Name of Mouse model or mutation:
CX3CL1-FLOX-EM1-B6N

Description:
Floxed mutant made using CRISPR/Cas9.

Type of mutation:
Floxed exon ENSEMBL:ENSMUSE00001312139 of Cx3cl1 gene.

Sequence details
Cx3cl1 WT
ACAACGCAGGCCGATAGAAACAGAATGCAAAAATGTTTATATCAAATAACTAGGTTTATCCACTCAC
AGAAATCCCTGCCCAGTGGCTTCTGCTACGTAGCTATCTGCATGTCCTTGCCCCCTACTTTGCCCCCCA
GCTTTTCTCCTGAACTCCTTATCCCAAACTCATTCTGCAGAACACAGGGGGATCTGCTCAGCAGACAC
TCACACACTTCCCAGGGAGGTAAGCTCCCCTATTCAACACAGACCCTTACCTGATCTATGCACCTGAT
CACATGTGTGTGGAGGAGGAGGATGCATGTGTAGGGTGATTGCTGAGCCCTTGACTTAATTGAGCA
CAAGACAGGGATGAGCAAGGAAGGCAATGCCGTAGGGGTGGAACAGGGCTGGAAGAGCGGGACT
GCACCATCATATCTCCCTAGATTCCTCTCCCCGCTATGACTTCATTTTAAAAATTAAACGTTTCCAGGC
CAGGAGTTGTGGTCCGCATCTTTAATCCCAGCACTTGGGAGGCAGTGGCAAGCAGATCTTGATGAG
CTCAAAGCCAGTCTGATTGTCTTAATGAGTACCAGGATAGCCAGAGCTACCTAGAGACCTTGTCTCA
AAATAACAACAACAACAACACAACAAAATGAAATGATTCCCAGGTATCAGGCAAAATGAGATTTATA
AATAAAGGCTAATTTTTTATCAGTCCAAAACAACCCCAGAGGCTGAGACTCTTGCTGTCTGGCAGGT
TATCACGGGTTGGGTTAGATGTGGATTCTGGCTTGGGAGTATAGCCTCGAGGAGCTGAAATGGGTA
TGGGGCACCAGATGCCCAGGGGTCCTACTGACTGCTGGCCTCTGTGGATCCAGGTCAGCACCTCGG
CATGACGAAATGCGAAATCATGTGCGACAAGATGACCTCACGAATCCCAGTGGCTTTGCTCATCCGC
TATCAGCTAAACCAGGAGTCCTGCGGCAAGCGTGCCATTGTGTAGGTATCCCTTTCAACCCCCAGAG
GCCCTAGGACATTCCAGCCCTCCCTCTCCCCCTCCTCACCCTGGGCTTCCTGCCAAAGGCCTTAGTAA
GTGTGGGCCTTGTTTCAGGCCCACAGGCTATCTCCTCGCTGACCTTAAGATCAGTGGTAAACAACAG
AGGTGGGAAATGGTTTCCTGGATGGCTCACCGTGGTTCAGGGCAGCACCCAGGATGGAGCCTCCTT
TCTTCAGCCCACTAGATCCCATGGGGGAGGTTGAGGGAAGTGGCCTACTAGAGCAGGATCCAGACT
CCAGCAGGAGATGGGTAGCAGCAGGGTGAGAGGAGGTGGAGGAGCTAAAATGATTGGCTAACCA
CAAGGATGGTCATCACCAAGTGACAGAAGAGCAGGGCCCTGGGAGCCTCTGGGGAGGAAACAGGC
TCAGTCAGTCATTTTCTGCCTGGCCAGTTTGGTCCCTTGTTCCTTCTGGGGCACATGGAGCGTACTGC
CTGTTTGCTCAGTCCCGAGT

Mutant CX3CL1-FLOX-EM1-B6N

ACAACGCAGGCCGATAGAAACAGAATGCAAAAATGTTTATATCAAATAACTAGGTTTATCCACTCAC
AGAAATCCCTGCCCAGTGGCTTCTGCTACGTAGCTATCTGCATGTCCTTGCCCCCTACTTTGCCCCCCA
GCTTTTCTCCTGAACTCCTTATCCCAAACTCATTCTGCAGAACACAGGGGGATCTGCTCAGCAGACAC
TCACACACTTCCCAGGGAGGTAAGCTCCCCTATTCAACACAGACCCTTACCTGATCTATGCACCTGAT
CACATGTGTGTGGAGGAGGAGGATGCATGTGTAGGGTGATTGCTGAGCCCTTGACTTAATTGAGCA
CAAGACAGGGATGAGCAAGGAAGGCAATGCCGTAGGGGTATCCGGGGGTACCGCGTCGAGGCGA
TCGCATAACTTCGTATAGCATACATTATACGAAGTTATAATTAAACGTTTCCAGGCCAGGAGTTGTG
GTCCGCATCTTTAATCCCAGCACTTGGGAGGCAGTGGCAAGCAGATCTTGATGAGCTCAAAGCCAGT
CTGATTGTCTTAATGAGTACCAGGATAGCCAGAGCTACCTAGAGACCTTGTCTCAAAATAACAACAA
CAACAACACAACAAAATGAAATGATTCCCAGGTATCAGGCAAAATGAGATTTATAAATAAAGGCTAA
TTTTTTATCAGTCCAAAACAACCCCAGAGGCTGAGACTCTTGCTGTCTGGCAGGTTATCACGGGTTG
GGTTAGATGTGGATTCTGGCTTGGGAGTATAGCCTCGAGGAGCTGAAATGGGTATGGGGCACCAG
ATGCCCAGGGGTCCTACTGACTGCTGGCCTCTGTGGATCCAGGTCAGCACCTCGGCATGACGAAAT
GCGAAATCATGTGCGACAAGATGACCTCACGAATCCCAGTGGCTTTGCTCATCCGCTATCAGCTAAA
CCAGGAGTCCTGCGGCAAGCGTGCCATTGTGTAGGTATCCCTTTCAACCCCCAGAGGCCCTAGGACA
TTCCAGCCCTCCCTCTCCCCCTCCTCACCCTGGGCTTCCTGCCAAAGGCCTTAGTAAGTGTGGGCCTT
GTTTCAGGCCCACAGGCTATCTCCTCGCTGACCTTAAGATCAGTGGTAAACAACAGAGGTGGGAAAT
GGTTTCCTGGATGATAACTTCGTATAGCATACATTATACGAAGTTATCGCCGGCGGGTCTGAGCTCG
CCATCAGTTAGATCCCATGGGGGAGGTTGAGGGAAGTGGCCTACTAGAGCAGGATCCAGACTCCAG
CAGGAGATGGGTAGCAGCAGGGTGAGAGGAGGTGGAGGAGCTAAAATGATTGGCTAACCACAAG
GATGGTCATCACCAAGTGACAGAAGAGCAGGGCCCTGGGAGCCTCTGGGGAGGAAACAGGCTCAG
TCAGTCATTTTCTGCCTGGCCAGTTTGGTCCCTTGTTCCTTCTGGGGCACATGGAGCGTACTGCCTGT
TTGCTCAGTCCCGAGT

Nucleotide Alignment: Orange is ENSMUSE00001312139, Red is LoxP sites, Yellow are universal primer and RE sites

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:

Universal PCR:

Geno_Cx3cl1 _F1	ACAACGCAGGCCGATAGAAA
Geno_Cx3cl1 _R1	ACTCGGGACTGAGCAAACAG
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	58
Elongation time (min)	1
WT product size (bp)	1488
Mutant product size (bp)	1483
Notes	Sequence with internal primers Geno_Cx3cl1 _F2 (GAACACAGGGGGATCTGCTC) and Geno_Cx3cl1 _R3 (ACTGACTGAGCCTGTTTCCTC)

LoxP PCR:

LoxPF	ATCCGGGGGTACCGCGTCGAG
LoxPR	ACTGATGGCGAGCTCAGACC
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	61
Elongation time (min)	3
Mutant product size (bp)	835
Notes	This is a generic LoxP PCR hence longer extension time than required for amplicon size.

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 Y chromosome.

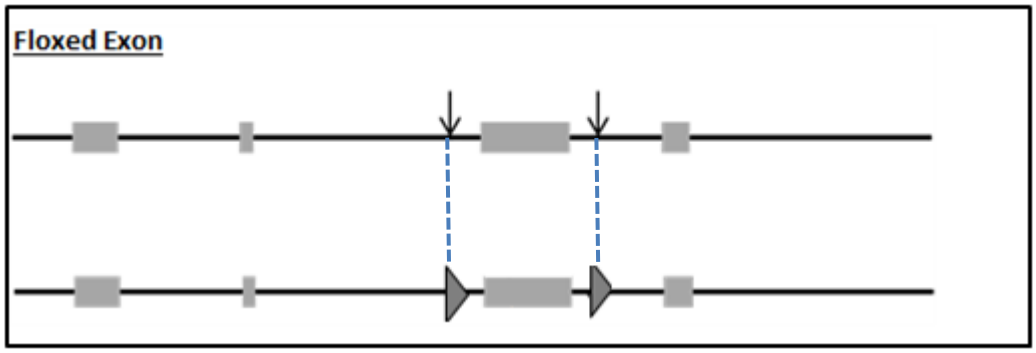
Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to detect the donor sequence:

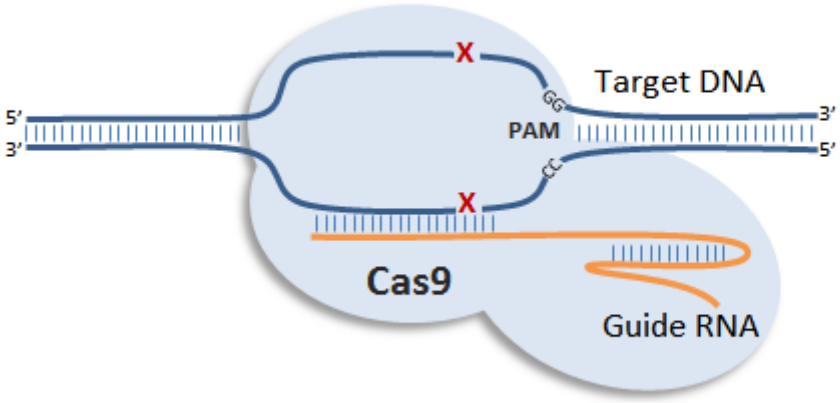
Assay name	Cx3cl1-CR-LOA-WT1
Forward Primer	CGGCATGACGAAATGCGAAATC
Reverse Primer	GCAGGACTCCTGGTTTAGCT
Probe	TGTGCGACAAGATGACCTCACGA
Label	FAM-BHQ1

The reference assay for the copy counting assay is a VIC-labelled assay that detects Dot1l on Chr10.

Cx3cl1-FLOX Genotyping Strategy

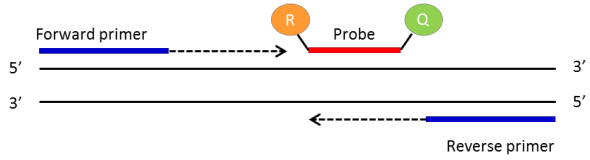
Animals have been engineered using the CRISPR/Cas9 technology. Most of the knockout alleles generated through this method will be obtained by deletion of a critical exon or by introduction of indel (insertion/deletion) within the coding sequence of a critical exon. There is also a possibility of inserting sequences like LoxP sites around critical exon to create floxed allele (see picture below).



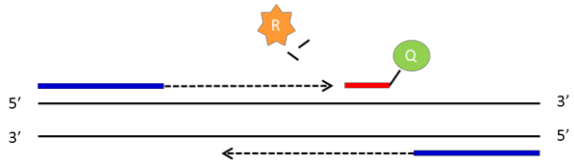


qPCR genotyping strategy

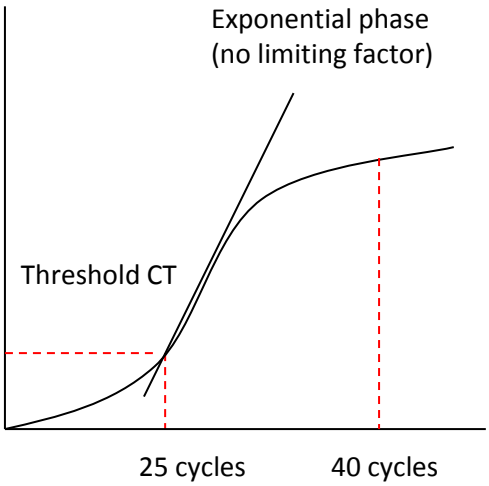
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that is complimentary to part of the DNA sequence between the forward and reverse primers. At the 5' end of the probe there is a fluorescent reporter (R) and at the 3' end a quencher (Q). Whilst they are in close contact with each other there is no fluorescent signal.



As the forward primer is extended the reporter is cleaved from the probe resulting in a fluorescent signal being detected. Once the primer extends enough to release the quencher this signal is blocked. By using probes with different fluorescent signals multiple PCR assays can be multiplexed and run together.



PCR reaction plot



The number of cycles the PCR takes to reach a set threshold is known as the CT value. This is inversely correlated to the amount of template DNA in the sample.

e.g. CT 25 = 2 x template DNA
CT 26 = 1 x template DNA
CT above 30 = no template represented in the sample

CT value can be used to determine how many copies of a particular allele samples have.

All our qPCR are run in duplicate. A FAM labelled genotyping assay is run in multiplex with a VIC labelled internal control Dot1l.

Cx3cl1-FLOX Genotyping Strategy

Samples are genotyped with a WT loss of allele (WT-LOA) assay. This is a FAM labelled assay that is designed to detect the critical exon that has been floxed. If the animal contains the modified allele the copy number of this assay should drop by 1. For autosomal genes that have been targeted this means the following

WT= 2 copies of the LOA assay
HET = 1 copy of the LOA assay
HOM = 0 copies of the LOA assay

Cx3cl1-FLOX-WT1 assay (FAM labelled probe)

Cx3cl1 WT
Sequence highlighted in blue letters is the BP-LOA (break point loss of allele) where the two loxp sites have been inserted

TGGAGGAGGAGGATGCATGTGTAGGGTGATTGCTGAGCCCTTGACTTAATTGAGCACAAGACAGGGATGAGCAAGGAAGGCAA
TGCCGTAGGGGTGGAACAGGGCTGGAAGAGCGGGACTGCACCATCATATCTCCCTAGATTCCTCTCCCCGCTATGACTTCATT
TTAAAAATTAAACGTTTCCAGGCCAGGAGTTGTGGTCCGCATCTTTAATCCCAGCACTTGGGAGGCAGTGGCAAGCAGATCTT
GATGAGCTCAAAGCCAGTCTGATTGTCTTAATGAGTACCAGGATAGCCAGAGCTACCTAGAGACCTTGTCTCAAAATAACAAC
AACAACAACACAACAAAATGAAATGATTCCCAGGTATCAGGCAAAATGAGATTTATAAATAAAGGCTAATTTTTTATCAGTCC
AAAACAACCCCAGAGGCTGAGACTCTTGCTGTCTGGCAGGTTATCACGGGTTGGGTTAGATGTGGATTCTGGCTTGGGAGTAT
AGCCTCGAGGAGCTGAAATGGGTATGGGGCACCAGATGCCCAGGGGTCCTACTGACTGCTGGCCTCTGTGGATCCAGGTCAGC
ACCTCGGCATGACGAAATGCGAAATCATGTGCGACAAGATGACCTCACGAATCCCAGTGGCTTTGCTCATCCGCTATCAGCTA
AACCAGGAGTCCTGCGGCAAGCGTGCCATTGTGTAGGTATCCCTTTCAACCCCCAGAGGCCCTAGGACATTCCAGCCCTCCCT
CTCCCCCTCCTCACCCTGGGCTTCCTGCCAAAGGCCTTAGTAAGTGTGGGCCTTGTTTCAGGCCCACAGGCTATCTCCTCGCT
GACCTTAAGATCAGTGGTAAACAACAGAGGTGGGAAATGGTTTCCTGGATGGCTCACCGTGGTTCAGGGCAGCACCCAGGATG
GAGCCTCCTTTCTTCAGCCCACTAGATCCCATGGGGGAGGTTGAGGGAAGTGGCCTACTAGAGCAGGATCCAGACTCCAGCAG

Cx3cl1-FLOX-EM1-B6N
Sequence highlighted in RED letters is the mutant sequence replaced by BP-LOA from above

TGGAGGAGGAGGATGCATGTGTAGGGTGATTGCTGAGCCCTTGACTTAATTGAGCACAAGACAGGGATGAGCAAGGAAGGCAA
TGCCGTAGGGGTatccgggggtaccgcgtcgaggcgatcgcataacttcgtatagcatacattatacgaagttatAATTAAAC
GTTTCCAGGCCAGGAGTTGTGGTCCGCATCTTTAATCCCAGCACTTGGGAGGCAGTGGCAAGCAGATCTTGATGAGCTCAAAG
CCAGTCTGATTGTCTTAATGAGTACCAGGATAGCCAGAGCTACCTAGAGACCTTGTCTCAAAATAACAACAACAACAACACAA
CAAAATGAAATGATTCCCAGGTATCAGGCAAAATGAGATTTATAAATAAAGGCTAATTTTTTATCAGTCCAAAACAACCCCAG
AGGCTGAGACTCTTGCTGTCTGGCAGGTTATCACGGGTTGGGTTAGATGTGGATTCTGGCTTGGGAGTATAGCCTCGAGGAGC
TGAAATGGGTATGGGGCACCAGATGCCCAGGGGTCCTACTGACTGCTGGCCTCTGTGGATCCAGGTCAGCACCTCGGCATGAC
GAAATGCGAAATCATGTGCGACAAGATGACCTCACGAATCCCAGTGGCTTTGCTCATCCGCTATCAGCTAAACCAGGAGTCCT
GCGGCAAGCGTGCCATTGTGTAGGTATCCCTTTCAACCCCCAGAGGCCCTAGGACATTCCAGCCCTCCCTCTCCCCCTCCTCA
CCCTGGGCTTCCTGCCAAAGGCCTTAGTAAGTGTGGGCCTTGTTTCAGGCCCACAGGCTATCTCCTCGCTGACCTTAAGATCA
GTGGTAAACAACAGAGGTGGGAAATGGTTTCCTGGATGataacttcgtatagcatacattatacgaagttatcgccggcgggt
ctgagctcgccatcagtTAGATCCCATGGGGGAGGTTGAGGGAAGTGGCCTACTAGAGCAGGATCCAGACTCCAGCAG

Cx3cl1-FLOX-WT1 primers and probe

Primer 1 = AAACAACAGAGGTGGGAAATGGT Primer 2 = GGGATCTAGTGGGCTGAAGAAAG
Probe = ACCGTGGTTCAGGGCAGCACC

Dot1l internal control (VIC labelled)

TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTCGTTTTCTG
TTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCTGGGGCAAGGCTTTATT
TCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG

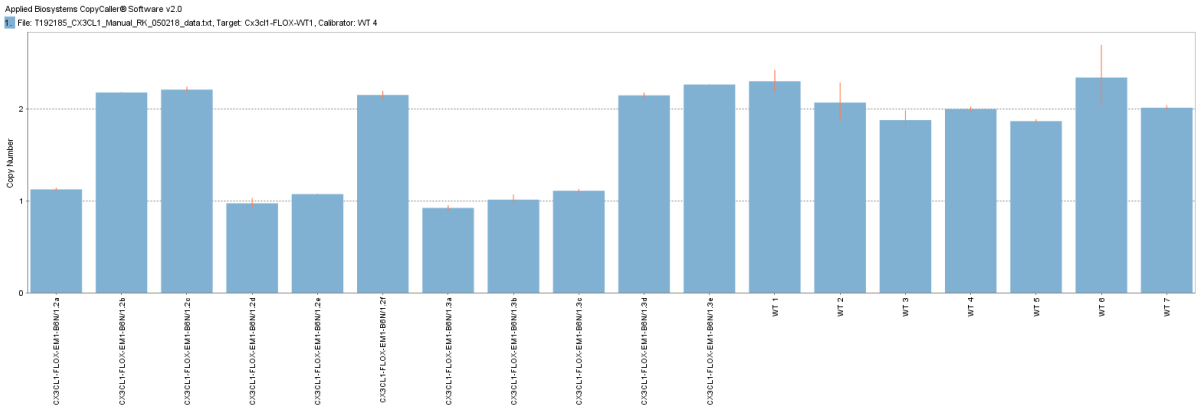
Primer 1 = GCCCCAGCACGACCATT Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

qPCR master mix

ABI GTX Taqman master mix	5µl
Primers Dot1L_2F (20µM)	0.225µl
Primers Dot1L_R (20µM)	0.225µl
Probe DotL_2M (5µM)	0.2µl
FAM Assay (probe 5µM & primers 15µM each)	0.3µl
ddH20	1.55µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

Cx3cl1-FLOX LOA copy called result, image showing both replicates and controls

Task 192185 Results



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