



Name of Mouse model or mutation:

Tasl-DEL10-EM3-B6J

Description:

Indel made by CRISPR/Cas9 gene editing.

Type of mutation:

Indel: 10 nt deleted in exon ENSMUSE00000697605.

Delivery method:

Electroporation into 1-cell stage embryo.

Genetic Background:

C57BL/6J

Nuclease:

Cas9 protein

sgRNAs:

Protospacer sequence	PAM sequence
TATTCATCAGTGCATGCAA	GGG

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/ μ l, 130 ng/ μ l each and 400 ng/ μ l, 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 F₀ progeny.

Sequence details

WT

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TTGCTGTCTAGACCCAGGAAAGAAATTTTTAAAATTGAGCAACATTACAGTTCTTGTTCAGTAGAA
TTTGCCACATATTTCTTCAGTCTTTTCTCATAACATACTAGCCATGTGGCTCTCCAGCTAGCAATGTCT
CCACTCCCGGCACCCCTCCGGGCACAACATAATTCTCTTGCCAATTCTTACAGCCCTTTGTAGTCATA
TTCCATGCTATTGTACCAGCCCCATTTCTTCTCAGCTTTTATTTTGTAGTGAATAGTCCATGCCACAGC
GTCTTCTTATAATTTAAGTCAGTAGCCCCAACTTCTGTTTACCCTTTATGCTTTTCGCTTCACTGCCACA
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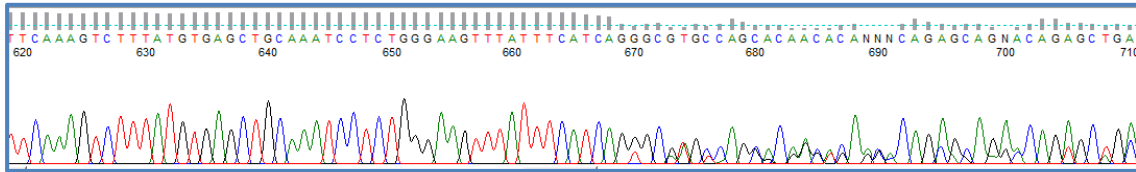
TGCCTTAAGCCACATCTTGTGAGTCTTTCTTTTATGCCCTTTTCTTTATGCTACCTTTGTGCCTACCACT
GTTATAATCCTCAGTATTATAATATTGGGATTATTTGTATCCAGTTTATTTTCTTCCATAAGAGAAGGA
TGGTCCTTTTCATCTTTGTATCTCTAGCATATAACATAGGTGTGGTATACATTAAGTATATAGTGAGTG
AATGAATTAATTTATCTTTCATTTTTCATATAGCAACTGTAAATTAATTTTTCTAGCCTTCTTTCCTCCC
ACTATTTCTCATGAATTCTTTTTCAGAATGCTATCAGAAGGATATCTCAGTGGACTTACCTACTGGA
ATGACATTCATTGGAATTGTGCATCTTATAATGAACCGGTGGCTGGGGACCAAGGCAAAGAGACAA
GTTCTGTTGCTGCTCTTTTCATATTCCTCTGTGGATGAAACACAAGTTCAAAGTCTTTATGTGAGCTGC
AAATCCTCTGGGAAGTTTATTTTCATCAGTGCATGCAAGGGCGAGTCAGCACAGCAGAAGCCAGAGC
AGAACAGTGCTGCAGGCAAACAGCAACCCTGTATTTGAAAGTCCAACCTTAGCTGCAGTTGGTATAT
GCAGAGATGTGATCAGGGAGACCTACTTGGTTCCACCTTCTTGAAAAGTATTTGCAAAAATTACAA
CGACTTACATATTGCAGGGGGACAGGTGATGGCCATTAECTCAGTAATGGCAAATTTCCCCTCTGAG
AGCAGCTTTGAAGATGGTCCTTTGCTAAAGTCATCTGAGATTTCTTTGTCCATGGAGGATTCCACTTC
CACTCAGCTCACTGAACTTCCCCTCAAACCTATCCAGCGGTACTCATCCTACTGGAGGATAACCAGCA
TCAAAGAGAAAAGCAGCCTGCAAATGCAGAAGCCTATTTCAAATGCAGTGCTCAATGAGTACCTGG
AGCAGAAGGTGGTGGAATTGTATAAGCAATATATTATGGACACTGTGTTTCATGACAGTTCTCCTAC
CCAGATTCTGGCATCAGAATTCATCATGACGAATGTAGATCAAATTAGTCTTCAAGTGTCTAAAGAG
AAGAACCTGGACACTTCAAAGTCAAGGACATAGTTATTAGCCACCTGTTGCAGTTGGTATCATCTG
AGATCAGCACCCCTAGTCTTCA

Tasl-DEL10-EM3-B6J

TTGCTGTCTAGACCCCAGGAAAGAAATTTTTAAAATTGAGCAACATTACAGTTCTTGTTCAGTAGAA
TTTGCCACATATTTCTTCAGTCTTTTCTCATAACATACTAGCCATGTGGCTCTCCAGCTAGCAATGTCT
CCACTCCCGGCACCCCTCCGGGCACAACATAATTCTCTTGCCAATTCTTACAGCCCTTTGTAGTCATA
TTCCATGCTATTGTACCAGCCCCATTTCTTCTCAGCTTTTATTTTGTAGTGAATAGTCCATGCCACAGC
GTCTTCTTATAATTTAAGTCAGTAGCCCCAACTTCTGTTTACCCTTTATGCTTTTCGTTCACTGCCACA
TGCCTTAAGCCACATCTTGTGAGTCTTTCTTTTATGCCCTTTTCTTTATGCTACCTTTGTGCCTACCACT
GTTATAATCCTCAGTATTATAATATTGGGATTATTTGTATCCAGTTTATTTTCTTCCATAAGAGAAGGA
TGGTCCTTTTCATCTTTGTATCTCTAGCATATAACATAGGTGTGGTATACATTAAGTATATAGTGAGTG
AATGAATTAATTTATCTTTCATTTTTCATATAGCAACTGTAAATTAATTTTTCTAGCCTTCTTTCCTCCC
ACTATTTCTCATGAATTCTTTTTCAGAATGCTATCAGAAGGATATCTCAGTGGACTTACCTACTGGA
ATGACATTCATTGGAATTGTGCATCTTATAATGAACCGGTGGCTGGGGACCAAGGCAAAGAGACAA
GTTCTGTTGCTGCTCTTTTCATATTCCTCTGTGGATGAAACACAAGTTCAAAGTCTTTATGTGAGCTGC
AAATCCTCTGGGAAGTTTATTTTCATCA[10_nt_del]GGGCGAGTCAGCACAGCAGAAGCCAGAGCA
GAACAGTGCTGCAGGCAAACAGCAACCCTGTATTTGAAAGTCCAACCTTAGCTGCAGTTGGTATATG
CAGAGATGTGATCAGGGAGACCTACTTGGTTCCACCTTCTTGAAAAGTATTTGCAAAAATTACAAC
GACTTACATATTGCAGGGGGACAGGTGATGGCCATTAECTCAGTAATGGCAAATTTCCCCTCTGAGA
GCAGCTTTGAAGATGGTCCTTTGCTAAAGTCATCTGAGATTTCTTTGTCCATGGAGGATTCCACTTCC
ACTCAGCTCACTGAACTTCCCCTCAAACCTATCCAGCGGTACTCATCCTACTGGAGGATAACCAGCAT
CAAAGAGAAAAGCAGCCTGCAAATGCAGAAGCCTATTTCAAATGCAGTGCTCAATGAGTACCTGGA
GCAGAAGGTGGTGGAATTGTATAAGCAATATATTATGGACACTGTGTTTCATGACAGTTCTCCTACC
CAGATTCTGGCATCAGAATTCATCATGACGAATGTAGATCAAATTAGTCTTCAAGTGTCTAAAGAGA

AGAACCTGGACACTTCAAAAAGTCAAGGACATAGTTATTAGCCACCTGTTGCAGTTGGTATCATCTGA
GATCAGCACCCCTAGTCTTCA

Heterozygous F1 animal sequence trace (female):



Nucleotide Alignment:

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                *      20      *      40      *      60      *      80      *      100     *      120     *      140     *
Tasl_WT : TTGCTGCTAGACCCAGGAAAGAAATTTTAAATTTAGCAACATTACAGTCTTGTTCAGTAGAATTTGCCACATATTTCTTCAGTCTTTTCTCATAACATACTAGCCATGTGGCTCTCCAGCTAGCAATGTCTCCACTCCCGGCAC
Tasl_EM3 : TTGCTGCTAGACCCAGGAAAGAAATTTTAAATTTAGCAACATTACAGTCTTGTTCAGTAGAATTTGCCACATATTTCTTCAGTCTTTTCTCATAACATACTAGCCATGTGGCTCTCCAGCTAGCAATGTCTCCACTCCCGGCAC

                160      *      180      *      200      *      220      *      240      *      260      *      280      *      300
Tasl_WT : CCCTCGGGCACAACATAATTTCTTGTCCAATTTCTTACAGCCCTTTGTAGTCATATTTCCATGCTATTTGTACCAGCCCCATTTCTTCTCAGCTTTTATTTTGTAGTGAATAGTCCATGCCACAGCGTCTTCTTATAATTTTAAAGTCAGTA
Tasl_EM3 : CCCTCGGGCACAACATAATTTCTTGTCCAATTTCTTACAGCCCTTTGTAGTCATATTTCCATGCTATTTGTACCAGCCCCATTTCTTCTCAGCTTTTATTTTGTAGTGAATAGTCCATGCCACAGCGTCTTCTTATAATTTTAAAGTCAGTA

                *      320      *      340      *      360      *      380      *      400      *      420      *      440      *
Tasl_WT : GCCCAACTTCTGTTTACCCTTTATGCTTTGCGCTTCACTGCCACATGCCTTAAGCCACATCTTGTGAGTCTTTCTTTTATGCCCTTTTCTTTATGCTACCTTTGTGCCCTACCCTGTTATAATCCTCAGTATTATAATTTGGGATTAAT
Tasl_EM3 : GCCCAACTTCTGTTTACCCTTTATGCTTTGCGCTTCACTGCCACATGCCTTAAGCCACATCTTGTGAGTCTTTCTTTTATGCCCTTTTCTTTATGCTACCTTTGTGCCCTACCCTGTTATAATCCTCAGTATTATAATTTGGGATTAAT

                460      *      480      *      500      *      520      *      540      *      560      *      580      *      600
Tasl_WT : TGTATCCAGTTTATTTTCTTCCATAAGAGAAGGATGGTCCCTTTCATCTTTGTATCTCTAGCATATAACATAGGTGTGGTATACATTAAGTATATAGTGAATGAATTAATTTATCTTTTCATTTTTCATATAGCAACTGTAAATTAAT
Tasl_EM3 : TGTATCCAGTTTATTTTCTTCCATAAGAGAAGGATGGTCCCTTTCATCTTTGTATCTCTAGCATATAACATAGGTGTGGTATACATTAAGTATATAGTGAATGAATTAATTTATCTTTTCATTTTTCATATAGCAACTGTAAATTAAT

                *      620      *      640      *      660      *      680      *      700      *      720      *      740      *
Tasl_WT : TTTTCTAGCCTTCTTTCCCTCCACTATTTCTCATGAATTTCTTTTTCAGAATGCTATCAGAAGGATATCTCAGTGGACTTACCTACTGGAATGACATTCATTGGAATTTGTGCATCTTATAATGAACCGGTGGCTGGGGACCAAGGCAAA
Tasl_EM3 : TTTTCTAGCCTTCTTTCCCTCCACTATTTCTCATGAATTTCTTTTTCAGAATGCTATCAGAAGGATATCTCAGTGGACTTACCTACTGGAATGACATTCATTGGAATTTGTGCATCTTATAATGAACCGGTGGCTGGGGACCAAGGCAAA

                760      *      780      *      800      *      820      *      840      *      860      *      880      *      900
Tasl_WT : GAGACAAGTTCTGTTGCTGCTCTTTCATATTCCTCTGTGGATGAAACACAAGTCAAGTCTTTATGTGAGCTGCAAATCTCTGGGAAGTTTATTTTCATCAGTGCATGCAAGGGCGAGTCAGCACAGCAGAAGCCAGAGCAGACAAGTCG
Tasl_EM3 : GAGACAAGTTCTGTTGCTGCTCTTTCATATTCCTCTGTGGATGAAACACAAGTCAAGTCTTTATGTGAGCTGCAAATCTCTGGGAAGTTTATTTTCATCAG-----GGCGAGTCAGCACAGCAGAAGCCAGAGCAGACAAGTCG

                *      920      *      940      *      960      *      980      *      1000     *      1020     *      1040     *
Tasl_WT : CTGCAGGCAACAGCAACCCTGTATTTGAAAGTCCAACCTTTAGCTGCAGTTGGTATATGCAGAGATGTGATCAGGGAGACCTACTTGGTCCACCTTCTTGTAAAAGTATTTGCAAAAATTACAACGACTTACATATTGCAGGGGGACAG
Tasl_EM3 : CTGCAGGCAACAGCAACCCTGTATTTGAAAGTCCAACCTTTAGCTGCAGTTGGTATATGCAGAGATGTGATCAGGGAGACCTACTTGGTCCACCTTCTTGTAAAAGTATTTGCAAAAATTACAACGACTTACATATTGCAGGGGGACAG

                1060      *      1080      *      1100      *      1120      *      1140      *      1160      *      1180      *      1200
Tasl_WT : GTGATGGCCATTAACCTCAGTAATGGCAAATTTCCCTCTGAGAGCAGCTTTGAAGATGGTCCCTTGTCAAAGTCACTGAGATTTCTTTGTCCATGGAGGATTCCTTCCACTCAGCTCACTGAACTTCCCTCAAACCTATCCAGCGG
Tasl_EM3 : GTGATGGCCATTAACCTCAGTAATGGCAAATTTCCCTCTGAGAGCAGCTTTGAAGATGGTCCCTTGTCAAAGTCACTGAGATTTCTTTGTCCATGGAGGATTCCTTCCACTCAGCTCACTGAACTTCCCTCAAACCTATCCAGCGG

                *      1220      *      1240      *      1260      *      1280      *      1300      *      1320      *      1340      *
Tasl_WT : TACTCATCTACTGGAGGATAACCAGCATCAAAGAGAAAAGCAGCCTGCAAATGCAGAAGCCATTTCAAATGCAGTGTCTCAATGAGTACCTGGAGCAGAAGGTGGTGGAAATGTATAAGCAATATATATGAGCACCTGTGTTTCATGAC
Tasl_EM3 : TACTCATCTACTGGAGGATAACCAGCATCAAAGAGAAAAGCAGCCTGCAAATGCAGAAGCCATTTCAAATGCAGTGTCTCAATGAGTACCTGGAGCAGAAGGTGGTGGAAATGTATAAGCAATATATATGAGCACCTGTGTTTCATGAC

                1360      *      1380      *      1400      *      1420      *      1440      *      1460      *      1480      *      1500
Tasl_WT : AGTTCTCCTACCAGATTTCTGGCATCAGAATTCATCATGACGAATGTAGATCAAATAGTCTTCAAGTGTCTAAAGAGAAGAACCTGGACACTTCAAAGTCAAGGACATAGTTATTAGCCACCTGTTGCAGTTGGTATCATCTGAGATC
Tasl_EM3 : AGTTCTCCTACCAGATTTCTGGCATCAGAATTCATCATGACGAATGTAGATCAAATAGTCTTCAAGTGTCTAAAGAGAAGAACCTGGACACTTCAAAGTCAAGGACATAGTTATTAGCCACCTGTTGCAGTTGGTATCATCTGAGATC

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Tasl_WT : AGCACCCCTAGTCTTCA
Tasl_EM3 : AGCACCCCTAGTCTTCA
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Predicted Protein Alignment:

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                *      20      *      40      *      60      *      80      *      100
Tas1_WT  : MLSEGYLSGLTYWNDIHWNCASYNEPVAGDQGKETSSVAALSYSSVDETQVQSLYVSKSSGKFISVHARASCHSRSCSRTVLCANSNEVFESPTLAAV
Tas1_EM3 : MLSEGYLSGLTYWNDIHWNCASYNEPVAGDQGKETSSVAALSYSSVDETQVQSLYVSKSSGKFISGRVSTAEARAEQCCRQTATLYLKVQL*-----

                *      120     *      140     *      160     *      180     *      200
Tas1_WT  : GICRDVIRETYLVPPSCKSICKNYNDLHIAGQVMAINSVMANFPSESSFEDGPLLSSEISLSMEDSTSTQLTELPLKPIQRYSSYWRITSIKEKSSLQ
Tas1_EM3 : -----

                *      220     *      240     *      260     *      280     *
Tas1_WT  : MQKPISNAVLNEYLEQKVVELYKQYIMDTVFHDSSPTQILASEFIMTNVDQISLQVSKEKNLDTSKVKDIVISHLLQLVSSEISTPSLHISQYSNITP*
Tas1_EM3 : -----
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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_5430427O19Rik_Flox_F1 (5'-3')	TTGCTGTCTAGACCCCAGGAA
Geno_5430427O19Rik_Flox_R1 (5'-3')	TGAAGACTAGGGGTGCTGATCT
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	1
WT product size (bp)	1517
Mutant product size (bp)	1507
Notes	Sequence with the following primers: Geno_5430427O19Rik_Flox_F2: CCTCCGGGCACAACATAATTC Geno_5430427O19Rik_Flox_R2: GAAGACTAGGGGTGCTGATCT

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.