Name of Mouse model or mutation:
RLIM-R575C-EM1-B6

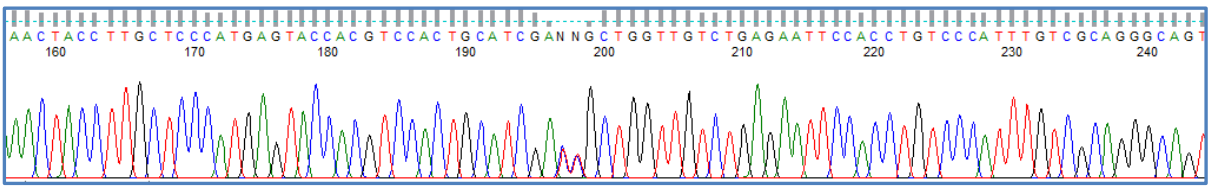
Description:
Point mutation model made using CRISPR/Cas9.

Type of mutation:
SNP: R575C

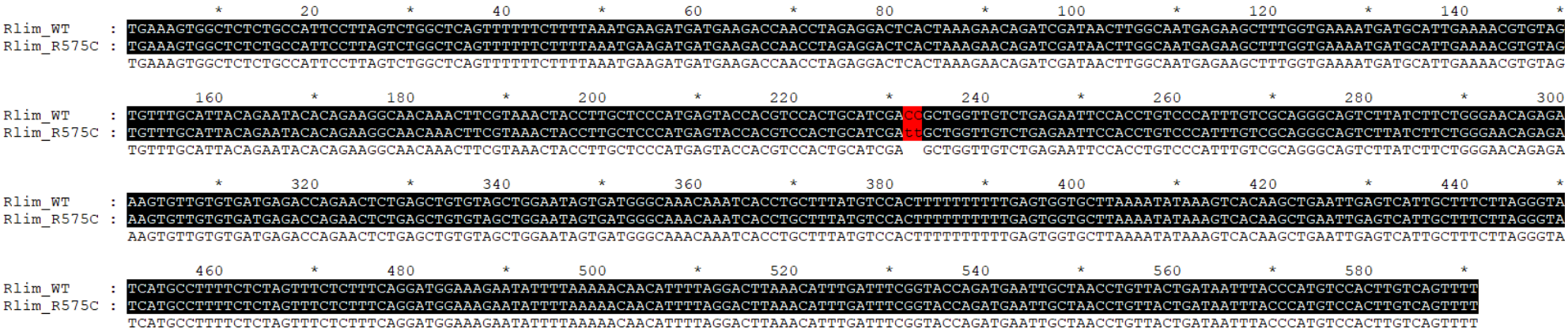
Sequence details
WT
TGAAAGTGGCTCTCTGCCATTCCTTAGTCTGGCTCAGTTTTTTCTTTTAAATGAAGATGATGAAGACC
AACCTAGAGGACTCACTAAAGAACAGATCGATAACTTGGCAATGAGAAGCTTTGGTGAAAATGATG
CATTGAAAACGTGTAGTGTTTGCATTACAGAATACACAGAAGGCAACAAACTTCGTAAACTACCTTG
CTCCCATGAGTACCACGTCCACTGCATCGACCGCTGGTTGTCTGAGAATTCCACCTGTCCCATTTGTC
GCAGGGCAGTCTTATCTTCTGGGAACAGAGAAAGTGTTGTGTGATGAGACCAGAACTCTGAGCTGT
GTAGCTGGAATAGTGATGGGCAAACAAATCACCTGCTTTATGTCCACTTTTTTTTTTGAGTGGTGCTT
AAAATATAAAGTCACAAGCTGAATTGAGTCATTGCTTTCTTAGGGTATCATGCCTTTTCTCTAGTTTCT
CTTTCAGGATGGAAAGAATATTTTAAAAACAACATTTTAGGACTTAAACATTTGATTTCGGTACCAGA
TGAATTGCTAACCTGTTACTGATAATTTACCCATGTCCACTTGTCAGTTTT

Mutant
TGAAAGTGGCTCTCTGCCATTCCTTAGTCTGGCTCAGTTTTTTCTTTTAAATGAAGATGATGAAGACC
AACCTAGAGGACTCACTAAAGAACAGATCGATAACTTGGCAATGAGAAGCTTTGGTGAAAATGATG
CATTGAAAACGTGTAGTGTTTGCATTACAGAATACACAGAAGGCAACAAACTTCGTAAACTACCTTG
CTCCCATGAGTACCACGTCCACTGCATCGAttGCTGGTTGTCTGAGAATTCCACCTGTCCCATTTGTCG
CAGGGCAGTCTTATCTTCTGGGAACAGAGAAAGTGTTGTGTGATGAGACCAGAACTCTGAGCTGTG
TAGCTGGAATAGTGATGGGCAAACAAATCACCTGCTTTATGTCCACTTTTTTTTTTGAGTGGTGCTTA
AAATATAAAGTCACAAGCTGAATTGAGTCATTGCTTTCTTAGGGTATCATGCCTTTTCTCTAGTTTCTC
TTTCAGGATGGAAAGAATATTTTAAAAACAACATTTTAGGACTTAAACATTTGATTTCGGTACCAGAT
GAATTGCTAACCTGTTACTGATAATTTACCCATGTCCACTTGTCAGTTTT

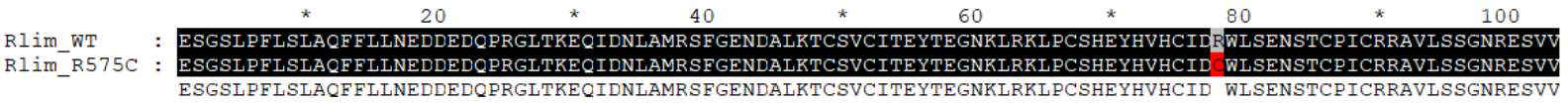
RLIM-R575C-EM1-B6 Heterozygous F1 animal sequence trace:



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_Rlim_F1 (5'-3')	TGAAAGTGGCTCTCTGCCAT
Geno_Rlim_R1 (5'-3')	TGAAAGTGGCTCTCTGCCAT
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	62
Elongation time (min)	1
WT product size (bp)	591
Mutant product size (bp)	591
Notes	

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 (highlighted in red below) for guide 3 used were checked with the following primers:

Off-target site	Sequence (protospacer plus PAM)	Type	Primers used (5'-3')
2:48191892-48191914	TGGAATTCTAAGAAAACCAG AGG	Intergenic	Geno_Rlim_OT1F1: GGCTCAGTGGGTATCTCTAGT Geno_Rlim_OT1R1: GGGCTTATCAATAATGGCTGGTACA
7:43476917-43476939	TGGAAATCTCAGACATCCAG CGG	Exonic	Geno_Rlim_OT2F1: ATCCTAGGCCCCTTTCCACT Geno_Rlim_OT2R1 ACAGAGGCCAGAGCACATTC

All amplicons were sent for Sanger sequencing to check for cutting at predicted off-target sites. No evidence of off-target activity was detected.

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	Rlim-R575C-UNIV1
Forward Primer	GGCAACAAACTTCGTAAACTACCT
Reverse Primer	GACTGCCCTGCGACAAATG
Probe	TGCTCCCATGAGTACCACGTCC
Label	FAM-BHQ1
Notes	The ddPCR assay is universal to the Rlim gene. WT female animals or correct female mutants with a single integration for a correct mutation are expected to call at 2 copies for F1 (HET) animals. Females with more than one donor integration are expected to call at 2+ copies. WT male animals or correct male mutants with a single integration for a correct mutation are expected to call at 1 copy for F1 animals. Males with more than one donor integration are expected to call at 2+ copies.

Reference Assay Name	Dot1l
Forward primer	GCCCCAGCACGACCATT
Reverse primer	TAGTTGGCATCCTTATGCTTCATC
Probe	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC