

Name of Mouse model or mutation:

Nars-R556C-EM1-B6N

Nars-R556C-EM2-B6N

Description:

Point mutation generated using CRISPR/Cas9 reagents.

Type of mutation:

Point mutation: R556C (Nars-R556C-EM1-B6N point mutation only, no silent mutation)

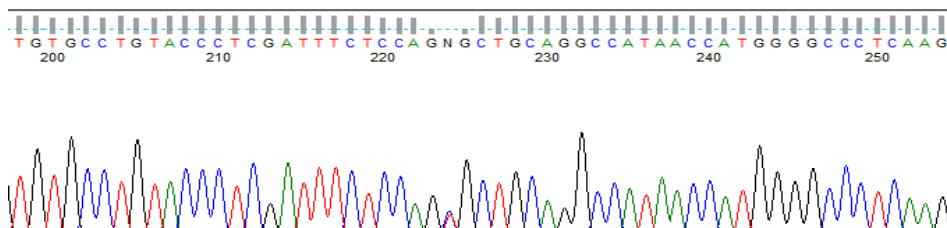
Sequence details**Nars WT:**

AGGCGTGTGGATTGTGTTGAATTACTACAGTGAGCTAACTGTTAGAGTGTGGAAAGTTAGCTCA
 TCGTAGTAATAATTTAATGTGATTAGATACAGTTTTAAATAAGCACTCTTCTCTCATTCAATTAAC
 AGAGAAAATATGGCACCTGTCCTCATGGAGGGTATGGCTTGGGCTTGGAACGATTTCTTAGCTGGA
 TTCTGAACAGGTATCACATCCGAGATGTGTGCCTGTACCCTCGATTTCTCCAGCGCTGCAGGCCATAA
 CCATGGGGCCCTCAAGCACGAAGGAAATGAAAAGTTAAGACTGCCTCTGCAAAAAGAACAACGCC
 AAATCGTCCCTTGACTTCCTGTTGGGGGTAGTAGCTTTCTCTTGACTTTCCATTATCAGAAAAAGCA
 GTAGGTATCACTTGAACATCAAGTGACATTGATGCTCCTTTAATGA

Nars-R556C-EM1-B6N:

AGGCGTGTGGATTGTGTTGAATTACTACAGTGAGCTAACTGTTAGAGTGTGGAAAGTTAGCTCA
 TCGTAGTAATAATTTAATGTGATTAGATACAGTTTTAAATAAGCACTCTTCTCTCATTCAATTAAC
 AGAGAAAATATGGCACCTGTCCTCATGGAGGGTATGGCTTGGGCTTGGAACGATTTCTTAGCTGGA
 TTCTGAACAGGTATCACATCCGAGATGTGTGCCTGTACCCTCGATTTCTCCAGIGCTGCAGGCCATAA
 CCATGGGGCCCTCAAGCACGAAGGAAATGAAAAGTTAAGACTGCCTCTGCAAAAAGAACAACGCC
 AAATCGTCCCTTGACTTCCTGTTGGGGGTAGTAGCTTTCTCTTGACTTTCCATTATCAGAAAAAGCA
 GTAGGTATCACTTGAACATCAAGTGACATTGATGCTCCTTTAATGA

Nucleotide changes highlighted in **red and underlined** = **nominated change**, silent changes highlighted in red only.

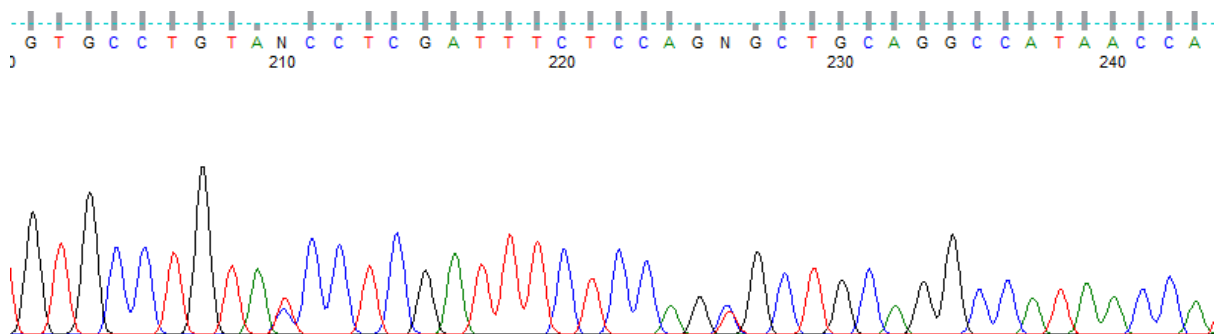
Nars-R556C-EM1-B6N Heterozygous F1 animal sequence trace:

Nars-R556C-EM2-B6N

AGGCGTGTGGATTGTGTTGAATTACTACAGTGAGCTAACTGTTAGAGTGTGGAAAGTTAGCTCA
TCGTAGTAATAATTTAATGTGATTAGATACAGTTTTAAAATAAGCACTCTTTCTCTCATTCAATTAAC
AGAGAAAATATGGCACCTGTCCTCATGGAGGGTATGGCTTGGGCTTGGAACGATTTCTTAGCTGGA
TTCTGAACAGGTATCACATCCGAGATGTGTGCCTGTATCCTCGATTTCTCCAGTGCTGCAGGCCATAA
CCATGGGGCCCTCAAGCACGAAGGAAATGAAAAGTTAAGACTGCCTCTGCAAAAGAACAAACGCC
AAATCGTCCCTTGACTTCCTGTTGGGGGTAGTAGCTTTCTCTTGACTTTCCATTATCAGAAAAAGCA
GTAGGTATCACTTGAACATCAAGTGACATTGATGCTCCTTAATGA

Nucleotide changes highlighted in red and underlined = nominated change, silent changes highlighted in red only.

Nars-R556C-EM2-B6N Heterozygous F1 animal sequence trace:



Nars-R556C-EM1-B6N and Nars-R556C-EM2-B6N Nucleotide Alignment:

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      *      20      *      40      *      60      *      80      *      100     *      120     *      140     *      160     *
Nars WT : AGGCGTGTGGATTGTGTTGAATTATTACTACAGTGAGCTAAGTCTAGAGTGTGGAAGTAGCTCATCGTAGTAATAATTTAATGTGATTAGATACAGTTTTAAAAATAAGCACTCTTCTCTCATTTCATTAAACAGAGAAAATATGGCACCTGTCTCATGGAGGGTATGGC : 174
Nars EM1 : AGGCGTGTGGATTGTGTTGAATTATTACTACAGTGAGCTAAGTCTAGAGTGTGGAAGTAGCTCATCGTAGTAATAATTTAATGTGATTAGATACAGTTTTAAAAATAAGCACTCTTCTCTCATTTCATTAAACAGAGAAAATATGGCACCTGTCTCATGGAGGGTATGGC : 174
Nars EM2 : AGGCGTGTGGATTGTGTTGAATTATTACTACAGTGAGCTAAGTCTAGAGTGTGGAAGTAGCTCATCGTAGTAATAATTTAATGTGATTAGATACAGTTTTAAAAATAAGCACTCTTCTCTCATTTCATTAAACAGAGAAAATATGGCACCTGTCTCATGGAGGGTATGGC : 174
      180      *      200      *      220      *      240      *      260      *      280      *      300      *      320      *      340
Nars WT : TTGGGCTTGGAACGATTTCTTAGCTGGATTCTGAACAGGTATCACAATCCGAGATGTGTGCCTGTA CCTCGATTTCTCCAG GCTGCAGGCCATAACCATGGGGCCCTCAAGCACGAAGGAAATGAAAAGTTAAGACTGCCTCTGCAAAAGAACAACGCCCAAATCGTCCCTT : 348
Nars EM1 : TTGGGCTTGGAACGATTTCTTAGCTGGATTCTGAACAGGTATCACAATCCGAGATGTGTGCCTGTA CCTCGATTTCTCCAG GCTGCAGGCCATAACCATGGGGCCCTCAAGCACGAAGGAAATGAAAAGTTAAGACTGCCTCTGCAAAAGAACAACGCCCAAATCGTCCCTT : 348
Nars EM2 : TTGGGCTTGGAACGATTTCTTAGCTGGATTCTGAACAGGTATCACAATCCGAGATGTGTGCCTGTA CCTCGATTTCTCCAG GCTGCAGGCCATAACCATGGGGCCCTCAAGCACGAAGGAAATGAAAAGTTAAGACTGCCTCTGCAAAAGAACAACGCCCAAATCGTCCCTT : 348
      *      360      *      380      *      400      *      420      *      440      *
Nars WT : GACTTCCTGTTGGGGGTAGTAGCTTTCTCTTGACTTTCCTTATCAGAAAAAGCAGTAGGTATCACTTGAACATCAAGTGACATTGATGCTCCTTTAATGA : 450
Nars EM1 : GACTTCCTGTTGGGGGTAGTAGCTTTCTCTTGACTTTCCTTATCAGAAAAAGCAGTAGGTATCACTTGAACATCAAGTGACATTGATGCTCCTTTAATGA : 450
Nars EM2 : GACTTCCTGTTGGGGGTAGTAGCTTTCTCTTGACTTTCCTTATCAGAAAAAGCAGTAGGTATCACTTGAACATCAAGTGACATTGATGCTCCTTTAATGA : 450
      GACTTCCTGTTGGGGGTAGTAGCTTTCTCTTGACTTTCCTTATCAGAAAAAGCAGTAGGTATCACTTGAACATCAAGTGACATTGATGCTCCTTTAATGA

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Nars-R556C-EM1-B6N and Nars-R556C-EM2-B6N Predicted Protein Alignment:

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      *      20      *      40
Nars WT : RKYGTCPHGGYGLGLERFLSWILNRYHIRDVCLYPRFLQ CRE* : 43
Nars EM1 : RKYGTCPHGGYGLGLERFLSWILNRYHIRDVCLYPRFLQ CRP* : 43
Nars EM2 : RKYGTCPHGGYGLGLERFLSWILNRYHIRDVCLYPRFLQ CRE* : 43
      RKYGTCPHGGYGLGLERFLSWILNRYHIRDVCLYPRFLQ CRP

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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_NARS_R556C_F1	AGGCGTGTGGATTGTGTTGA
Geno_NARS_R556C_R1	TCATTAAAGGAGCATCAATGTCAC
Taq Polymerase used	Roche Expand Long Range DNTPack
Annealing Temperature (°C)	58
Elongation time (min)	1
WT product size (bp)	450
Mutant product size (bp)	450
Notes	

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.

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 Dot11:

Assay name	NARS-R556C-UNI2
Forward Primer (5'-3')	TTAGCTGGATTCTGAACAGGTATCAC
Reverse Primer (5'-3')	GCAGAGGCAGTCTTAACTTTTCATT
Probe (5'-3')	CATGGGGCCCTCAAGCACGAAG
Label	FAM-BHQ1

The ddPCR assay recognises sequence common to both the WT Nars and the R556C mutant. Therefore, WT controls and correctly targeted F1 R556C heterozygote animals will call at 2 copies.

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.