

Two novel alleles in *C. elegans mir-1822* gene.

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A



B

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wt          ATTCTGATTCTTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAACTCTTGGCTCATCGAGAAATTTCTAAA'
zen100      ATTCTGATTCTTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAACTCTTGGCTCATCGAGAAATTTCTAAA'
pre-miR-1822  ATTCTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAACTCTTGGCTCATCGAGAAA
miR-1822*   AGTTTCTCTGGGAAAGCTATCGGC
miR-1822    GAGCTGCCCTCAGAAAACTCT
    
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C

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wt          'ATTCTGATTCTTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAACTCTTGGCTCATCGAGAAATTTCTAAA'
zen101      'ATTCTGATTCTTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAACTCTTGGCTCATCGAGAAA'
pre-miR-1822  ATTCTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAACTCTTGGCTCATCGAGAAA
miR-1822*   AGTTTCTCTGGGAAAGCTATCGGC
miR-1822    GAGCTGCCCTCAGAAAACTCT
    
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(A) *zen100* and *zen101* are novel alleles of *mir-1822*. (B,C) Sequence information for the two *mir-1822* deletions. (B) *zen100* removes 43 bps from the *mir-1822* locus. (C) *zen101* removes 41 bps from the *mir-1822* locus.

Description

microRNAs are small noncoding RNAs of ~22 nucleotides in length that regulate gene expression by degrading target mRNAs or inhibiting their translation. To our knowledge *mir-1822* currently lacks deletion alleles, impeding *mir-1822* functional characterization. We generated two new deletions of the *C. elegans mir-1822* locus, using the CRISPR-Cas9 genome editing technique. The following *mir-1822* specific Alt-R crRNAs were ordered from IDT: gRNA1, 5'-AGTTTCTCTGGGAAAGCTAT-3' and gRNA2: 5'-TGAGCCAAGAGTTTTTCTGA-3'. To create the deletions, Cas9 (Alt-R Cas9, IDT) was loaded with the two *mir-1822* guide RNAs, *dpy-10* guide RNA (Arribere et al, 2014) (IDT), and tracer RNA (IDT) and the mixture was injected into *C. elegans*. The resulting progeny were screened for CRISPR-Cas9 positive animals as previously described (Arribere et al, 2014). The following PCR primers were used to screen for deletions of interest: *mir-1822.for1*: 5'-CGGAAGGACACCTGCCACCAATG-3' and *mir-1822.rev1*: 5'-GAGGGCAATCTTCTTCTGGTCGCC-3'.

Using PCR screening, we identified two independent deletions of approximately 40 nucleotides each, with the positions of each deletion schematized in Figure 1A. *mir-1822(zen100)* removes 43 base pairs (Fig. 1B), and *mir-1822(zen101)* deletion removes 41 base pairs from the *mir-1822* precursor region (Fig. 1C). Each deletion was sequenced twice for confirmation. Both *mir-1822* alleles are homozygous viable and appear to be superficially wild type, with no obvious phenotypes observed in either strain.

Reagents

UY265 *mir-1822(zen100)* and UY266 *mir-1822(zen101)* strains are available upon request.

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References

Arribere JA, Bell RT, Fu BX, Artiles KL, Hartman PS, Fire AZ (2014). “Efficient Marker-Free Recovery of Custom Genetic Modifications with CRISPR/Cas9 in *Caenorhabditis elegans*.” *GENETICS* 198(3): 837-846. DOI: <https://doi.org/10.1534/genetics.114.169730> | PMID: 25161212.

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