Mary Lyon Centre at MRC Harwell

Name of Mouse model or mutation:
TARDBP-N390D-EM3-B6J
TARDBP-N390D-EM4-B6J
TARDBP-N390D-EM5-B6J

Please note that all these alleles have the same sequence and were produced using the same reagents. EM3 and EM4 were derived from different founders from the same session. EM5 was produced from a separate session/founder.

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

Type of mutation:
SNP: N390D

Delivery method:
Electroporation into 1-cell stage embryo.

Genetic Background:
C57BL/6J

Nuclease:
Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
tggggatcagcatccaatgc	agg
ggggatcagcatccaatgca	ggg

ssODN donor sequence (5'-3'):
cctactaaccacccccaccacctacattccccagccagaagacttagaatccatgcttgagccaaagcctccattaaaaccactgcccgaccct gcatCAgatgctgatccccaaccaattgctgcaccagaattagagccactataagagttatttccagaaccgaaggcctggtttggctccctctg catgttgcct

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

Existing transgenic allele aka 'WT'

ttgggggtttaaatgaaatgagtgttcattgcttatttttcctctggctttagataaattaatgcttgtaatctaagttttgttgctacttt
aaatatatgaatcagtggtttaatcttctttgtttacatcccttatttcttatagattgcgcagtctctttgtggagaggacttgatcatta
aaggaatcagcgttcatatatccaatgccgaacctaagcacaatagcaatagacagttagaaagaagtggaagatttggtggtaat
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agtggttttaatggaggctttggctcaagcatggattctaagtcttctggctggggaatgtaggtggtgggggtggttagtaggttg
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ataagttttgttatataaagggttaaaatactgagtgggtgaaagtgaactgctgtttgcctaattggtaaaccaacactacaattga
tctcagaaggtttctctgtaatattctatcattgaaattgttaatgaattctttgcatgttcagagtagaaacca

TARDBP-N390D-EM3-B6J/ TARDBP-N390D-EM4-B6J/TARDBP-N390D-EM5-B6J

ttgggggtttaaatgaaatgagtgttcattgcttatttttcctctggctttagataaattaatgcttgtaatctaagttttgttgctacttt
aaatatatgaatcagtggtttaatcttctttgtttacatcccttatttcttatagattgcgcagtctctttgtggagaggacttgatcatta
aaggaatcagcgttcatatatccaatgccgaacctaagcacaatagcaatagacagttagaaagaagtggaagatttggtggtaat
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tggtgggatgaactttggtgcgttcagcattaatccagccatgatggctgccgcccaggcagcactacagagcagttggggtatgat
gggcatgttagccagccagcagaaccagtcaggcccatcgggtaataaccaaaaccaaggcaacatgcagagggagccaaacca
ggccttcggttctggaaataactcttatagtggctctaattctggtgcagcaattggttggggatcagcatc**TG**atgcagggtcgggc
agtggttttaatggaggctttggctcaagcatggattctaagtcttctggctggggaatgtaggtggtgggggtggttagtaggttg
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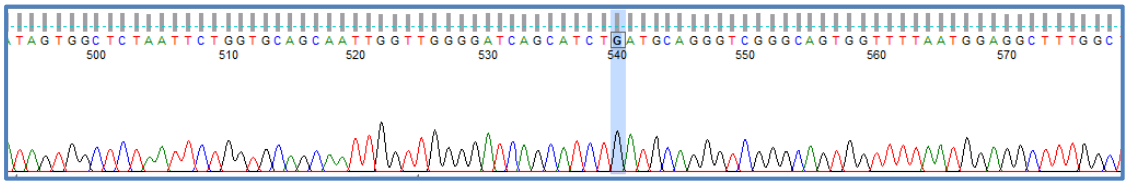
tctcagaaggtttctctgtaatattctatcattgaaattgttaatgaattctttgcatgttcagagtagaaacca

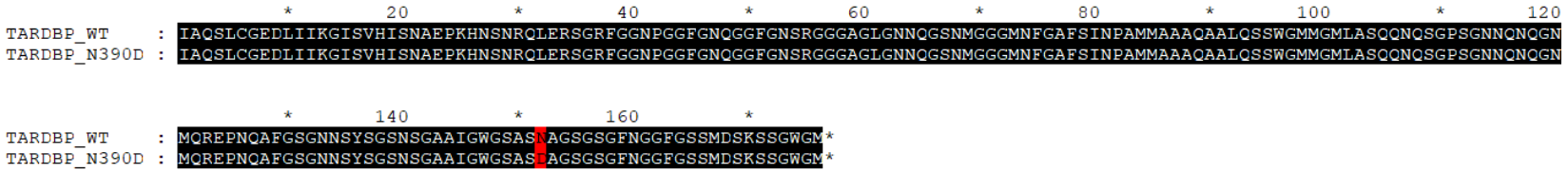
Red and bold = silent change to prevent re-processing by CRISPR/Cas9.
Red, bold and underlined = N390D change

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_N390D_F1 (5'-3')	ttgggggtttaaatgaaatgagtgt
Geno_TARDBP_N390D_R1 (5'-3')	tggtttctactctgaacatgcaaa
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.6
WT product size (bp)	1046
Mutant product size (bp)	1046
Notes	Amplicons sequenced with: Geno_TARDBP_M337V_F2 (ttcattgcttatttttcctctggc), Geno_TARDBP_M337V_R2(agcagttcactttcacccactc)

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 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: 148702788-148702810	TGGGGGTCAGCATCAAATGCAGG	exon: Tardbp/Masp2	Geno_TARDBP_N390D_OT1F1: AGCAGTTCACTTTCACCCACTC Geno_TARDBP_N390D_OT1R1: ATCCTTGTTTCTTACAGGTTGCCCA
18: 27351590-27351612	AGAGGAGCAGCATCCAATGCTGG	intergenic: Celf4-4930474G06Rik	Geno_TARDBP_N390D_OT2F1: ATTGCATCCTTCCTCGGCTC Geno_TARDBP_N390D_OT2R1: GGATCTCAGCAGGCTTCTCC
16: 51036513-51036535	GGGAAACAGCATCCAATGTAAGG	intergenic: 1700116B05Rik-Gm29686	Geno_TARDBP_N390D_OT3F1: TGGAATAAAAGGCAGATGGCATAC Geno_TARDBP_N390D_OT3R1: CTGGGCATGCTTTTCATGCTAC
8: 12131096-12131118	GGAGAGCAGCATCCAATGCCAGG	intergenic: Tex29-Sox1ot	Geno_TARDBP_N390D_OT4F1: TCAGTGGACTCAGAGTATTCAGT Geno_TARDBP_N390D_OT4R1: AATTGAACATGCGCCTTCCG

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-N390D-MUT1
Forward Primer (5'-3')	GGTTGGGGATCAGCATCTG
Reverse Primer (5'-3')	CCCAGCCAGAAGACTTAGAATC
Probe (5'-3')	AATGGAGGCTTTGGCTCAAGCATG
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-N390D

Allele Description

This is a CRISPR/Cas9 induced mutation creating a point mutation, N390D 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 reverse primer binding to the G601R, F606Y and R609H 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-N390D

Mm00735064 WT assay (FAM labelled)

The Mm00735064 assay can be procured directly from Thermo Fischer.

TARDBP WT sequence

GTCAGGCCCATCGGGTAATAACCAAAACCAAGGCAACATGCAGAGGGAGCCAAACCAGGCCTTCG
GTTCTGGAAATAACTCTTATAGTGGCTCTAATTCTGGTGCAGCAATTGGTTGGGGATCAGCATCc**a**AT
GCAGGGTCGGGCAGTGGTTTTAATGGAGGCTTTGGCTCAAGCATGGATTCTAAGTCTTCTGGCTGG
GGAATGTAGGTGGTGGGGGGTGGTTAGTAGGTTGGTTATTAGGTTAGGTAGATTTAGAATGGTGG

Lower case letters denote bases changed in the mutant allele.
Lower case and bold letter denote the N390D base change.

TARDBP-N390D-MUT1 assay (FAM labelled)

GTCAGGCCCATCGGGTAATAACCAAAACCAAGGCAACATGCAGAGGGAGCCAAACCAGGCCTTCG
GTTCTGGAAATAACTCTTATAGTGGCTCTAATTCTGGTGCAGCAATT**GGTTGGGGATCAGCATCt*g***AT
GCAGGGTCGGGCAGTGGTTTT**AATGGAGGCTTTGGCTCAAGCATG**GATTCTAAGTCTTCTGGCTGG
GGAATGTAGGTGGTGGGGGGTGGTTAGTAGGTTGGTTATTAGGTTAGGTAGATTTAGAATGGTGG

Lower case letters denote bases changed in the mutant allele.
Lower case and italicized letter denote the N390D base change.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo TARDBP-N390D	**5' label**	**Sequence 5' → 3'**	**3' label**	**Oligo Type**
TARDBP-N390D-MUT_F	n/a	GGTTGGGGATCAGCATCTG	n/a	Mutant Forward
TARDBP-N390D-MUT_PROBE	FAM	AATGGAGGCTTTGGCTCAAGCATG	BHQ	Mutant Probe
TARDBP-N390D-MUT_R	n/a	CCCAGCCAGAAGACTTAGAATC	n/a	Mutant Reverse

TARDBP-N390D

Dot1l internal control (VIC labelled)

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

Oligo TARDBP-N390D	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

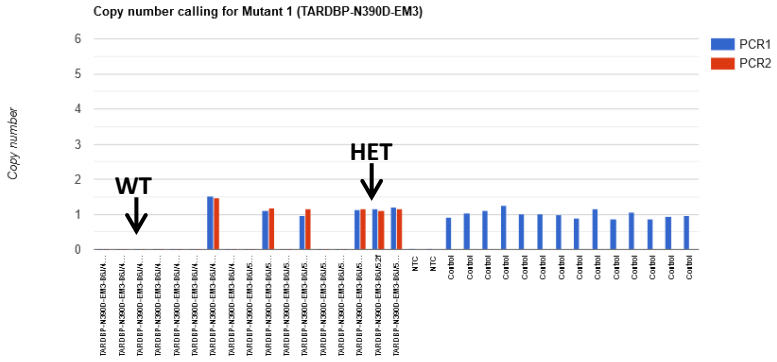
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

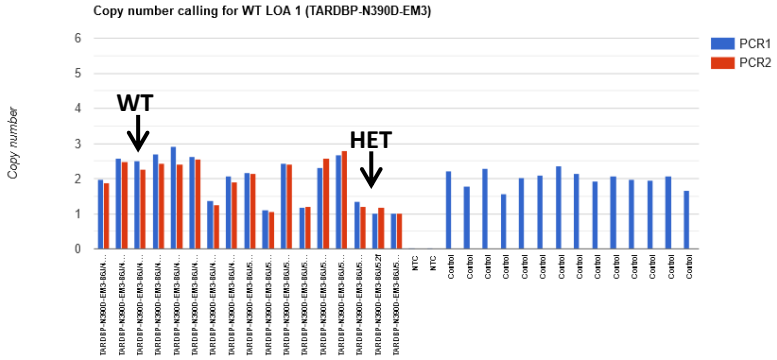
TARDBP-N390D

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-N390D -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 384886 results)





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