WDRCTCF-DEL11828

Allele Description

This is a CRISPR/Cas9 induced mutation deleting 11828 nucleotides of the *WDR25* gene, an intronic deletion removing two CTCF binding domains. The stock was generated at MRC Harwell via microinjection of CRISPR/Cas9 reagents into 1-cell stage embryos.

qPCR Copy Counting Genotyping Strategy

The genotyping strategy presented here has been optimized for reagents and conditions used by the Genotyping Core at MRC Harwell. To genotype animals, we recommend researchers validate the assay independently. PCR cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

Samples are genotyped using qPCR copy counting with both a wildtype loss of allele (WT-LOA) and a mutant assay against a known reference assay (*Dot1l* on chromosome 10; 2 copies present). Samples for this line are genotyped using the following primers and probe:

- Universal probe and Universal primer designed 5' of the deleted region.
- Wildtype specific primer situated within the deleted region.
- Mutant specific primer that either bridges the junction designed for the CRISPR mutant allele or is 3' of the junction

For autosomal genes that have been targeted, the following results would be expected:

Genotype of the Modified allele	WT Assay	Mutant Assay
Wildtype	2	0
Heterozygous	1	1
Homozygous mutant	0	2

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WDRCTCF-DEL970-WT1 assay (FAM labelled)

CTCTTCCTTCAGAGTGCTAAGAGAAAGGCAAGTGCCACTATGCTCCATGTTGTTTTTTAAGAAAGAG
TGAGTTGGCACCTTGGCT**CTGGGTGTGGTGAAGAAAAGTG**TGCCAGGACTGGTGTGACACTTCAC
GGC**CAGAAGAGGTCGCAGTTGGG**CTGCCTGGGATGACGAGAAGCCTGCGTTTAGGGCTGTGTGTG
GGATGCTGATGTGTGGCCTGTCCCAGGAGGCGTGTGGGCCGGAGGTGGTGATGCTAGTGTGAGAC

Lower case letters denote the deleted sequence in the mutant allele.
Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo WDRCTCF-DEL11828	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
WDRCTCF-DEL11828-WT _F	n/a	**CTGGGTGTGGTGAAGAAAAGTG**	n/a	WT Forward
WDRCTCF-DEL11828-WT_PROBE	FAM	**TGCCAGGACTGGTGTGACACT**	BHQ	WT Probe
WDRCTCF-DEL11828-WT_R	n/a	**CCCAACTGCGACCTCTTCTG**	n/a	WT Reverse

WDRCTCFDEL11828EM4-MUT1 assay (FAM labelled)

GCAGAGACTCCTGTTTGGCTCTAGCAAGGCTGATATGTTCTCTTCAATTCTTTGAGCCAGGCTTTGGT
GCTTCTGTGGTGTTGGGCTGTGTAATCTATGTGAGGGACGTTCAGTGGTCTG**GGTGGCTCATGGCA**
ATCCTGGACTGACCCAGAGTTT**TGAGAGCC[11828_nt_deletion]CACTATAGGTCCCACC**ATCAA
AGGACTCTGCTCTTTTTTCA**TGTAGTGCTAGTCCTGGAGGA**GTATAGGACACATAGTCAGTGCTAAG
AAGACATTTGTGAATGAGCCCTTTGAGCCGCAGGGTCTGGGAGACCTCCCAGAGGTCTCAATTACAC

Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo WDRCTCF-DEL11828	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
WDRCTCF-DEL11828-MUT _F	n/a	**GGTGGCTCATGGCAATCCT**	n/a	Mutant Forward
WDRCTCF-DEL11828-MUT _PROBE	FAM	**TGAGAGCCCACTATAGGTCCCACC**	BHQ	Mutant Probe
WDRCTCF-DEL11828-MUT_R	n/a	**TCCTCCAGGACTAGCACTACA**	n/a	Mutant Reverse

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Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT CACCCTATGA

Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo WDRCTCF-DEL11828	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	**GCCCCAGCACGACCATT**	n/a	WT Forward
Dot1l_Probe	VIC	**CCAGCTCTCAAGTCG**	BHQ	WT Probe
Dot1l_Reverse	n/a	**TAGTTGGCATCCTTATGCTTCATC**	n/a	WT Reverse

DNA extraction method

DNA is extracted from ear clips using Applied Biosystems Taqman Sample-to-SNP Kit and qPCR run using 1:10 dilution from the crude preparation.

qPCR master mix 1X

Applied Biosystems GTX Taqman master mix	5 µl
Dot1l_Forward (20 µM)	0.225 µl
Dot1l_Reverse (20 µM)	0.225 µl
Dot1l_Probe (5 µM)	0.2 µl
FAM Assay (probe 5µM & primers 15µM each)	0.3 µl
ddH20	1.55 µl
DNA (1:10 dilution of ABI Sample-to-SNP prep)	2.5 µl

Each sample is ran in technical duplicate. Seven WT and/or mutant controls are also included in duplicate. Non-template controls are also run.

qPCR cycling conditions

qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

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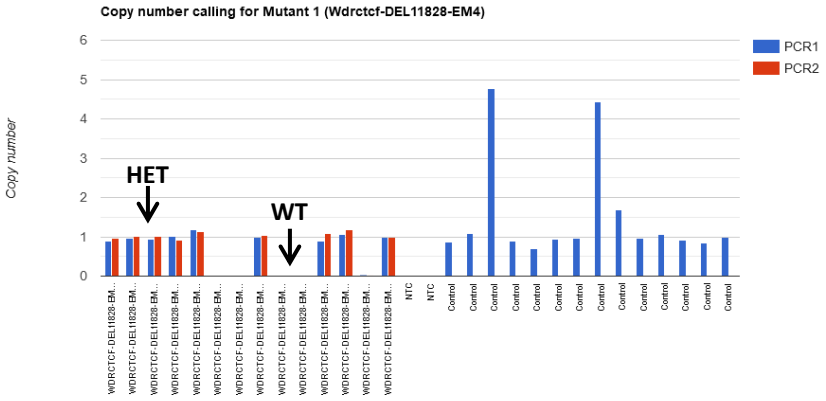
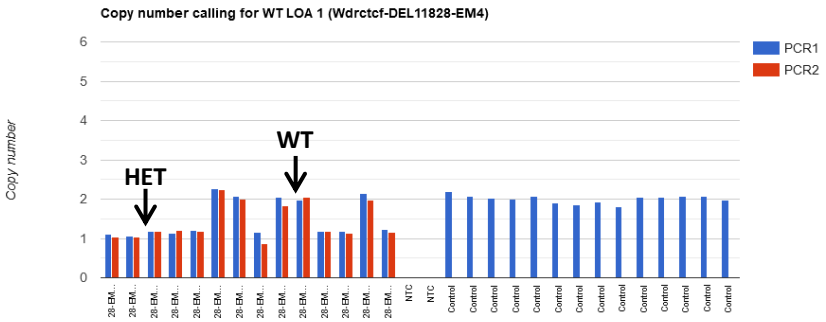
Analysis

The results are analysed using CopyCaller Software v2.0 from Applied Biosystems or in-house software that is based on CopyCaller v2.0.

WDRCTCF-DEL11828-WT1 and WDRCTCF-DEL11828-MUT1 copy called result, image showing copy number chart for WT and Mutant assays (Task 253749 results)





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