

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

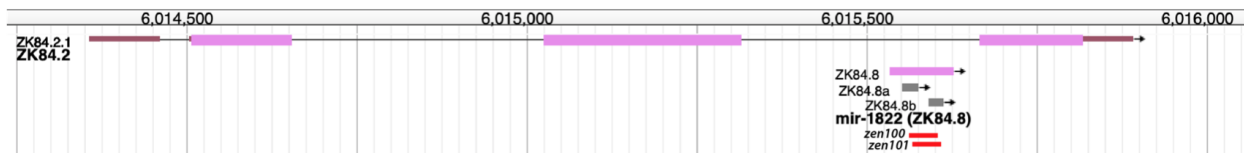
Marcy Anderson^{1*}, Maddy Mash^{1*}, Dustin Haskell¹, Shilpa Hebbar¹ and Anna Y Zinovyeva^{1§}

¹Division of Biology, Kansas State University, Manhattan, KS, USA

[§]To whom correspondence should be addressed: zinovyeva@ksu.edu

*These authors contributed equally.

A



B

```

wt          ATTCTGATTCTTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAAACTCTTGGCTCATCGAGAAATTTCTAAA'
zen100      ATTCTGATTCTTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAAACTCTTGGCTCATCGAGAAATTTCTAAA'
pre-miR-1822  ATTCTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAAACTCTTGGCTCATCGAGAAA
miR-1822*   AGTTTCTCTGGGAAAGCTATCGGC
miR-1822    GAGCTGCCCTCAGAAAAACTCT
  
```

C

```

wt          'ATTCTGATTCTTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAAACTCTTGGCTCATCGAGAAATTTCTAAA'
zen101      'ATTCTGATTCTTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAAACTCTTGGCTCATCGAGAAATTTCTAAA'
pre-miR-1822  ATTCTGAAAACCTCAATAGTTTCTCTGGGAAAGCTATCGGCCAATTTAACTGTCCGAGCTGCCCTCAGAAAAACTCTTGGCTCATCGAGAAA
miR-1822*   AGTTTCTCTGGGAAAGCTATCGGC
miR-1822    GAGCTGCCCTCAGAAAAACTCT
  
```

(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.

Acknowledgments: These deletions were created during the BIOL676 Molecular Genetics course at Kansas State University. We thank all the students of the Fall 2019 BIOL676 course for help with screening.

References

1/16/2020 - Open Access

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.

Funding: Funding for BIOL676 is provided by Kansas State University. This work was in part supported by R35GM124828 to A.Z.

Author Contributions: Marcy Anderson: Investigation, Writing - original draft. Maddy Mash: Investigation, Writing - original draft. Dustin Haskell: Investigation, Supervision. Shilpa Hebbar: Investigation, Supervision. Anna Y Zinovyeva: Supervision, Writing - review and editing, Funding acquisition, Resources.

Reviewed By: Katherine McJunkin

History: Received December 28, 2019 Accepted January 9, 2020 Published January 16, 2020

Copyright: © 2020 by the authors. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Citation: Anderson, M; Mash, M; Haskell, D; Hebbar, S; Zinovyeva, AY (2020). Two novel alleles in *C. elegans mir-1822* gene.. microPublication Biology. <https://doi.org/10.17912/micropub.biology.000212>