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

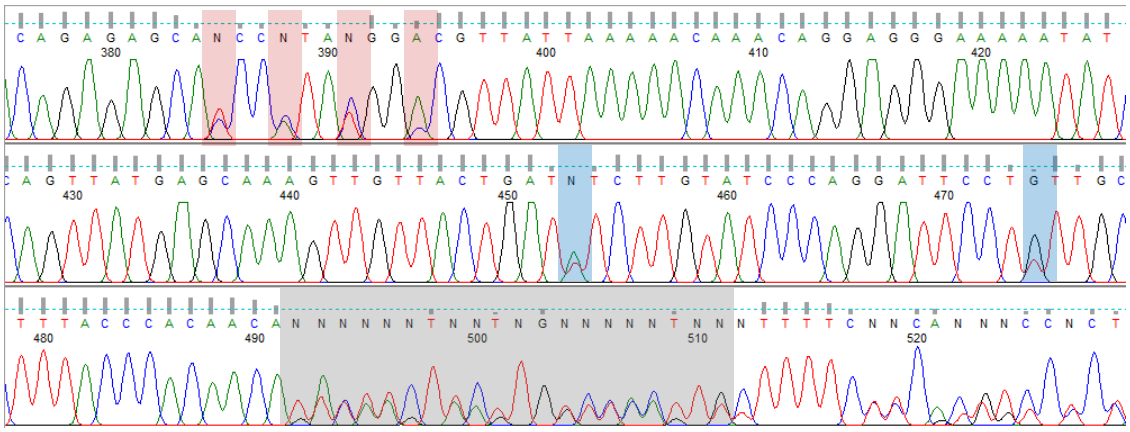
Description:
Deletion of regulatory element made using CRISPR/Cas9.

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:

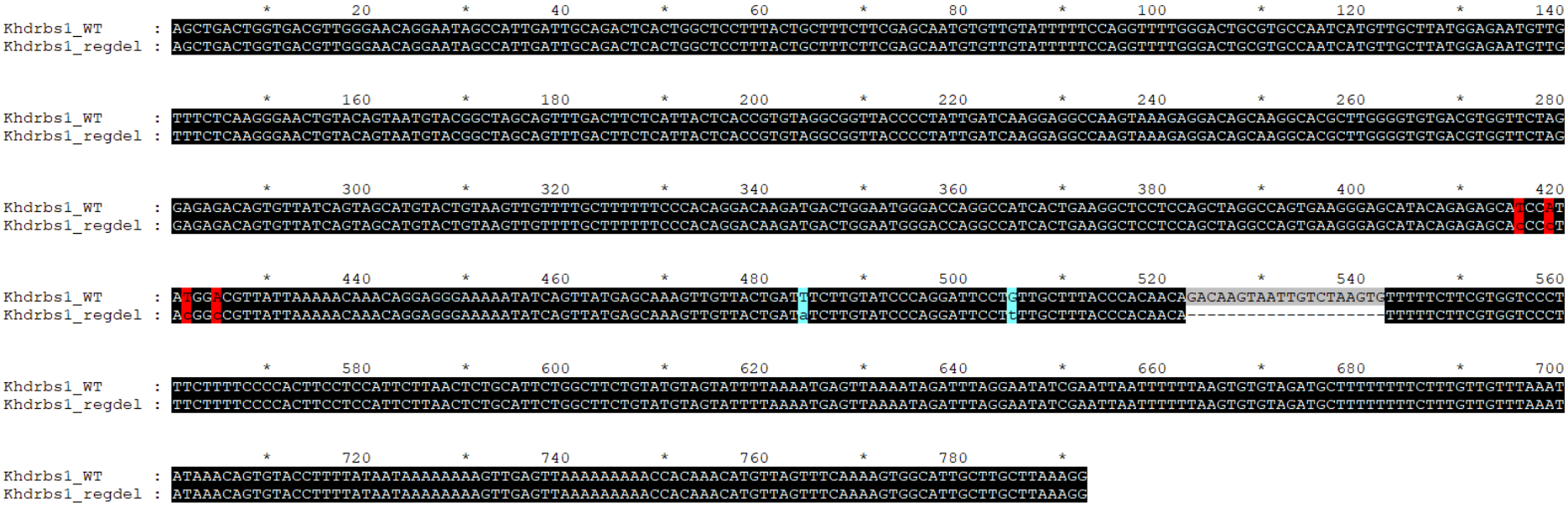


Top row: red highlights the four silent changes incorporated into the donor to prevent re-processing of the engineered allele by CRISPR/Cas9 as the embryo develops.

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')	

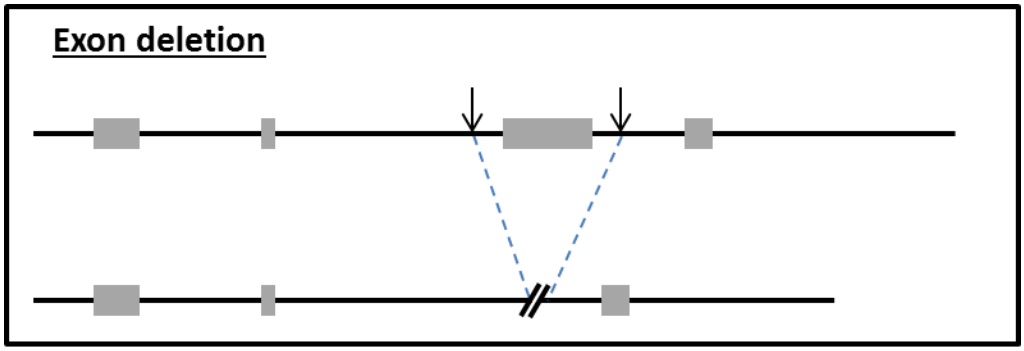
Label	FAM-BHQ1

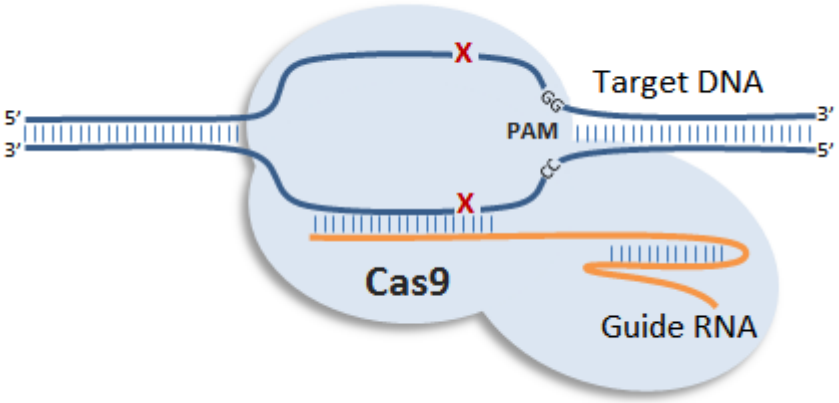
Reference Assay Name	Dot1l
Forward primer (5’-3’)	GCCCCAGCACGACCATT
Reverse primer (5’-3’)	TAGTTGGCATCCTTATGCTTCATC
Probe (5’-3’)	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

VIC-labelled reference assay for Dot1l gene.

Khdrbs1-REGDEL Genotyping Strategy

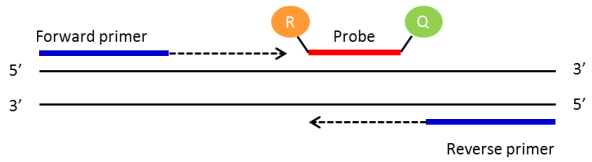
Animals have been engineered using the CRISPR/Cas9 technology. Most of the knockout alleles generated through this method will be obtained by deletion of a critical exon or by introduction of an indel (insertion/deletion) within the coding sequence of a critical exon (see picture below).



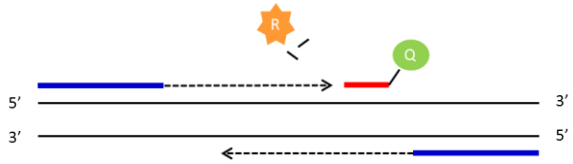


qPCR genotyping strategy

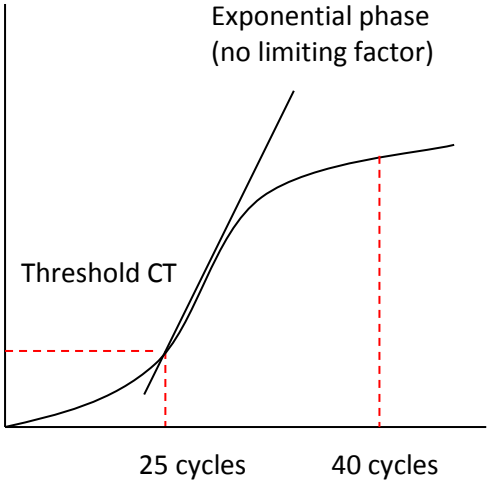
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that 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.

Khdrbs1-REGDEL Genotyping Strategy

Samples are genotyped with both WT loss of allele (WT-LOA) and Mutant assays. Samples for this line are genotyped using the following primers and probe (see Figure1)

- Universal probe and Universal primer designed near the CRISPR deletion for both alleles.
- Wildtype specific primer in the deletion designed for the WT allele.
- Mutant specific primer that bridges the junction designed for the CRISPR mutant allele.

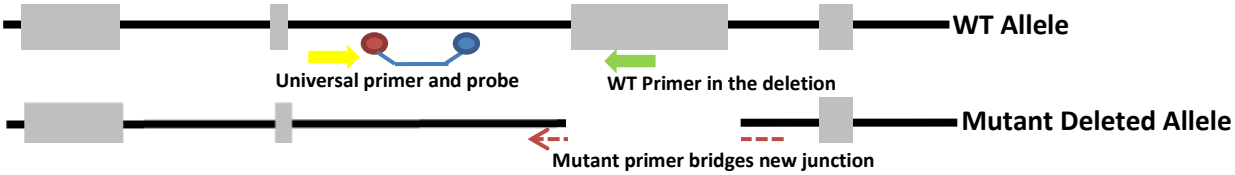


Figure1: Showing position of primers and probes

~~Khdrbs1-REGDEL WT2 assay (FAM labelled) Not Working~~

~~Khdrbs1-REGDEL-Probe (5nmol)~~	~~TGTCTAAGTGTTTTTCTTCGTGGTCCC~~
~~Khdrbs1-REGDEL-R (15nmol)~~	~~GAAGCCAGAATGCAGAGTTAAGAATG~~
~~Khdrbs1-REGDEL-F (15nmol)~~	~~TGCTTTACCCACAACAGACAAG~~

Khdrbs1-REGDEL MUT1 assay (FAM labelled)

Khdrbs1-REGDEL-Univ-Probe (5nmol)	CTCCTCCAGCTAGGCCAGTGAAGG
Khdrbs1-REGDEL-MUT-R (15nmol)	GAAAGGGACCACGAAGAAAAATGT
Khdrbs1-REGDEL-Univ-F (15nmol)	GGACCAGGCCATCACTGAAG

Dot1l internal control (VIC labelled)

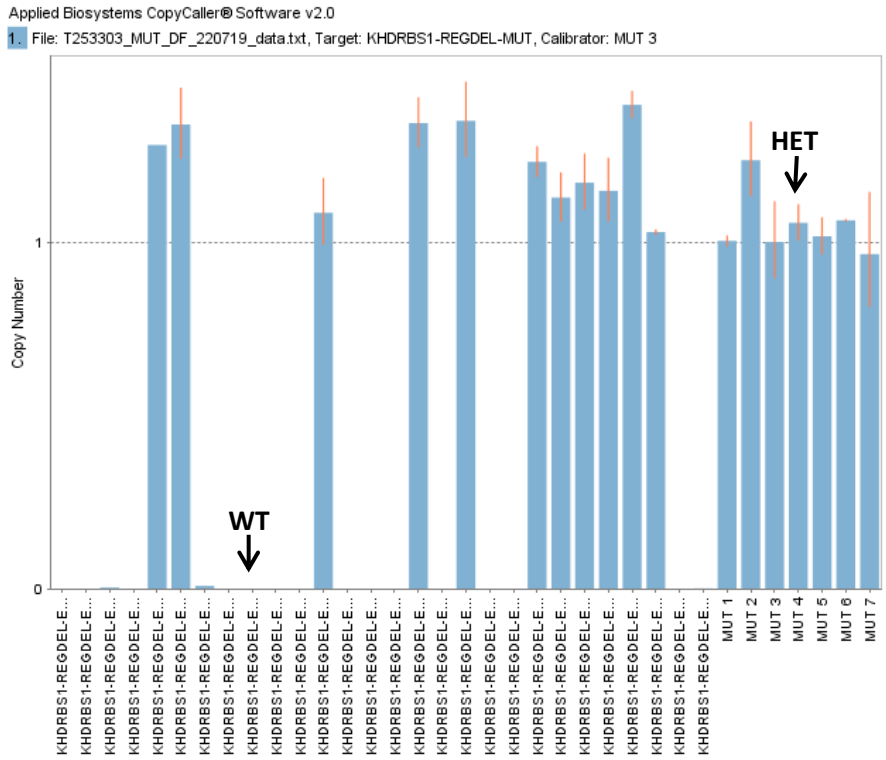
TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTTCCCCTCTAGTC
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCT
GGGGCAAGGCTTTATTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCCACACCCATCAG

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

Khdrbs1-REGDEL Assay copy called result, image showing both replicates and controls for Mutant assay (T253303)



Version No.	2
Date:	14/02/2020
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman