Name of Mouse model or mutation:
WDRENHDEL-DEL1203-EM1-B6

Description:
Intronic deletion to remove specific enhancer region.

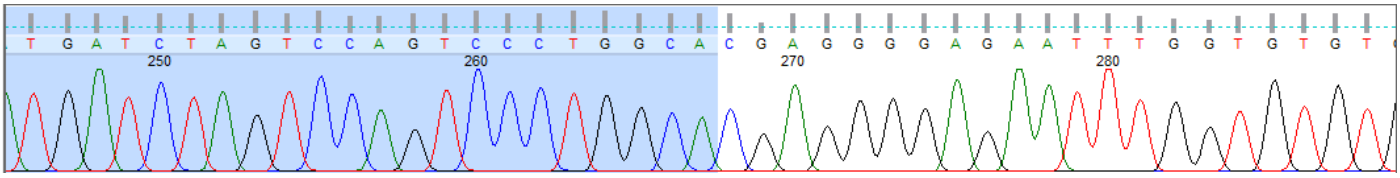
Type of mutation:
Deletion: 1203 nt

Sequence details
WT
GCAAGTAGTCCTGCCGTGATCACACTGTGCATAAATGCCATCTTTTCTGCTGCTTAATGTAGTGAAGA
TGGGCTTTGCACTGTCGGCCGGCAGAAGGCCTGTGTGTGGGAAATGGAACCTTACACTGGCCGGCC
TTTGGTGCGTGACTCTACTGCTGCTCAGTGCTGCCGGGACTTTGCATACGATTACTTGGTCTCTCTGA
TCCCCAGGTATCACGGTAAAGGAGAGAGAATGGCCATCAATGAGGACTGTTGCCACAGATATGGGA
AGAAATGATCTAGTCCAGTCCCTGGCATAAGCGAGAATTTCCCCCCTTGTACCCATATTGATTGGTTA
AAACATAAATGTGCAAAAAACACCTCAGTTAGAGTCTTCTGACTTAATGTAGTTCTGCTTTTGTGAAT
CTCTTCATAGTCTGTTTTTGTACATATGGATTTTTAAAGAGTTTCATCCTCAGTGTTCCTGAGATAAAT
CCCTTTAAAATGCATGGAGTTTCTGAGATCAATGCTACTGAGCTGCCTTTGTCGTCCTTTGGAGTGCT
TTGTGCATCCGCCAGGTCCTGCTGAGGACGGCCCAGTTGCCAGGCGCTCACACTGGCCACTGGATG
GTGGACGGTTGCTTGAGTCCAAAGTCAAGCCTCAGCAGCGCTGGGGAAGCAACCTCTTAGGGGACC
CAGGAGGGAGCTATGCTGCCTCCCTCAGGCAAGCTGCCTTCGGAGGTAGCTGAAGGCTCTGCGATG
GAGGCAGGTGACCCCAGCTGGTGCTTTCTTGTGCTGATCACCGGCCCTGTGGTGACCAGGAGCACA
TGAGGGAGCAGGGCGTCAGCAGCTGCACCGGGTCCACCACATGCTGCTAGGCACAGCTTTTAACAC
ACTTTACCACAGAGCTGAAACTTGTTCCCAGTTACACAGCAGGCAACATGGGGCGGGCAATAGGCT
ACAGAGACTGAGCCAAACAGGCAAGCTGTTCCTCCTCCACCTCTGGAAATGCCCAGTTGAAAAGCA
GCGCTTTCTCCCAGATGGGCCCAGGCTCTCTTCAGCCTCACTACTTGGGCTTTGTTAAGGGCAGGGG
GAGCAGAGATGATTTCCACGGTGGCTTGGATTTGCATTTTTATATATTACAGGATCTCGCTGATTTAT
CATTGTTAGTGGATCTGATTATATTAGGGTATCTTGGATGTGCTTTGTAAAAAGTGATATATTTCACA
TTCAAAGACAAGGAATTTATGGATCCAAGTGCCAGAGCGAGGGTTTAGTTCAGTTTTGAAAGAATTA
CTCTTGATGGATTTTTACGAAATGAGGTTGAGAAGTGATTTGGTGCGTGGAGGAAGGAGCTCGGGA
GCATTATGCAGATGATTGGCAATTTATTCCGAGCACGAGGCAGATCTGTTGGAAATGAGAGGCGCA
GCTGGGTAGGAGGGGCGGGTTGTGGTGGTGGGTGTCTGGTATTTGGGGAGTGAATGATTAACAGT
GAGTGCATGGAGGGTAATTAGGTGAAGGCGAGGGGAGAATTTGGTGTGTGTGTTAACATGCACAC
ACGCCGGCACATGGAGAGATCATCTGGTCCTTCTTCAGGTGGCGGAGACCAAGGGAGAGCAGGAG
GTGCCCTGGGGCACGCAGTTCTGGAGTCTGCTTGCCCCTCTCTTCCCTCTCACCTTCCTGGGAAGGTT
GAGAGTCCCCGTGTTGTCCTGCGGTGGCTTAGGTGTCAGTAGCCAGGCTGCAGAGCCTCTGGGAAG
AGTTGGCAG

WDRENHDEL-DEL1203-EM1-B6N

GCAAGTAGTCCTGCCGTGATCACACTGTGCATAAATGCCATCTTTTCTGCTGCTTAATGTAGTGAAGA
TGGGCTTTGCACTGTCGGCCGGCAGAAGGCCTGTGTGTGGGAAATGGAACCTTACACTGGCCGGCC
TTTGGTGCGTGACTCTACTGCTGCTCAGTGCTGCCGGGACTTTGCATACGATTACTTGGTCTCTCTGA
TCCCCAGGTATCACGGTAAAGGAGAGAGAATGGCCATCAATGAGGACTGTTGCCACAGATATGGGA
AGAAATGATCTAGTCCAGTCCCTGGCA[1203 nt
deletion]CGAGGGGAGAATTTGGTGTGTGTGTTAACATGCACACACGCCGGCACATGGAGAGATCA
TCTGGTCCTTCTTCAGGTGGCGGAGACCAAGGGAGAGCAGGAGGTGCCCTGGGGCACGCAGTTCT
GGAGTCTGCTTGCCCCTCTCTTCCCTCTCACCTTCCTGGGAAGGTTGAGAGTCCCCGTGTTGTCCTGC
GGTGGCTTAGGTGTCAGTAGCCAGGCTGCAGAGCCTCTGGGAAGAGTTGGCAG

WDRENHDEL-DEL1203-EM1-B6N Heterozygous F1 animal sequence trace:



Blue highlights sequence 5’ of deletion, unhighlighted is downstream i.e. breakpoint of deletion visualised as per mark up of mutant sequence above.

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_Wdr25_ENHdel_F2 primer (5'-3')	GCAAGTAGTCCTGCCGTGAT
Geno_Wdr25_ENHdel_R2 primer (5'-3')	CTGCCAACTCTTCCCAGAGG
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	57
Elongation time (min)	2
WT product size (bp)	1913
Mutant product size (bp)	710
Notes	

All amplicons were sent for Sanger sequencing to check the integrity of the target site. F1 sequences should be heterozygous unless on sex chromosome.

As one of sgRNAs used in this project had a potential off-target sites with two or fewer mismatches, the site was amplified in a PCR reaction using the following conditions/primer sequences:

Geno_WdrENHdel_OT1_F1 primer (5'-3')	AGCCTGGTACCCAATTCAGC
Geno_WdrENHdel_OT1_R1 primer (5'-3')	CATGTGTGTAAGCCCTGGGT
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	61
Elongation time (min)	0.5
WT product size (bp)	463
Mutant product size (bp)	n/a
Notes	

All amplicons were sent for Sanger sequencing to check the integrity of the target site.

Copy counting of the excised sequence was carried out by ddPCR at the F1 stage to confirm if this region inserted elsewhere into the genome. The following Universal probe library (https://lifescience.roche.com/en_gb/brands/universal-probe-library.html) assay was used to copy count the excised sequence compared against a VIC-labelled reference Taqman assay for Dot1l:

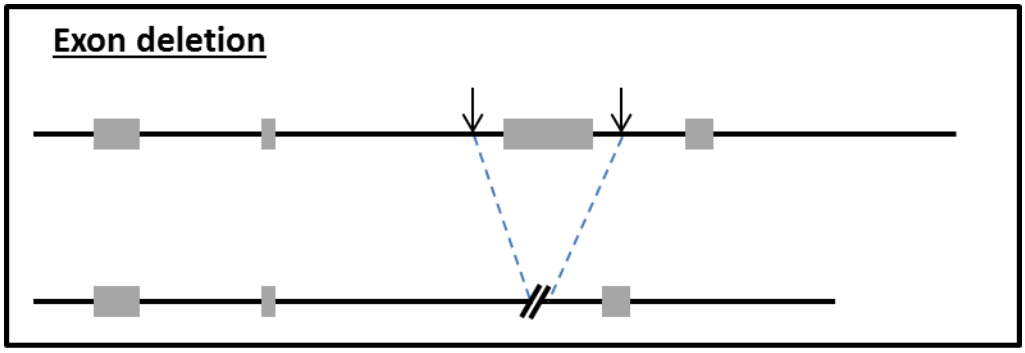
Assay name	WdrENHdel_UPL25
Forward Primer (5'-3')	GACTGAGCCAAACAGGCAAG
Reverse Primer (5'-3')	CTGCTTTTCAACTGGGCATT
UPL Probe (5'-3')	UPL25
Label	FAM-BHQ1

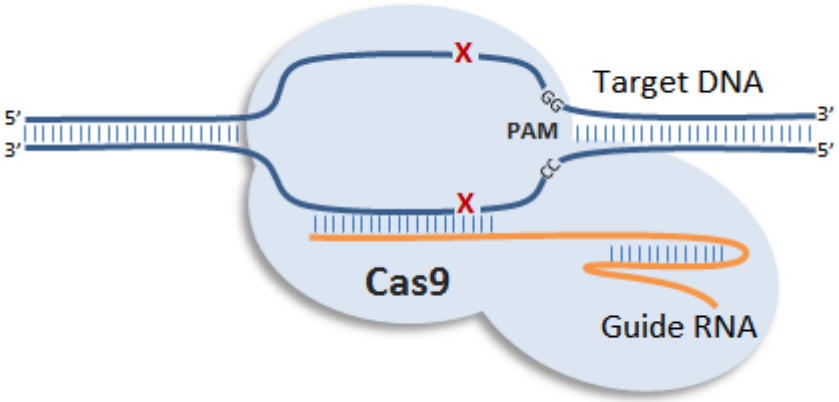
Reference Assay Name	Dot1l
Forward primer (5'-3')	GCCCCAGCACGACCATT
Reverse primer (5'-3')	TAGTTGGCATCCTTATGCTTCATC
Probe (5'-3')	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

VIC-labelled reference assay (2 copies) for Dot1l gene.

WdrEHN-DEL1203-EM1 Genotyping Strategy

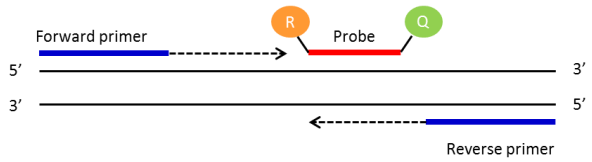
Animals have been engineered using the CRISPR/Cas9 technology. Most of the knockout alleles generated through this method will be obtained by deletion of a critical exon or by introduction of an indel (insertion/deletion) within the coding sequence of a critical exon (see picture below).



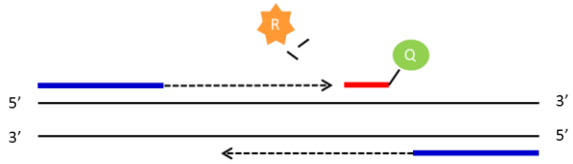


qPCR genotyping strategy

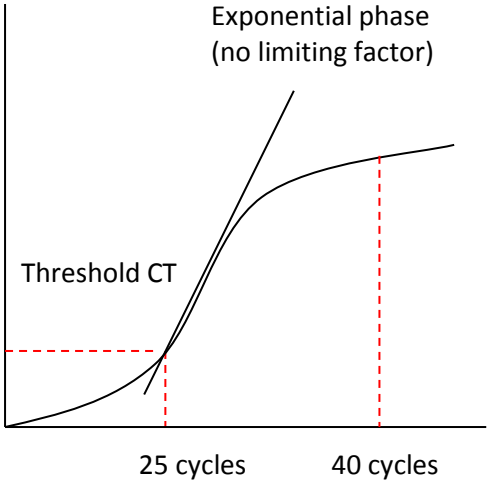
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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

WdrEHN-DEL1203-EM1 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 the WT allele.
- Wildtype specific primer in the deletion designed for the WT allele.
- Mutant specific primer that bridges the junction designed for the CRISPR mutant allele.

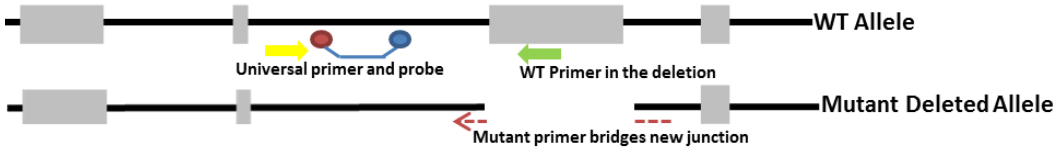


Figure1: Showing position of primers and probes

WdrEHN-DEL1203-WT1 assay (FAM labelled Universal probe)

GCAAGTAGTCCTGCCGTGATCACACTGTGCATAAATGCCATCTTTTCTGCTGCTTAATGTAGTGAAGATGGGCTTTGCACTGTCGGCCGGCAGA
AGGCCTGTGTGTGGGAAATGGAACCTTACACTGGCCGGCCTTTGGTGCGTGACTCTACTGCTGCTCAGTGCTGCCGGGACTTTGCATACGATTA
CTTGGTCTCTCTGATCCCCAGGTATCACGGTAAAGGAGAGAGAATGGCCATCAATGAGGACTGTTGCCACAGATATGGGAAGAAATGATCTAGT
CCAGTCCCTGGCATAAGCGAGAATTTCCCCCCTTGTACCCATATTGATTGGTTAAAACATAAATGTGCAAAAAACACCTCAGTTAGAGTCTTCT
GACTTAATGTAGTTCTGCTTTTGTGAATCTCTTCATAGTCTGTTTTTGTACATATGGATTTTTAAAGAGTTTCATCCTCAGTGTTCCTGAGATA
AATCCCTTTAAAATGCATGGAGTTTCTGAGATCAATGCTACTGAGCTGCCTTTGTCGTCCTTTGGAGTGCTTTGTGCATCCGCCAGGTCCTGCT
GAGGACGGCCCAGTTGCCAGGCGCTCACACTGGCCACTGGATGGTGGACGGTTGCTTGAGTCCAAAGTCAAGCCTCAGCAGCGCTGGGGAAGCA
ACCTCTTAGGGGACCCAGGAGGGAGCTATGCTGCCTCCCTCAGGCAAGCTGCCTTCGGAGGTAGCTGAAGGCTCTGCGATGGAGGCAGGTGACC
CCAGCTGGTGCTTTCTTGTGCTGATCACCGGCCCTGTGGTGACCAGGAGCACATGAGGGAGCAGGGCGTCAGCAGCTGCACCGGGTCCACCACA
TGCTGCTAGGCACAGCTTTTAACACACTTTACCACAGAGCTGAAACTTGTTCCCAGTTACACAGCAGGCAACATGGGGCGGGCAATAGGCTACA
GAGACTGAGCCAAACAGGCAAGCTGTTCCTCCTCCACCTCTGGAAATGCCCAGTTGAAAAGCAGCGCTTTCTCCCAGATGGGCCCAGGCTCTCT
TCAGCCTCACTACTTGGGCTTTGTTAAGGGCAGGGGGAGCAGAGATGATTTCCACGGTGGCTTGGATTTGCATTTTTATATATTACAGGATCTC
GCTGATTTATCATTGTTAGTGGATCTGATTATATTAGGGTATCTTGGATGTGCTTTGTAAAAAGTGATATATTTCACATTCAAAGACAAGGAAT
TTATGGATCCAAGTGCCAGAGCGAGGGTTTAGTTCAGTTTTGAAAGAATTACTCTTGATGGATTTTTACGAAATGAGGTTGAGAAGTGATTTGG
TGCGTGGAGGAAGGAGCTCGGGAGCATTATGCAGATGATTGGCAATTTATTCCGAGCACGAGGCAGATCTGTTGGAAATGAGAGGCGCAGCTGG
GTAGGAGGGGCGGGTTGTGGTGGTGGGTGTCTGGTATTTGGGGAGTGAATGATTAACAGTGAGTGCATGGAGGGTAATTAGGTGAAGGCGAGGG
GAGAATTTGGTGTGTGTGTTAACATGCACACACGCCGGCACATGGAGAGATCATCTGGTCCTTCTTCAGGTGGCGGAGACCAAGGGAGAGCAGG
AGGTGCCCTGGGGCACGCAGTTCTGGAGTCTGCTTGCCCCTCTCTTCCCTCTCACCTTCCTGGGAAGGTTGAGAGTCCCCGTGTTGTCCTGCGG
TGGCTTAGGTGTCAGTAGCCAGGCTGCAGAGCCTCTGGGAAGAGTTGGCAG

WdrEHN-DEL1203 -Univ-Probe	ATGGCCATCAATGAGGACTGTTGCC
WdrEHN-DEL1203 -WT-R	GCAGAACTACATTAAGTCAGAAGACTC
WdrEHN-DEL1203 -Univ-F	GATCCCCAGGTATCACGGTAAAG

WdrEHN-DEL1203-MUT1 assay (FAM labelled Universal probe)

CTTGGTCTCTCTGATCCCCAGGTATCACGGTAAAGGAGAGAGAATGGCCATCAATGAGGACTGTTGCCACAGATATGGGAAGAAATGATCTAGT
CCAGTCCCTGGCA[1203ntDEL]CGAGGGGAGAATTTGGTGTGTGTGTTAACATGCACACACGCCGGCACATGGAGAGATCATCTGGTCC
TTCTTCAGGTGGCGGAGACCAAGGGAGAGCAGGAGGTGCCCTGGGGCACGCAGTTCTGGAGTCTGCTTGCCCCTCTCTTCCCTCTCACCTTC

WdrEHN-DEL1203 -Univ-Probe	ATGGCCATCAATGAGGACTGTTGCC
WdrEHN-DEL1203 -mut-R	CAAATTCTCCCCTCGTGCCA
WdrEHN-DEL1203 -Univ-F	GATCCCCAGGTATCACGGTAAAG

Dot1l internal control (VIC labelled)

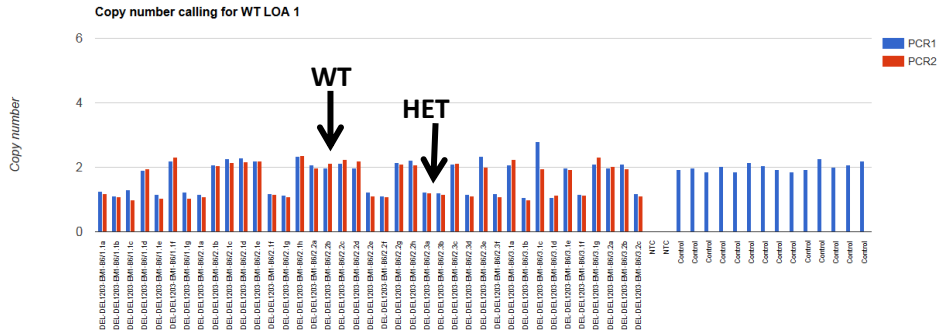
GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTCGACTTGAGAGCTGGCCCTGAATGGTCGTGCTGGGGCAAGGCTTTATTTCA

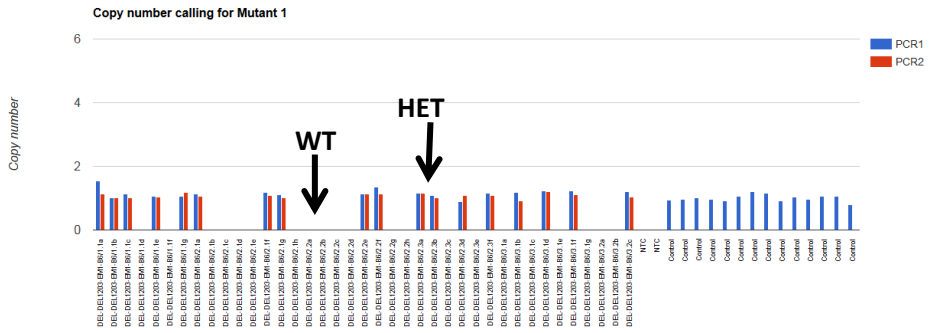
Primer 1 = GCCCCAGCACGACCATT Primer 2 = TAGTTGGCATCCTTATGCTTCATC
Probe = CCAGCTCTCAAGTCG

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

WdrEHN-DEL1203 Assay copy called result, image showing both replicates and controls for Mutant and WT assays (Task 247685)





Version No. 1

Date: 29/05/19

Created/Updated by: Daniel Ford

Approved by: