Mary Lyon Centre at MRC Harwell

Name of Mouse model or mutation:
TARDBP-M337V-EM2-B6J

Description:
Point mutation made by CRISPR/Cas9 gene editing introduced onto existing transgenic.

Type of mutation:
SNP: M337V

Delivery method:
Electroporation into 1-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
cagagcagttggggtatgat	ggg
ACAGAGCAGTTGGGGTATGA	TGG

ssODN donor sequence (5'-3'):
cagaaccgaaggcctggtttggctccctctgcatgttgccttggttttggttattacccgatgggcctgactggttctgctggctggctaacatgccc
aCcatTccccaactgctctgtagtgctgcctgggcggcagccatcatggctggattaatgctgaacgcaccaaagttcatcccaccacccatatt
actacct

Electroporation mixes:
Cas9 protein, sgRNAs and ssODNs were diluted and mixed in Electroporation buffer (EB; Gibco Opti-MEM I Reduced Serum Media – (Thermo Fisher Scientific)) to the working concentrations of 650 ng/µl, 130 ng/µl each and 400 ng/µl, respectively. Embryos were electroporated using the following conditions: 30 V, 3 ms pulse length, 100 ms pulse interval, 12 pulses. Electroporated embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F_0 progeny.

Sequence details

WT

ttgggggtttaaatgaaatgagtgttcattgcttatttttcctctggctttagataaattaatgcttgtaatctaagttttgttgctacttt
aaatatatgaatcagtggtttaatcttctttgtttacatcccttatttcttatagattgcgcagtctctttgtggagaggacttgatcatta
aaggaatcagcgttcatatatccaatgccgaacctaagcacaatagcaatagacagttagaaagaagtggaagatttggtggtaat
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tggtgggatgaactttggtgcgttcagcattaatccagccatgatggctgccgcccaggcagcactacagagcagttggggtatgat
gggcatgttagccagccagcagaaccagtcaggcccatcgggtaataaccaaaaccaaggcaacatgcagagggagccaaacca
ggccttcggttctggaaataactcttatagtggctctaattctggtgcagcaattggttggggatcagcatccaatgcagggtcgggc
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ataagttttgttatataaagggttaaaatactgagtgggtgaaagtgaactgctgtttgcctaattggtaaaccaacactacaattga
tctcagaaggtttctctgtaatattctatcattgaaattgttaatgaattctttgcatgttcagagtagaaacca

TARDBP-M337V-EM2-B6J

ttgggggtttaaatgaaatgagtgttcattgcttatttttcctctggctttagataaattaatgcttgtaatctaagttttgttgctacttt
aaatatatgaatcagtggtttaatcttctttgtttacatcccttatttcttatagattgcgcagtctctttgtggagaggacttgatcatta
aaggaatcagcgttcatatatccaatgccgaacctaagcacaatagcaatagacagttagaaagaagtggaagatttggtggtaat
ccaggtggctttgggaatcagggtggatttggtaatagcagaggggtggagctggtttgggaaacaatcaaggtagtaatatggg
tggtgggatgaactttggtgcgttcagcattaatccagccatgatggctgccgcccaggcagcactacagagcagttgggg**A**atg**G**
tgggcatgttagccagccagcagaaccagtcaggcccatcgggtaataaccaaaaccaaggcaacatgcagagggagccaaacc
aggccttcggttctggaaataactcttatagtggctctaattctggtgcagcaattggttggggatcagcatccaatgcagggtcggg
cagtggttttaatggaggctttggctcaagcatggattctaagtcttctggctggggaatgtaggtggtgggggtggttagtaggtt
ggttattaggttaggtagatttagaatggtgggattcaaatttttctaaactcatggtaagtatattgtaaaatacatatgtactaaaa
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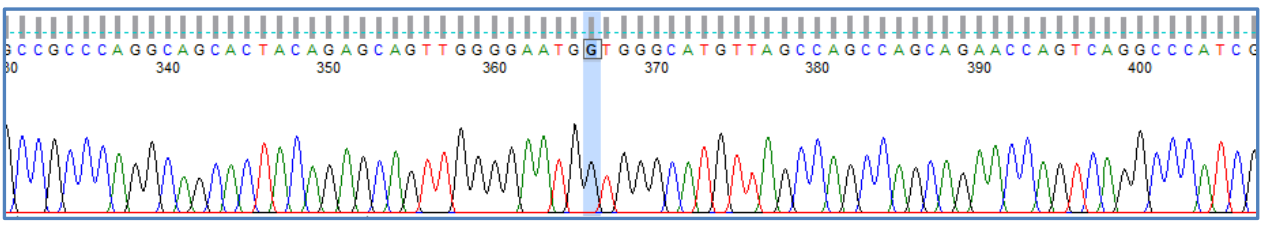
atctcagaaggtttctctgtaatattctatcattgaaattgttaatgaattctttgcatgttcagagtagaaacca

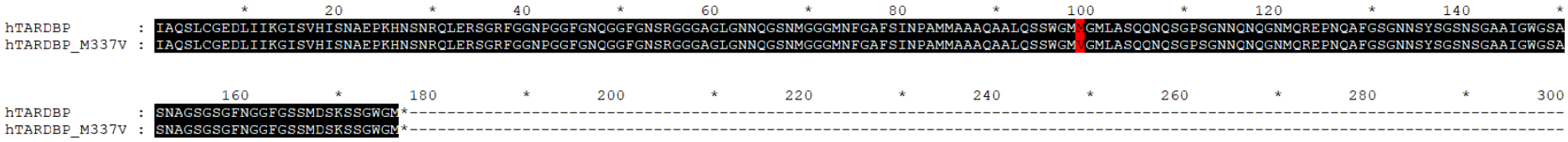
Red and bold = silent change to prevent re-processing by CRISPR/Cas9.
Red, bold and underlined = M337V change

F1 animal sequence trace:



Nucleotide Alignment:

Predicted Protein 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_ TARDBP_M337V_F1 (5'-3')	ttgggggtttaaatgaaatgagtgt
Geno_ TARDBP_M337V_R1 (5'-3')	tggtttctactctgaacatgcaaa
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	1046
Mutant product size (bp)	1046
Notes	Sequenced with Geno_TARDBP_M337V_F2 (ttcattgcttatttttcctctggc) Geno_TARDBP_M337V_R2 (gcaaacagcagttcactttcac)

All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site. F1 sequences appear homozygous as the PCR is centralised in the humanised region.

Off-target sites with ≤2 mismatches for guide(s) or considered risk sites used were checked with the following primers:

Off-target site	Sequence	Type	Primers used (5'-3')
4: 120573544-120573566	AAGTACAGTTGGGGTGTGATGGG	intron: Kcnq4	Geno_TARDBP_M337V_OT1F1: TGAATTTTCGTGGACCCTCCC Geno_TARDBP_M337V_OT1R1: CCTGTCAGGGGAAAAGGCTAC
4: 109122965-109122987	CACCGCTATTGGGGTATGATTGG	Intergenic: Calr4-Eps15	Geno_TARDBP_M337V_OT2F1: ACTACATAGCCAAGGACGAC Geno_TARDBP_M337V_OT2R1: TGAAAGGAAGACCAACCCAC
4: 148704543-148704565	CAGAGCAGTTGAGGTCAGTTTGG	intron: Tardbp	Geno_TARDBP_M337V_OT3F1: CAACAAATACTATTTGCCAGCC Geno_TARDBP_M337V_OT3R1: CTGCCTGCCTGTCTTCTAAG
4: 148702951-148702973	GCAGAGCAGTTGGGGTATGATGG	exon: Tardbp/Masp2	Geno_TARDBP_M337V_OT4F1: ACTCCACACTGAACAAACCA Geno_TARDBP_M337V_OT4R1: TGCCACCTTCCCTTTTCACA

4: 84826853-84826875	AGGGAGGAGATGGGGTATGAAGG	intron: Cntln	Geno_TARDBP_M337V_OT5F1: GAACTCACATGGGGCTCACT Geno_TARDBP_M337V_OT5R1: AGCAGAGGATCGCCTAGTTG

All amplicons were sent for Sanger sequencing.

No off-target activity was detected in the animals selected to establish the colony.

Additional integrations of the donor sequence

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	Tardbp-M337V-MUT1
Forward Primer (5'-3')	CAGAGCAGTTGGGGAATGG
Reverse Primer (5'-3')	TCTGCATGTTGCCTTGGT
Probe (5'-3')	TTAGCCAGCCAGCAGAACCAGTC
Label	FAM

This ddPCR assay is specific to the donor used to create the engineered mutation and only mutant alleles are expected to be recognised by this assay. Therefore, WT controls are expected to call at 0 copies and a single integration for a correct mutation is expected to call at 1 copy for F1 (HET) animals.

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.

No additional donor integrations were detected in the animals taken forward to establish the colony.

TARDBP-M337V

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; M337V in *TARDBP*. 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 wild type 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:

- Wild type (WT) assay with probe and reverse primer binding to the WT bases mutated in the mutant allele.
- Mutant assay with probe and forward primer binding to the M337V point mutations.

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

TARDBP-M337V

Mm00735064 WT assay (FAM labelled)

The Mm00735064 assay can be procured directly from Thermo Fischer.

TARDBP WT sequence

TCAAGGTAGTAATATGGGTGGTGGGATGAACTTTGGTGCGTTCAGCATTAATCCAGCCATGATGGCT
GCCGCCCAGGCAGCACTACAGAGCAGTTGGGGtATG**a**TGGGCATGTTAGCCAGCCAGCAGAACCAG
TCAGGCCCATCGGGTAATAACCAAAACCAAGGCAACATGCAGAGGGAGCCAAACCAGGCCTTCGGT
TCTGGAAATAACTCTTATAGTGGCTCTAATTCTGGTGCAGCAATTGGTTGGGGATCAGCATCCAA

Lower case letters denote bases changed in the mutant allele.
Lower case and bold letter denote the M337V base change.

TARDBP-M337V-MUT1 assay (FAM labelled)

TCAAGGTAGTAATATGGGTGGTGGGATGAACTTTGGTGCGTTCAGCATTAATCCAGCCATGATGGCT
GCCGCCCAGGCAGCACTA**CAGAGCAGTTGGGGaATG*g***TGGGCATG**TTAGCCAGCCAGCAGAACCA**
GTCAGGCCCATCGGGTAATAACCAAA**ACCAAGGCAACATGCAGA**GGGAGCCAAACCAGGCCTTCG
GTTCTGGAAATAACTCTTATAGTGGCTCTAATTCTGGTGCAGCAATTGGTTGGGGATCAGCATCCAA

Lower case letters denote bases changed in the mutant allele.
Lower case and italicized letter denote the M337V base change.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo TARDBP-M337V	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
TARDBP-M337V-MUT_F	n/a	**CAGAGCAGTTGGGGAATGG**	n/a	Mutant Forward
TARDBP-M337V-MUT_PROBE	FAM	**TTAGCCAGCCAGCAGAACCAGTC**	BHQ	Mutant Probe
TARDBP-M337V-MUT_R	n/a	**TCTGCATGTTGCCTTGGT**	n/a	Mutant Reverse

TARDBP-M337V

Dot1l internal control (VIC labelled)

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

Oligo TARDBP-M337V	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

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

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 5 µM & primers 15 µM 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 along with non-template controls.

qPCR cycling conditions

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

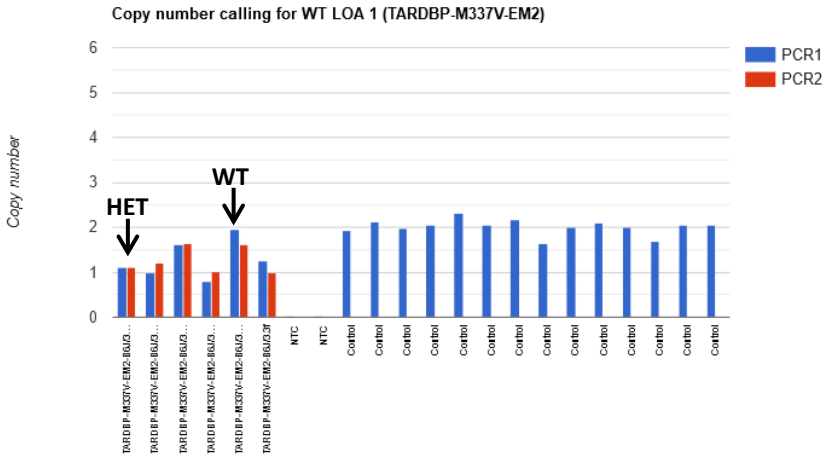
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

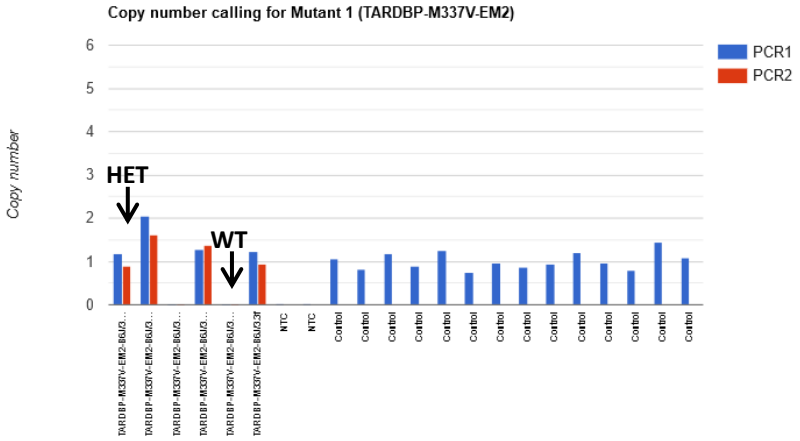
TARDBP-M337V

Analysis

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

Mm00735064 and TARDBP-M337V -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 384886 results)





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