microPublication BIOLOGY

1/16/2020 - *Open Access*

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

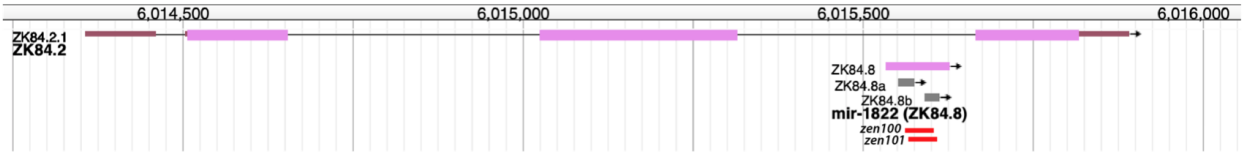
Marcy Anderson[1*], Maddy Mash[1*], Dustin Haskell[1], Shilpa Hebbar[1] and Anna Y Zinovyeva[1§]

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

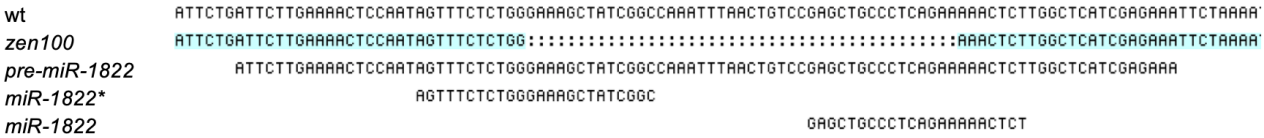
[§]To whom correspondence should be addressed: zinovyeva@ksu.edu

[*]These authors contributed equally.

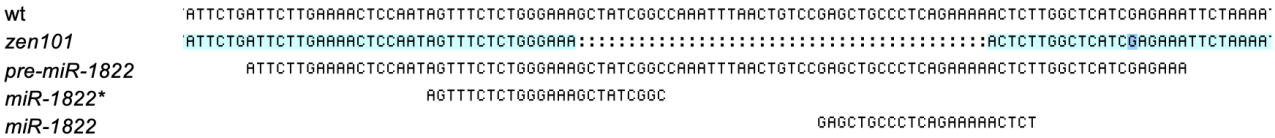
A



B



C



(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

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

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