATTGAGATTGGTTGGGCAGGCTGTGGAGAGCCCTTGACTGACAT
GGTACTGTCTTGTCAGGCCCGCCTGCCTGTAAAGTGGATGGCCCCAGAGAGCATCTTTGACTGCGTC
TACACAGTTCAGAGTGATGTGTGGTCCTACGGCATCCTCCTCTGGGAGATCTTCTCGCTTGGTGAGC
TGCTAAGCCTGTTTTCAGGTGCAGCCTAGGGCTGACCCGTCTTTGGCTATGCCATCGGTGTCTTGAAC
CTCATGGACGAAATCTACTCAAAGATGTCGGTGTCCAAGACAGAGCAAGGGCTGGCAGGAGCAATG
GGATGGTGAGGGCTCTTGAACCAG

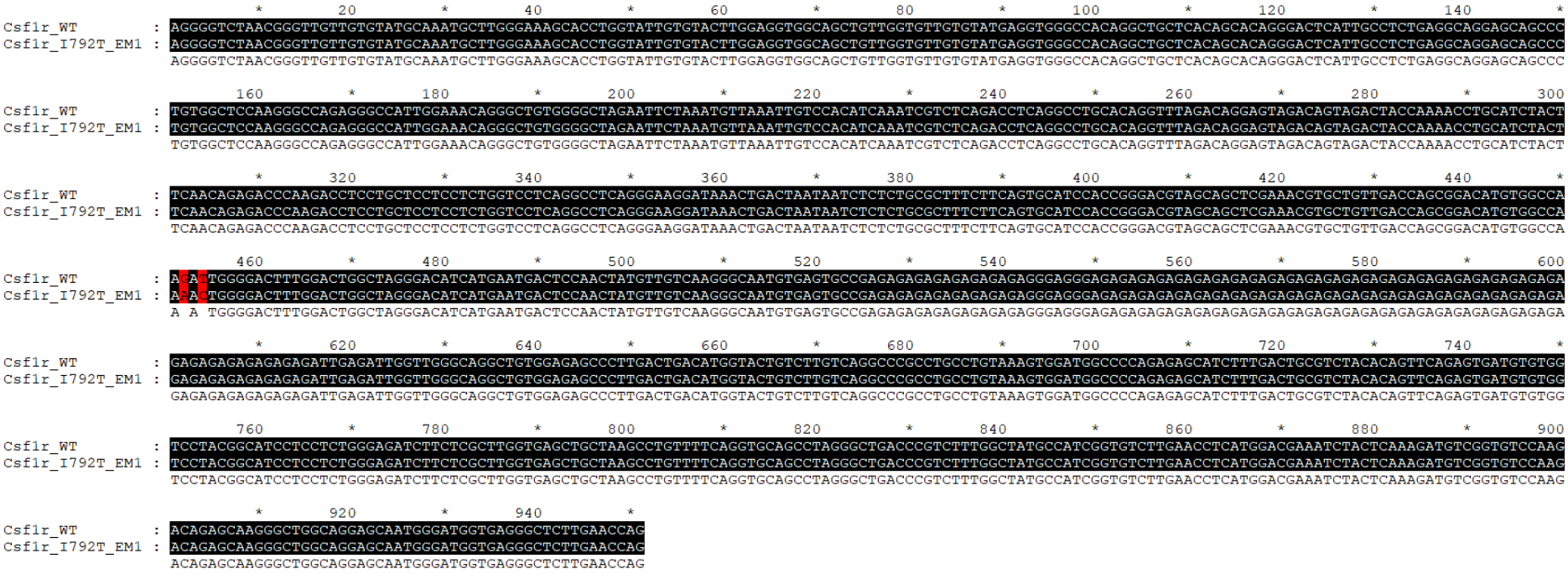
Mutant
AGGGGTCTAACGGGTTGTTGTGTATGCAAATGCTTGGGAAAGCACCTGGTATTGTGTACTTGGAGG
TGGCAGCTGTTGGTGTTGTGTATGAGGTGGGCCACAGGCTGCTCACAGCACAGGGACTCATTGCCT
CTGAGGCAGGAGCAGCCCTGTGGCTCCAAGGGCCAGAGGGCCATTGGAAACAGGGCTGTGGGGCT
AGAATTCTAAATGTTAAATTGTCCACATCAAATCGTCTCAGACCTCAGGCCTGCACAGGTTTAGACAG
GAGTAGACAGTAGACTACCAAAACCTGCATCTACTTCAACAGAGACCCAAGACCTCCTGCTCCTCCTC
TGGTCCTCAGGCCTCAGGGAAGGATAAACTGACTAATAATCTCTCTGCGCTTTCTTCAGTGCATCCAC
CGGGACGTAGCAGCTCGAAACGTGCTGTTGACCAGCGGACATGTGGCCAA**A**A**C**TGGGGACTTTGG
ACTGGCTAGGGACATCATGAATGACTCCAACTATGTTGTCAAGGGCAATGTGAGTGCCGAGAGAGA
GAGAGAGAGAGGGAGGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
GAGAGAGAGAGAGAGAGAGAGATTGAGATTGGTTGGGCAGGCTGTGGAGAGCCCTTGACTGACAT

GGTACTGTCTTGTCAGGCCCGCCTGCCTGTAAAGTGGATGGCCCCAGAGAGCATCTTTGACTGCGTC
TACACAGTTCAGAGTGATGTGTGGTCCTACGGCATCCTCCTCTGGGAGATCTTCTCGCTTGGTGAGC
TGCTAAGCCTGTTTTCAGGTGCAGCCTAGGGCTGACCCGTCTTTGGCTATGCCATCGGTGTCTTGAAC
CTCATGGACGAAATCTACTCAAAGATGTCGGTGTCCAAGACAGAGCAAGGGCTGGCAGGAGCAATG
GGATGGTGAGGGCTCTTGAACCAG

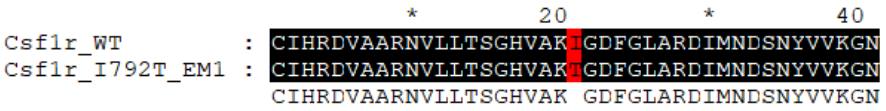
CSF1R-I792T-EM1-B6 Heterozygous F1 animal sequence trace:



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_Csf1r_I792T_F2 (5'-3')	AGGGGTCTAACGGGTTGTTG
Geno_Csf1r_I792T_R2 (5'-3')	CTGGTTCAAGAGCCCTCACC
Taq Polymerase used	ThermoFisher SuperFi Taq
Annealing Temperature (°C)	64
Elongation time (min)	0.5
WT product size (bp)	951
Mutant product size (bp)	951
Notes	Sequence with primers Geno_Csf1r_I792T_F1 (5'-3'; TCAGGCCTGCACAGGTTTAG) and Geno_Csf1r_I792T_R3 (5'-3'; CACTCACATTGCCCTTGACAACATA)

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	CSF1R-I792T-UNI1
Forward Primer (5'-3')	GCGCTTTCTTCAGTGCATCC
Reverse Primer (5'-3')	TCCCTAGCCAGTCCAAAGTC
Probe (5'-3')	CCGGGACGTAGCAGCTCGAAAC
Label	FAM-BHQ1

The ddPCR assay recognises both the WT and the mutant allele. 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	CSF1R-I792T-MUT1
Forward Primer (5’-3’)	AGCGGACATGTGGCCAAAAC
Reverse Primer (5’-3’)	GCCCTTGACAACATAGTTGGA
Probe (5’-3’)	TTTGGACTGGCTAGGGACATCATGA
Label	FAM-BHQ1

The ddPCR assay is specific to the mutant I792T allele of the gene. 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.

CSF1R-I792T

Allele Description

This is a CRISPR/Cas9 induced mutation creating a point mutations; I792T in CSF1R. 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

CSF1R-I792T

CSF1R-I792T-WT1 assay (FAM labelled)

TGGTCCTCAGGCCTCAGGGAAGGATAAACTGACTAATAATCTCTCT**GCGCTTTCTTCAGTGCATCC**A**C**
CGGGACGTAGCAGCTCGAAACGTGCTGTTGACCAGCGGACATGTGGCCAA**gAtTGGGGACTTTGG**
ACTGGCTAGGGACATCATGAATGACTCCAACTATGTTGTCAAGGGCAATGTGAGTGCCGAGAGAGA

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 CSF1R-I792T	5' label	Sequence 5' → 3'	3' label	Oligo Type
CSF1R-I792T-UNI_F	n/a	**GCGCTTTCTTCAGTGCATCC**	n/a	Universal Forward
CSF1R-I792T-UNI_PROBE	FAM	**CCGGGACGTAGCAGCTCGAAAC**	ZEN/IBFQ	Universal Probe
CSF1R-I792T-WT_R	n/a	**CCAGTCCAAAGTCCCCAATC**	n/a	Wild type Reverse

CSF1R-I792T-MUT1 assay (FAM labelled)

TGGTCCTCAGGCCTCAGGGAAGGATAAACTGACTAATAATCTCTCT**GCGCTTTCTTCAGTGCATCC**A**C**
CGGGACGTAGCAGCTCGAAACGTGCTGTTGACCAGCGGACATGTGGCCAA**aAcTGGGGACTTTGG**
ACTGGCTAGGGACATCATGAATGACTCCAACTATGTTGTCAAGGGCAATGTGAGTGCCGAGAGAGA

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 CSF1R-I792T	5' label	Sequence 5' → 3'	3' label	Oligo Type
CSF1R-I792T-UNI_F	n/a	**GCGCTTTCTTCAGTGCATCC**	n/a	Universal Forward
CSF1R-I792T-UNI_PROBE	FAM	**CCGGGACGTAGCAGCTCGAAAC**	BHQ	Universal Probe
CSF1R-I792T-MUT_R	n/a	**CCAGTCCAAAGTCCCCAGTT**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo CSF1R-I792T	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

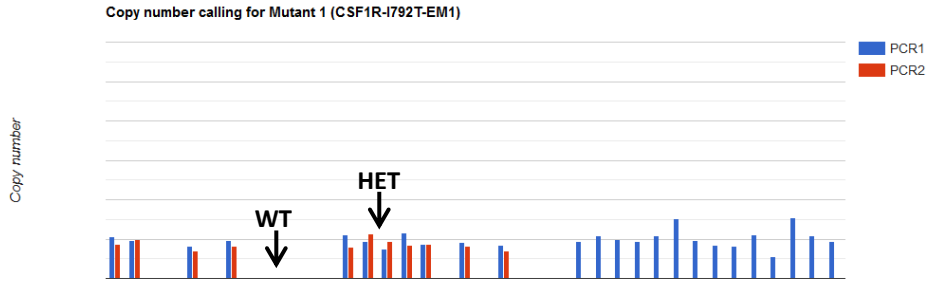
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

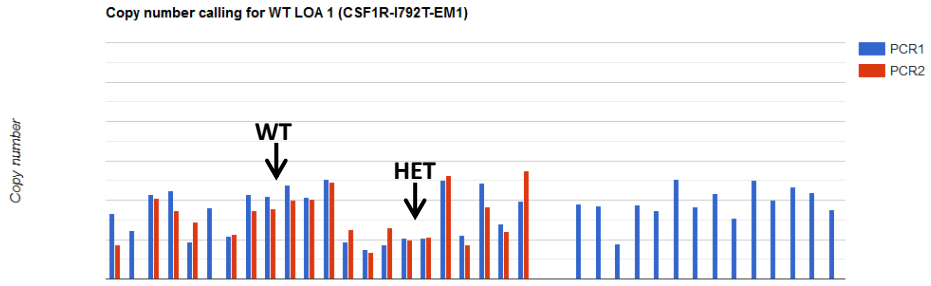
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

CSF1R-I792T-WT1 and CSF1R-I792T -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 278187 results)





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