Name of Mouse model or mutation:
Khdrbs1-REGDEL-EM1-B6N

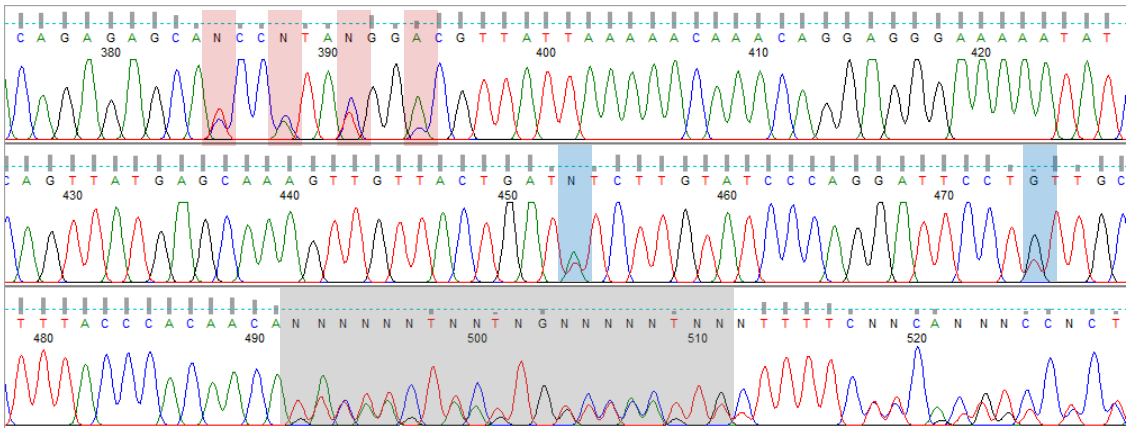
Description:
Deletion of regulatory element made using CRISPR/Cas9.

Type of mutation:
20 nt deletion in 3' UTR of Khdrbs1.

Sequence details
WT
Khdrbs1 WT:
AGCTGACTGGTGACGTTGGGAACAGGAATAGCCATTGATTGCAGACTCACTGGCTCCTTTACTGCTTTCTTCG
AGCAATGTGTTGTATTTTTCCAGGTTTTGGGACTGCGTGCCAATCATGTTGCTTATGGAGAATGTTGTTTCTCA
AGGGAACTGTACAGTAATGTACGGCTAGCAGTTTGACTTCTCATTACTCACCGTGTAGGCGGTTACCCCTATTG
ATCAAGGAGGCCAAGTAAAGAGGACAGCAAGGCACGCTTGGGGTGTGACGTGGTTCTAGGAGAGACAGTGT
TATCAGTAGCATGTACTGTAAGTTGTTTTGCTTTTTTCCCACAGGACAAGATGACTGGAATGGGACCAGGCCAT
CACTGAAGGCTCCTCCAGCTAGGCCAGTGAAGGGAGCATACAGAGAGCATCCATATGGACGTTATTAAAAAC
AAACAGGAGGGAAAAATATCAGTTATGAGCAAAGTTGTTACTGATTTCTTGTATCCCAGGATTCCTGTTGCTTT
ACCCACAACAGACAAGTAATTGTCTAAGTGTTTTTCTTCGTGGTCCCTTTCTTTTCCCCACTTCCTCCATTCTTAA
CTCTGCATTCTGGCTTCTGTATGTAGTATTTTAAAATGAGTTAAAATAGATTTAGGAATATCGAATTAATTTTTT
AAGTGTGTAGATGCTTTTTTTTCTTTGTTGTTTAAATATAAACAGTGTACCTTTTATAATAAAAAAAAGTTGAGT
TAAAAAAAAACCACAAACATGTTAGTTTCAAAAGTGGCATTGCTTGCTTAAAGG

KHDRBS1-REGDEL-EM1-B6N (Regulatory element deletion plus 2 SNPs)
AGCTGACTGGTGACGTTGGGAACAGGAATAGCCATTGATTGCAGACTCACTGGCTCCTTTACTGCTTTCTTCG
AGCAATGTGTTGTATTTTTCCAGGTTTTGGGACTGCGTGCCAATCATGTTGCTTATGGAGAATGTTGTTTCTCA
AGGGAACTGTACAGTAATGTACGGCTAGCAGTTTGACTTCTCATTACTCACCGTGTAGGCGGTTACCCCTATTG
ATCAAGGAGGCCAAGTAAAGAGGACAGCAAGGCACGCTTGGGGTGTGACGTGGTTCTAGGAGAGACAGTGT
TATCAGTAGCATGTACTGTAAGTTGTTTTGCTTTTTTCCCACAGGACAAGATGACTGGAATGGGACCAGGCCAT
CACTGAAGGCTCCTCCAGCTAGGCCAGTGAAGGGAGCATACAGAGAGCAcCCcTAcGGcCGTTATTAAAAACA
AACAGGAGGGAAAAATATCAGTTATGAGCAAAGTTGTTACTGATaTCTTGTATCCCAGGATTCCTtTTGCTTTA
CCCACAACA_20nt_TTTTTCTTCGTGGTCCCTTTCTTTTCCCCACTTCCTCCATTCTTAACTCTGCATTCTGGCTTC
TGTATGTAGTATTTTAAAATGAGTTAAAATAGATTTAGGAATATCGAATTAATTTTTTAAGTGTGTAGATGCTTT
TTTTTCTTTGTTGTTTAAATATAAACAGTGTACCTTTTATAATAAAAAAAAGTTGAGTTAAAAAAAAACCACAAA
CATGTTAGTTTCAAAAGTGGCATTGCTTGCTTAAAGG
*Red text highlights silent mutations incorporated into the donor to prevent re-processing of the engineered allele. The blue highlights the two erroneous bases incorporated by unwanted deviations in the donor template used.

KHDRBS1-REGDEL-EM1-B6N Heterozygous F1 animal sequence trace:

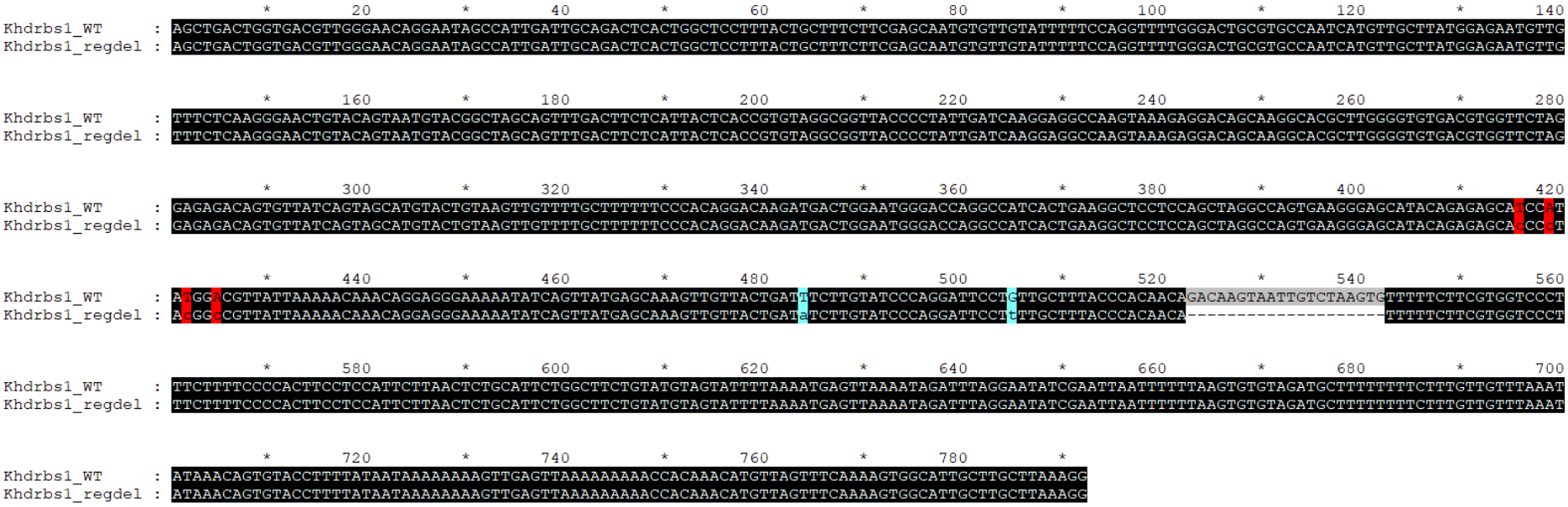


Top row: red highlights the four silent changes incorporated into the donor to prevent re-processing of the engineered allele by CRISPR/Cas9 as the embryo develops.

Middle row: blue highlights the two unintended SNPs incorporated into the allele as a result of errors in the donor template.

Bottom row: grey highlights the regulatory element deletion.

Nucleotide 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_Khdrbs1_Regdel_F1 (5'-3')	CAGTGAGGCACACTACAAGAGTT
Geno_Khdrbs1_Regdel_R1 (5'-3')	AGTTAACAAGCCTTCAGAACCT
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	61
Elongation time (min)	1
WT product size (bp)	998
Mutant product size (bp)	978
Notes	Sequence with Geno_Khdrbs1_Regdel_F2 (AGCTGACTGGTGACGTTGG) & Geno_Khdrbs1_Regdel_R2 (CCTTTAAGCAAGCAATGCCACTT)

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 for guide X were checked with the following primers:

Off-target site	Sequence	Type	Primers used

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	
Forward Primer (5'-3')	
Reverse Primer (5'-3')	
Probe (5'-3')	

Label	FAM-BHQ1

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.