Name of Mouse model or mutation:
WDRCTCF-DEL970-EM1-B6 & WDRCTCF-DEL970-EM2-B6

Description:
Intronic deletion to remove two CTCF binding domains.

Type of mutation:
Deletion: 970 nt

Delivery method:
Cytoplasmic injection into 1-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
Cas9 mRNA

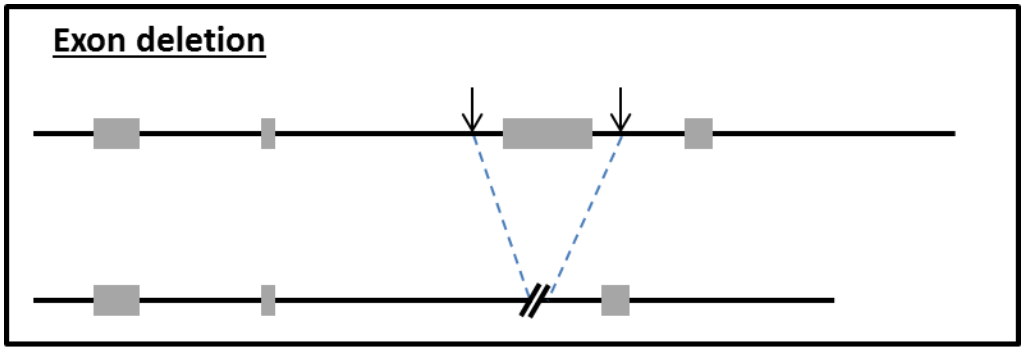
sgRNAs:

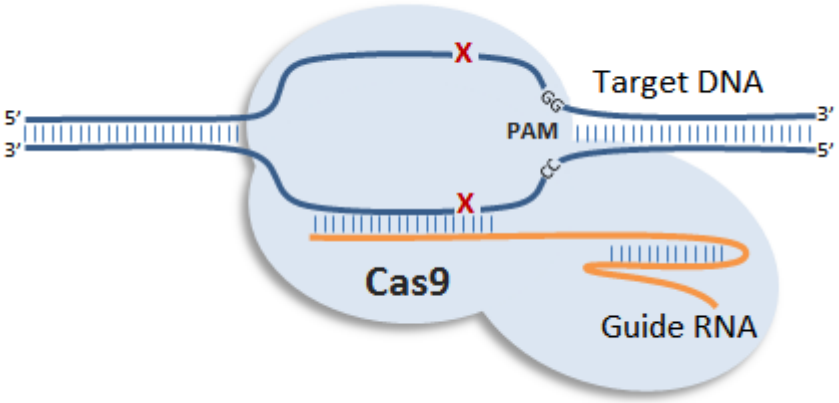
Protospacer sequence	PAM sequence
TGACACTTCACGGCCAGAAG	AGG
AGCCCAACTGCGACCTCTTC	TGG
AAAATCCTGCTCACCTCTAG	GGG
AAATCCTGCTCACCTCTAGG	GGG

Microinjection mixes:
Microinjection buffer (MIB; 10 mM Tris–HCl, 0.1 mM EDTA, 100 mM NaCl, pH7.5) was prepared and filtered through a 2 nm filter and autoclaved. Cas9 mRNA and sgRNAs were diluted and mixed in MIB to the working concentrations of 50 ng/µl and 6.5 ng/µl respectively. Injected embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Wdrctcf-DEL970 Genotyping Strategy

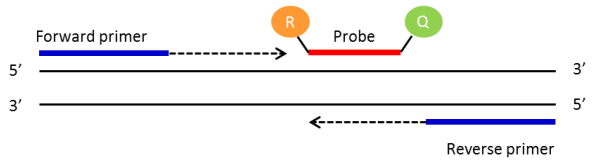
Animals have been engineered using the CRISPR/Cas9 technology. Most of the knockout alleles generated through this method will be obtained by deletion of a critical exon or by introduction of an indel (insertion/deletion) within the coding sequence of a critical exon (see picture below).



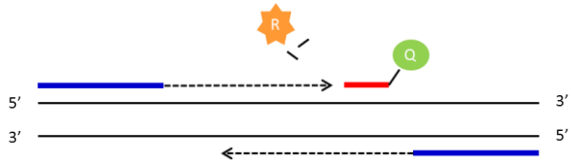


qPCR genotyping strategy

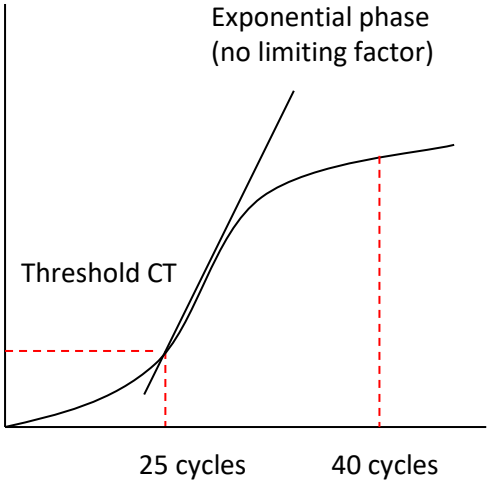
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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

Wdrctcf-DEL970 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.

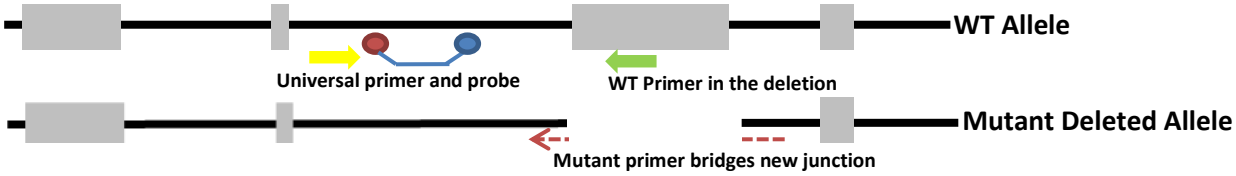


Figure1: Showing position of primers and probes

Wdrctcf-DEL970 WT1 assay (FAM labelled)

GTGCTAAGAGAAAGGCAAGTGCCACTATGCTCCATGTTGTTTTTTAAGAAAGAGTGAGTTGGCACCTTGGCTCTG
GGTGTGGTGAAGAAAAGTGTGCCAGGACTGGTGTGACACTTCACGGCCAGAAGAGGTCGCAGTTGGGCTGCCTGG
GATGACGAGAAGCCTGCGTTTAGGGCTGTGTGTGGGATGCTGATGTGTGGCCTGTCCCAGGAGGCGTGTGGGCCG
GAGGTGGTGATGCTAGTGTGAGACTCCTTGGCTGCCAGGAAGCTTGAGGGAGTGCTCTACGCCCCTGTCTAGTTC
CCCAGTACATCCAAAGGATGGCTTCGGGCAAAGACACTTGCTATAGTTGAGTTGTTGTCAGTACTGACCATGCAT
GCTAGGAGCAGAGTCTCCTGATCTTTATGAAAATTCAGGTTTCTATAAGCCAGGGGTGACTATCAAGCTGGAACC
CGAGCAGAGGCTTTAATAAGATGTCTCATAGTTTTAGTGATTAGGGTTGTTTGGAAAATCATTTTGTGTAAGTGG
GACTCTCAGTAAGTTCGGAATGCAGAATGAGTAGTAGGAACCAATAAGCTGTGGTGGGAAGATGGGTTAGGGCAT
GGAGGGCTCAGGTGTGCACCTGAGGTTGGAAGTCCTACCCTAGGCCTGCAGGTTGGTATGTGGCCTGCTGAGTGG
TGGCTGCTTTTCCCTCCCTGATGTCAGAGATGCCTGTGGTTATAAACCAAGCCCCAGTATTCCTGTGAGGTGGAG
GTATTATGTGTGTGCTTGGTCTTCCTGGCCTGGGTGCTGGCACTCAGGATGTGAACAACTGGGTGGGACCTGGCA
TGTGCTCCTGTCACTCAGGGTGCTGGGGCTTTGCTCCCGTCCCTGCCAGCGGTGGCTTACTGTATCTGAGATGTT
CAACAGGCGGTAAGCTTTGTGAATGCAGAAGTCACACCAGACCCATTTTTATATTCTTTGTAGCATCTGGCATGA
GATTTGGTCCCTGTTAAGTGATCAATGAAGGTTTAAAGACATTTTTTGGATTTCACTGCTTTGATTTAAGAGGGT
TTGTGTGTGCACATGCAAAAATCCTGCTCACCTCTAGGGGGCACAGTAAGACTTGCTATCTTTTGCCCATCCAAG

Wdrctcf-DEL970-Univ-Probe (5nmol)	TGCCAGGACTGGTGTGACACT
Wdrctcf-DEL970-WT-R (15nmol)	CCCAACTGCGACCTCTTCTG
Wdrctcf-DEL970-Univ-F (15nmol)	CTGGGTGTGGTGAAGAAAAGTG

Wdrctcf-DEL970 MUT1 assay (FAM labelled)

GTGCTAAGAGAAAGGCAAGTGCCACTATGCTCCATGTTGTTTTTTAAGAAAGAGTGAGTTGGCACCTTGGCTCTG
GGTGTGGTGAAGAAAAGTGTGCCAGGACTGGTGTGACACTTCACGG [970nt DEL]
CACAGTAAGACTTGCTATCTTTTGCCCATCCAAG

Wdrctcf-DEL970-Univ-Probe (5nmol)	TGCCAGGACTGGTGTGACACT
Wdrctcf-DEL970-MUT-R (15nmol)	GCAAGTCTTACTGTGCCGTGA
Wdrctcf-DEL970-Univ-F (15nmol)	CTGGGTGTGGTGAAGAAAAGTG

Dot1l internal control (VIC labelled)

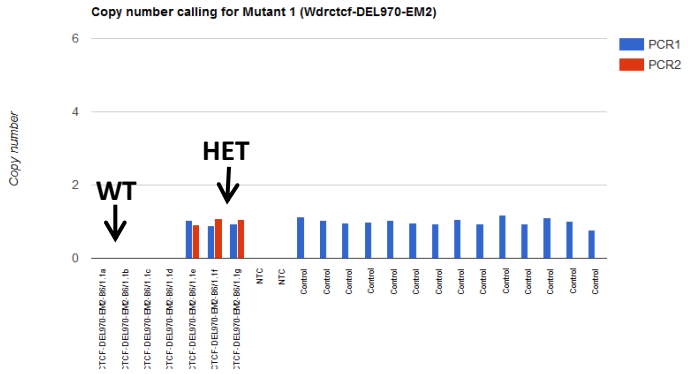
CTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCTGGGGC

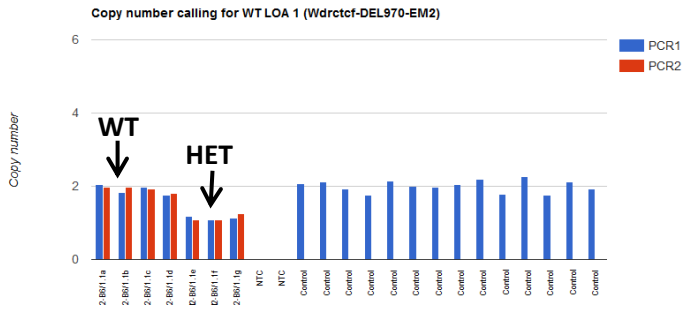
Primer 1 = GCCCCAGCACGACCATT
Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

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

Wdrctcf-DEL970 Assay copy called result, image showing both replicates and controls for WT and Mutant assays (T238495)





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Created/Updated by:	Daniel Ford
Approved by:	Debbie Williams