

**Name of Mouse model or mutation:****KHDRBS1-REGDEL-EM2-B6N****KHDRBS1-REGDEL-EM3-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:**

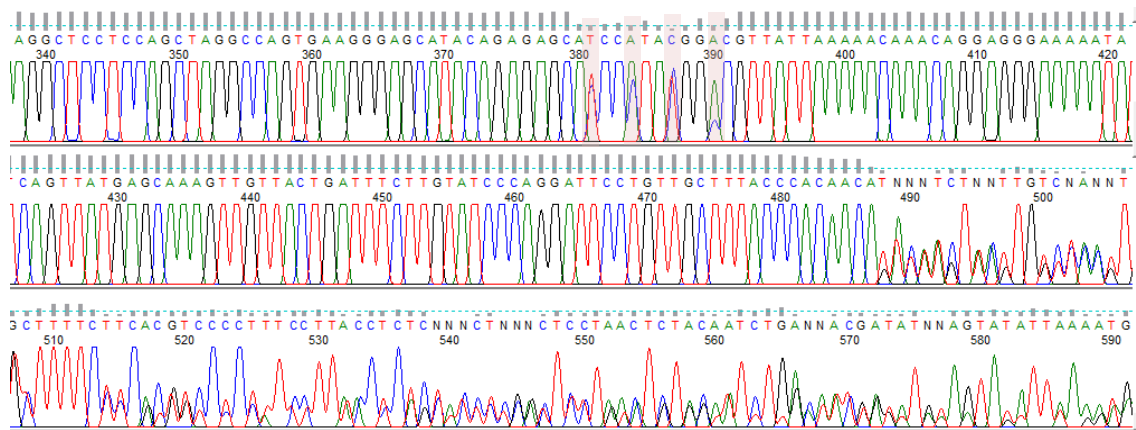
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 ATCAAGGAGGCCAAGTAAAGAGGACAGCAAGGCACGCTTGGGGTGTGACGTGGTTCTAGGAGAGACAGTGT  
 TATCAGTAGCATGTAAGTTGTTTTGCTTTTTCCACAGGACAAGATGACTGGAATGGGACCAGGCCAT  
 CACTGAAGGCTCCTCCAGCTAGGCCAGTGAAGGGAGCATAACAGAGAGCATCCATATGGACGTTATTA AAAAC  
 AAACAGGAGGGAAAAATATCAGTTATGAGCAAAGTTGTTACTGATTTCTTGATCCCAGGATTCTGTTGCTTT  
 ACCACAACAGACAAGTAATTGTCTAAGTGTTTTCTTCGTGGTCCCTTTCTTTCCCACTTCCTCCATTCTTAA  
 CTCTGCATTCTGGCTTCTGTATGTAGTATTTTAAAATGAGTTAAAATAGATTTAGGAATATCGAATTAATTTTT  
 AAGTGTGTAGATGCTTTTTTTCTTTGTTGTTAAATATAAACAGTGTACCTTTTATAATAAAAAAAGTTGAGT  
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**KHDRBS1-REGDEL-EM1-B6N (Regulatory element deletion plus 2 SNPs)**

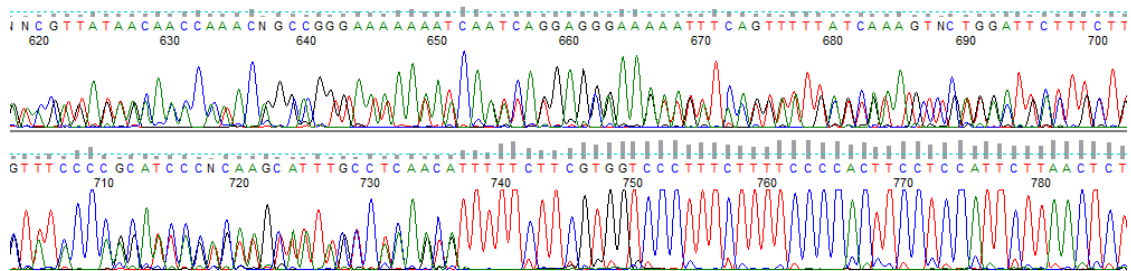
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 AGGGAAGTGTACAGTAATGTACGGCTAGCAGTTTGACTTCTCATTACTCACCGTGTAGGCGGTTACCCCTATTG  
 ATCAAGGAGGCCAAGTAAAGAGGACAGCAAGGCACGCTTGGGGTGTGACGTGGTTCTAGGAGAGACAGTGT  
 TATCAGTAGCATGTAAGTTGTTTTGCTTTTTCCACAGGACAAGATGACTGGAATGGGACCAGGCCAT  
 CACTGAAGGCTCCTCCAGCTAGGCCAGTGAAGGGAGCATAACAGAGAGCAcCCcTAcGGcCGTTATTA AAAACA  
 AACAGGAGGGAAAAATATCAGTTATGAGCAAAGTTGTTACTGATTTCTTGATCCCAGGATTCTGTTGCTTTA  
 CCCACAACA\_20nt\_TTTTTCTTCGTGGTCCCTTTCTTTCCCACTTCCTCCATTCTTAACTCTGCATTCTGGCTTC  
 TGTATGTAGTATTTTAAAATGAGTTAAAATAGATTTAGGAATATCGAATTAATTTTTTAAAGTGTGTAGATGCTTT  
 TTTTTCTTTGTTGTTAAATATAAACAGTGTACCTTTTATAATAAAAAAAGTTGAGTTAAAAAAAACCACAAA  
 CATGTTAGTTTCAAAGTGCCATTGCTTGCTTAAAGG

\*Red text highlights silent mutations incorporated into the donor to prevent re-processing of the engineered allele.

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



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. The breakpoint of the 20 nt deletion can then clearly be seen as sequence becomes two traces.



Bottom panel: The breakpoint of the 20 nt deletion can then clearly be seen from the reverse primer (sequence complemented) as sequence becomes two traces.

## Nucleotide Alignment:

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*          20          *          40          *          60          *          80          *          100         *          120         *          140         *
Khdrbs1_WT   : AGCTGACTGGTGACGTTGGGAACAGGAATAGCCATTGATTCGAGACTCACTGGCTCCTTTACTGCTTTCTTCGAGCAATGTGTTGATTTTTCCAGGTTTTGGGACTGCGTGCCAATCATGTTGCTTATGGAGAAATGTTGTTTCTCAAGG
Khdrbs1_EM2_EM3 : AGCTGACTGGTGACGTTGGGAACAGGAATAGCCATTGATTCGAGACTCACTGGCTCCTTTACTGCTTTCTTCGAGCAATGTGTTGATTTTTCCAGGTTTTGGGACTGCGTGCCAATCATGTTGCTTATGGAGAAATGTTGTTTCTCAAGG
AGCTGACTGGTGACGTTGGGAACAGGAATAGCCATTGATTCGAGACTCACTGGCTCCTTTACTGCTTTCTTCGAGCAATGTGTTGATTTTTCCAGGTTTTGGGACTGCGTGCCAATCATGTTGCTTATGGAGAAATGTTGTTTCTCAAGG

          160          *          180          *          200          *          220          *          240          *          260          *          280          *          300
Khdrbs1_WT   : GAACTGTACAGTAATGTACGGCTAGCAGTTTGACTTCTCATTACTCACCGTGTAGGCGGTTACCCCTATTGATCAAGGAGGCCAAGTAAAGAGGACAGCAAGGCACGCTTGGGGTGTGACGTGGTTCTAGGAGAGACAGTGTATCAGTA
Khdrbs1_EM2_EM3 : GAACTGTACAGTAATGTACGGCTAGCAGTTTGACTTCTCATTACTCACCGTGTAGGCGGTTACCCCTATTGATCAAGGAGGCCAAGTAAAGAGGACAGCAAGGCACGCTTGGGGTGTGACGTGGTTCTAGGAGAGACAGTGTATCAGTA
GAACTGTACAGTAATGTACGGCTAGCAGTTTGACTTCTCATTACTCACCGTGTAGGCGGTTACCCCTATTGATCAAGGAGGCCAAGTAAAGAGGACAGCAAGGCACGCTTGGGGTGTGACGTGGTTCTAGGAGAGACAGTGTATCAGTA

*          320          *          340          *          360          *          380          *          400          *          420          *          440          *
Khdrbs1_WT   : GCATGTAAGTTGTTTTGCTTTTTCCACAGGACAAGATGACTGGAATGGGACCAGGCCATCACTGAAGGCTCCTCCAGCTAGGCCAGTGAAGGGAGCATAACAGAGAGCA CC TA GG CGTTATTA AAAACAACAGGAGGGA
Khdrbs1_EM2_EM3 : GCATGTAAGTTGTTTTGCTTTTTCCACAGGACAAGATGACTGGAATGGGACCAGGCCATCACTGAAGGCTCCTCCAGCTAGGCCAGTGAAGGGAGCATAACAGAGAGCA CC TA GG CGTTATTA AAAACAACAGGAGGGA
GCATGTAAGTTGTTTTGCTTTTTCCACAGGACAAGATGACTGGAATGGGACCAGGCCATCACTGAAGGCTCCTCCAGCTAGGCCAGTGAAGGGAGCATAACAGAGAGCA CC TA GG CGTTATTA AAAACAACAGGAGGGA

          460          *          480          *          500          *          520          *          540          *          560          *          580          *          600
Khdrbs1_WT   : AAAATATCAGTTATGAGCAAAGTTGTTACTGATTTCTTGTATCCAGGATTCCTGTGCTTTACCCACAAGA GACAAGTAATTGCTAAGTC TTTTCTTCGTTGGTCCCTTCTTTCCCCACTTCTCCATTCTTA ACTCTGCATTCTG
Khdrbs1_EM2_EM3 : AAAATATCAGTTATGAGCAAAGTTGTTACTGATTTCTTGTATCCAGGATTCCTGTGCTTTACCCACAACA ----- TTTTCTTCGTTGGTCCCTTCTTTCCCCACTTCTCCATTCTTA ACTCTGCATTCTG
AAAATATCAGTTATGAGCAAAGTTGTTACTGATTTCTTGTATCCAGGATTCCTGTGCTTTACCCACAACA TTTTCTTCGTTGGTCCCTTCTTTCCCCACTTCTCCATTCTTA ACTCTGCATTCTG

*          620          *          640          *          660          *          680          *          700          *          720          *          740          *
Khdrbs1_WT   : GCTTCTGTATGTAGTATTTTAAAATGAGTTAAAATAGATTTAGGAATATCGAATTAATTTTTTAAGTGTGTAGATGCTTTTTTTCTTTGTTGTTTAAATATAAACAGTGACCTTTTATAATAAAAAAAGTTGAGTTAAAAAAAACC
Khdrbs1_EM2_EM3 : GCTTCTGTATGTAGTATTTTAAAATGAGTTAAAATAGATTTAGGAATATCGAATTAATTTTTTAAGTGTGTAGATGCTTTTTTTCTTTGTTGTTTAAATATAAACAGTGACCTTTTATAATAAAAAAAGTTGAGTTAAAAAAAACC
GCTTCTGTATGTAGTATTTTAAAATGAGTTAAAATAGATTTAGGAATATCGAATTAATTTTTTAAGTGTGTAGATGCTTTTTTTCTTTGTTGTTTAAATATAAACAGTGACCTTTTATAATAAAAAAAGTTGAGTTAAAAAAAACC

          760          *          780          *
Khdrbs1_WT   : ACAAAACATGTTAGTTTCAAAGTGGCATTGCTTGCTTAAAGG
Khdrbs1_EM2_EM3 : ACAAAACATGTTAGTTTCAAAGTGGCATTGCTTGCTTAAAGG
ACAAACATGTTAGTTTCAAAGTGGCATTGCTTGCTTAAAGG
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### 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 $\leq 2$ mismatches for guides used were checked with the following primers:

Off-target site	Sequence	Type	Primers used
<a href="#">8:102534873-102534895</a>	TGTTTTTAATAACTTCCATA TGG	Intergenic	Geno_Khdrbs1_OT1F1 primer (5'-3') ACCTTTAAATAAGCAATGCCACTT Geno_Khdrbs1_OT1R1 primer (5'-3') TTGGACCTTTACATGAGGTAGCC
<a href="#">9:68999906-68999928</a>	TGTTTTTAATAACATCCATC AGG	Intronic	Geno_Khdrbs1_OT2F2 primer (5'-3') GTGTATGAGACATGGCACGGA Geno_Khdrbs1_OT2R2 primer (5'-3') CAAAGCATTACCCTCCTCCT
<a href="#">8:102534989-102535011</a>	TGTCTAAGTGTTTTTCTTTG TGG	Intergenic	Geno_Khdrbs1_OT3F1 primer (5'-3') GTCAAGGGGAGTCGGAGTATT Geno_Khdrbs1_OT3R1 primer (5'-3') AACTATGACTTTACGGGGTGCC
<a href="#">12:43917824-43917846</a>	TGTCTAAGTGTTTATCTTCA GGG	Intergenic	Geno_Khdrbs1_OT4F1 primer (5'-3') TGGAAGTATGCTGTTTTCTAGCC Geno_Khdrbs1_OT4R1 primer (5'-3') TTACGGAACCTTAGGGGTGAG

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

Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligo(s) 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:

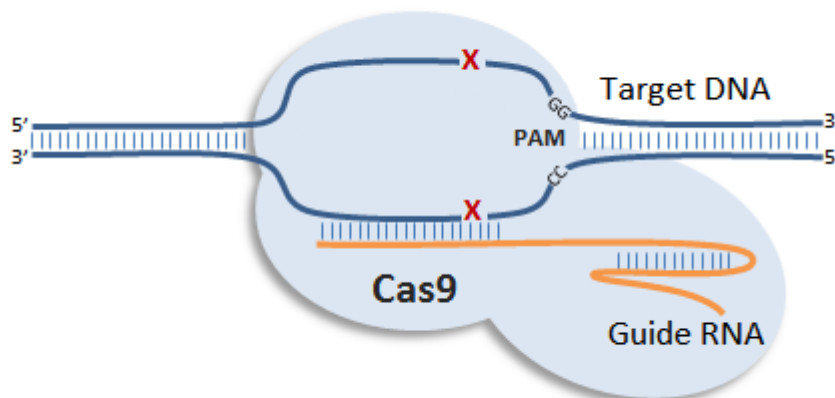
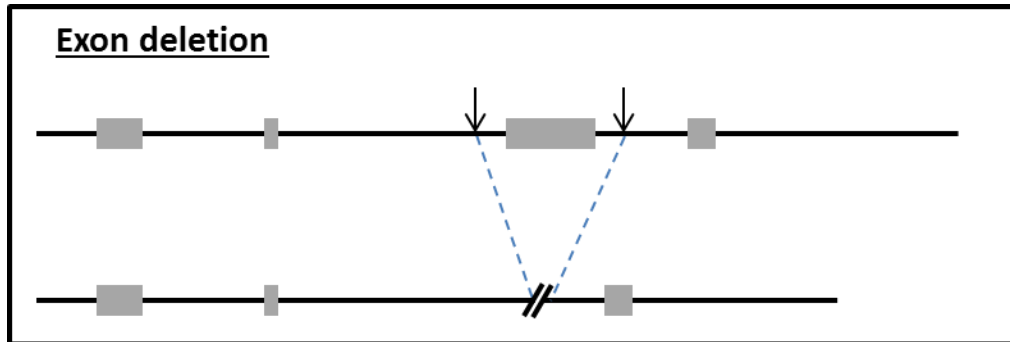
Assay name	Khdrbs1REGDELDONORMUT1
Forward Primer (5'-3')	GCCAGTGAAGGGAGCATACAG
Reverse Primer (5'-3')	AAGGGACCACGAAGAAAAATGTTGTGG
Probe (5'-3')	AGAGCACCCCTACGGCCGTTATTA
Label	FAM-BHQ1

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.

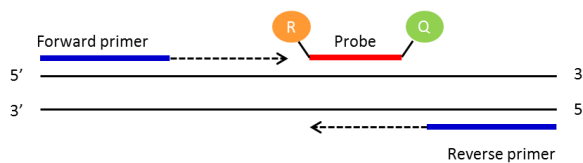
## Khdrbs1-REGDEL Genotyping Strategy

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).

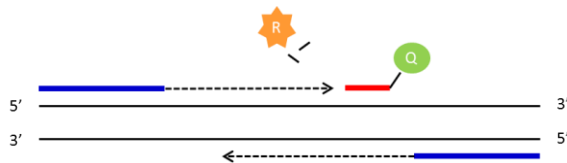


## qPCR genotyping strategy

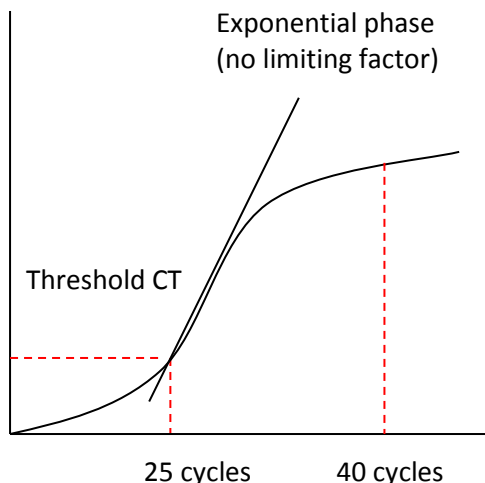
Standard PCR is the amplification of DNA between a pair of primers. Quantitative PCR employs the same principal as standard PCR, although it actually monitors the progress of the DNA synthesis as it occurs. The progress of the reaction is measured by using a Taqman probe. This is a short DNA oligo that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

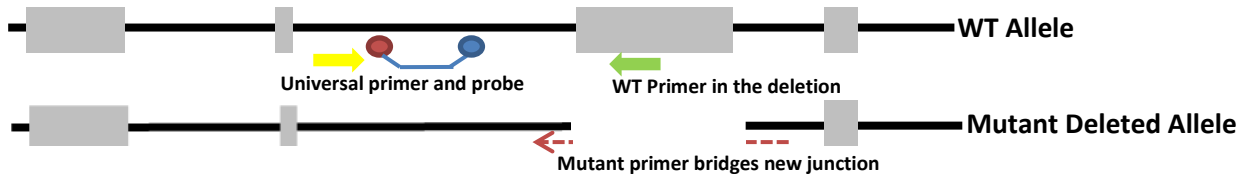
CT value can be used to determine how many copies of a particular allele samples have.

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) ————— TGTCTAAGTGTTTTCTTCGTGGTCCC  
 Khdrbs1-REGDEL-R (15nmol) ————— GAAGCCAGAATGCAGAGTTAAGAATG  
 Khdrbs1-REGDEL-F (15nmol) ————— TGCTTACCCACAACAGACAAG

## Khdrbs1-REGDEL MUT1 assay (FAM labelled)

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

## Dot1l internal control (VIC labelled)

TCATAGGGTGACTGGCCAACCCAGGGAAGCCGGAGTGCTGCGTCTTCTGTTTCCTTGTTCTTTCCCTCTAGTC  
 GTTTTCTGTTAGTAGTTGGCATCCTTATGCTTCATCTTACAGTGGACTTGAGAGCTGGCCCTGAATGGTCGTGCT  
 GGGGCAAGGCTTTATTTTCAGGCGTAGCACACATGGTGGCCAATGGGACTCTGTAGGATCTGCCACACCCATCAG

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
ddH2O	1.55µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

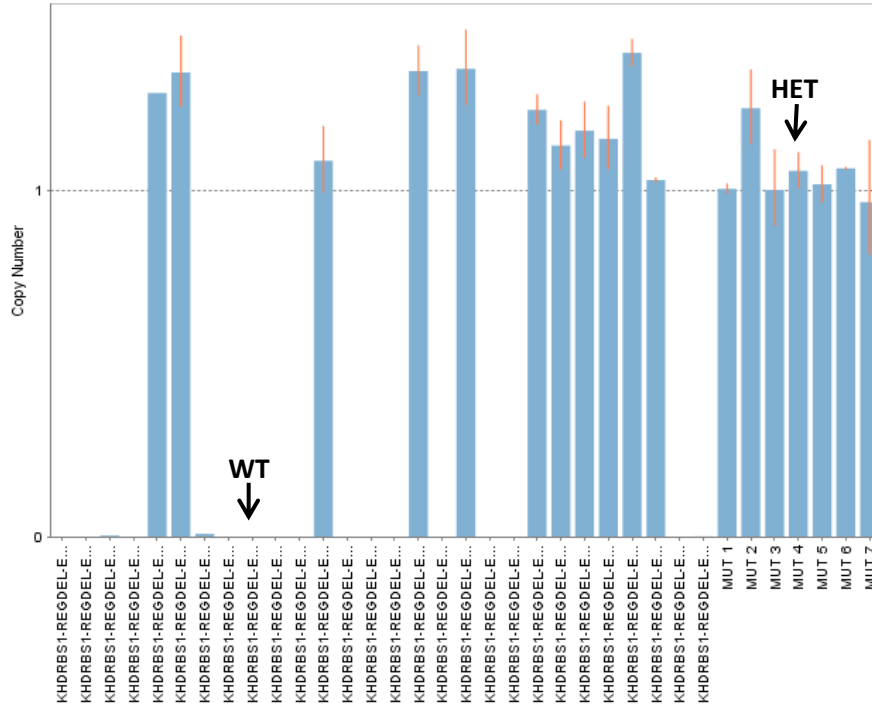




Khdrbs1-REGDEL Assay copy called result, image showing both replicates and controls for Mutant assay (T253303)

Applied Biosystems CopyCaller® Software v2.0

1. File: T253303\_MUT\_DF\_220719\_data.txt, Target: KHDRBS1-REGDEL-MUT, Calibrator: MUT 3



Version No. 2  
Date: 14/02/2020  
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