Two Deletion Alleles in the *C. elegans* mir-49 gene.

Cassandra Delich1*, Annabelle Dillon1*, Noah Winans1*, Shilpa Hebbar1*, Dustin Haskell1 and Anna Zinovyeva1§

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

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

*These authors contributed equally.

**Figure 1:** (A) A schematic of the *mir-49* locus and the location of the newly generated *mir-49*(zen99) and *mir-49*(zen102) deletion alleles. (B) *zen99* removes 56 base pairs from the *mir-49* precursor. (C) *zen102* removes 58 base pairs from the *mir-49* precursor.

**Description**

MicroRNAs (miRNAs) are small, non-coding RNAs that post-transcriptionally repress gene expression (Gebert and MacRae, 2018). While many miRNA genes and their families have been analyzed for function (Miska et al. 2007, Alvarez-Saavedra and Horvitz 2010), there are microRNA genes for which loss of function alleles have not yet been generated. There are no available alleles for the *C. elegans* mir-49 gene.

Using CRISPR-Cas9 genome editing, we generated two deletion alleles, *zen99* and *zen102*, that disrupt the *C. elegans* mir-49 gene (Fig 1A). *mir-49*(zen99) and *mir-49*(zen102) delete 56 base pairs and 58 base pairs from the *mir-49* locus, respectively (Fig 1B and Fig 1C). Each deletion nearly completely removes both strands generated by the *mir-49* locus, *mir-49*-3p and *mir-49*-5p. Both *mir-49* alleles are homozygous viable and appear to be superficially wild type. Careful phenotypic analysis will be important to characterize the effects of the two *mir-49* deletions.

**Methods**

To generate the *mir-49* deletion alleles, N2 animals were injected with the CRISPR-Cas9 components as an RNA-protein complex (Paix et al. 2015). The following components were used: Alt-R Cas9 (IDT, cat# 1081058) loaded with *mir-49* crRNAs (IDT, custom) (*mir-49* crRNA1 sequence: 5’-GAGCACATCACAACAAACTG-3’, *mir-49* crRNA2 sequence: 5’-GCACCACGAGAAGCTGCAGA-3’), dpy-10 targeting guide RNA (IDT, custom) (5’-GCUACCAUAGGCACCACGAG-3’), Arribere et al. 2014 and tracer RNA (IDT, cat# 1072532) (AGCAUAGCAUGUAAAUAUGGCCUGUUUAUCAACUGUGAAAAAGUGGACCGAGUCGGUGCUU). Briefly, to load the Alt-R Cas9, the following mixture was incubated at 37°C for 15 minutes: 0.5µL of Alt-R Cas9, 2.4µL of tracrRNA (0.4µg/µL), 0.8µL of *mir-49* crRNA1 (0.4µg/µL), 0.8µL of *mir-49* crRNA2 (0.4µg/µL), 1.3 µL of dpy-10 crRNA (0.1µg/µL), 1µL IDT annealing buffer (provided with Alt-R Cas9), and 3.2µL of water. Following the incubation, the mixture was spun for 2 minutes at top speed (~10,000rpm). The progeny of the injected animals was first screened for the presence of dumpy worms to identify parents positive for Cas9 activity (Arribere et al. 2014). F1 offspring of the Cas9-positive parents were then genotyped for the presence of potential *mir-49* deletions using the following primers: mir-
49.for1 (5’-AGGCACCACCTACCACTTATTACCAT-3’) and mir-49.rev1 (5’-GATGACTTACGTCGCTTTCTT-3’), which generate a wild type product of ~430 bps. Independent mir-49 deletions were identified, homozygosed, and sequenced. The resultant strains, UY264 (mir-49[zen99]) and UY267 (mir-49 [zen102]) were not outcrossed, but appear to be free of background dpy-10 mutations. Sequencing was repeated in the next generation to ensure the stability of the generated alleles.

Reagents
UY264 mir-49[zen99] and UY267 mir-49 (zen102) are available upon request.

Acknowledgments: This deletion was generated during the Molecular Genetics Laboratory Class (BIOL 676) at Kansas State University’s Division of Biology. We thank the Fall BIOL676 class for help with screening during this experiment: Astrid Altamirano, Marcy Anderson, Marielena Banos Del Mazo, Elise Barna, Colin Ferrel, Connor Horn, Jason Leftwich, Joshua Lingo, Maddison Mash, Robert Sholl, and Ce (Lily) Zheng.

References


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

Author Contributions: Cassandra Delich: Investigation, Writing - original draft. Annabelle Dillon: Investigation, Writing - original draft. Noah Winans: Investigation, Writing - original draft. Shilpa Hebbar: Investigation, Supervision, Writing - review and editing. Dustin Haskell: Supervision. Anna Zinovyeva: Supervision, Writing - review and editing, Resources, Funding acquisition.

Reviewed By: Anonymous

History: Received February 20, 2020 Accepted April 1, 2020 Published April 1, 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: Delich, C; Dillon, A; Winans, N; Hebbar, S; Haskell, D; Zinovyeva, A (2020). Two Deletion Alleles in the C. elegans mir-49 gene.. microPublication Biology. https://doi.org/10.17912/micropub.biology.000236