

## Two new CRISPR-generated alleles in the *C. elegans mir-1022* gene.

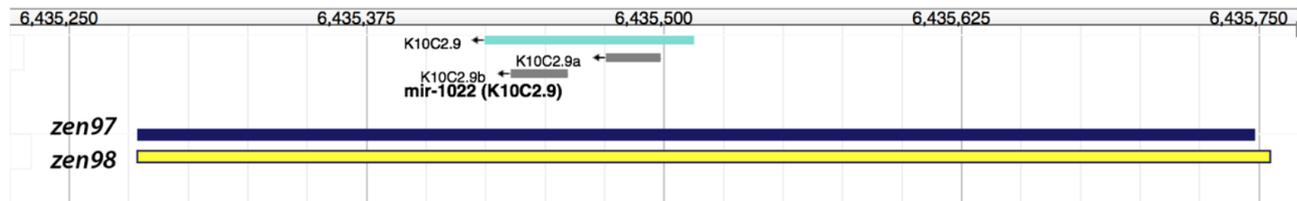
Connor Horn<sup>1\*</sup>, Robert Sholl<sup>1\*</sup>, Dustin Haskell<sup>1</sup>, Shilpa Hebbar<sup>1</sup> and Anna Zinovyeva<sup>1§</sup>

<sup>1</sup>Division of Biology, Kansas State University, Manhattan, KS

<sup>§</sup>To whom correspondence should be addressed: zinovyeva@ksu.edu

\*These authors contributed equally.

A



B

**zen97** CGGACTATGTCGGGCCAACACATTT..... [GAGGGGATT] ..... TGAGGGGATATACATTGTGAAAAATGGG  
**zen98** CGGACTATGTCGGGCCAACACA... [CGGTGTACAATGTATAACAATGT] ...ATATACATTGTGAAAAATGGG

**Figure 1:** Using CRISPR/Cas9, two *mir-1022* deletions/insertions (indels), *zen97* and *zen98*, were generated. (A) A schematic of the *C. elegans mir-1022* locus. The location of *zen97* and *zen98* deletions are designated by the blue and yellow rectangle, respectively. (B) The genetic sequence of the *zen97* and *zen98* deletions' endpoints in the *mir-1022* locus, with the deletions represented by the ellipsis. Insertion sequence within each allele is in brackets.

### Description

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression during animal development. Large scale efforts to identify functions of individual miRNAs or miRNA families provide invaluable insight into the roles these miRNAs play in organism development (Miska *et al.* 2007, Alvarez-Saavedra and Horvitz 2010), however, some miRNA genes lack deletion alleles. To our knowledge, no alleles in *C. elegans mir-1022* gene are currently available.

Using CRISPR/Cas9 genome editing technique, we generated two novel alleles in the *mir-1022* locus in *C. elegans* (Fig 1A). The *zen97* allele is a 498 pair deletion of the *mir-1022* locus which also inserts GAGGGGATT into that locus (Fig 1B). The *zen98* allele is a 508 base pair deletion from the *mir-1022* locus, inserting CGGTGTACAATGTATAACAATGT in that location (Fig 1B).

To specifically target the *mir-1022* locus for editing, we used the following guide RNAs: *mir-1022\_gRNA* 1: 5'-TCGGGCCAACACATTTTCAG-3' and *mir-1022\_gRNA* 2: 5'-TTTGCCTGCGAAAGTGGTGA-3'. The primers used for PCR genotyping were *mir-1022.for3*: 5'-CTAGTGCATTGTCCAGGCAG-3' and *mir-1022.rev3*: 5'-CGTGATCTCTTGGTGCACAT-3'. The Co-CRISPR marker *dpy-10* was used in order to more easily identify worms with active CRISPR/Cas9 as previously described (Arribere *et al.* 2014). CRISPR components were injected into N2 animals as an RNP complex (Paix *et al.* 2015). Alt-R Cas9 (cat# 1081058), *mir-1022* and *dpy-10* Alt-R® CRISPR-Cas9 crRNAs (custom), and tracer RNA (cat# 1072532) were purchased from IDT. PCR screening identified two independent mutations, each disrupting the *mir-1022* locus (Fig 1), which were subsequently homozygosed and sequenced. UY262 *mir-1022(zen97)* did not segregate any dumpy, dumpy roller, or roller animals and was not outcrossed. *mir-1022(zen98)* was outcrossed once to wild type (N2) males to remove a background *dpy-10* mutation, generating the 1x outcrossed UY286 *mir-1022(zen98)* strain. Sequencing was repeated to confirm the mutation.

Both alleles are homozygous viable. While not extensively characterized, no gross morphological phenotype was produced by either *mir-1022* allele. Further phenotypic analysis will be necessary to determine the exact effect of the two *mir-1022* mutations.

### Reagents

UY262 *mir-1022(zen97)* and UY286 *mir-1022(zen98)* (1x outcrossed) are available upon request.

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

### References

Miska EA, Alvarez-Saavedra E, Abbott AL, Lau NC, Hellman AB, McGonagle SM, et al. (2007). “Most *Caenorhabditis elegans* microRNAs Are Individually Not Essential for Development or Viability.” *PLoS Genet* 3(12): e215. DOI: 10.1371/journal.pgen.0030215 | PMID: 18085825.

Alvarez-Saavedra E, Horvitz HR (2010). “Many families of *C. elegans* microRNAs are not essential for development or viability.” *Curr Biol* 20(4):367-73. DOI: 10.1016/j.cub.2009.12.051 | PMID: 20096582.

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: 10.1534/genetics.114.169730 | PMID: 25161212.

Paix A, Folkmann A, Rasoloson D, Seydoux G (2015). “High Efficiency, Homology-Directed Genome Editing in *Caenorhabditis elegans* Using CRISPR-Cas9 Ribonucleoprotein Complexes.” *GENETICS* 201(1): 47-54. DOI: 10.1534/genetics.115.179382 | PMID: 26187122.

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

**Author Contributions:** Connor Horn: Investigation, Writing - original draft. Robert Sholl: Investigation, Writing - original draft. Dustin Haskell: Investigation, Supervision. Shilpa Hebbar: Investigation, Supervision. Anna Zinovyeva: Supervision, Writing - review and editing, Resources, Funding acquisition.

**Reviewed By:** Katherine McJunkin

**History:** Received February 5, 2020 Accepted March 27, 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:** Horn, C; Sholl, R; Haskell, D; Hebbar, S; Zinovyeva, A (2020). Two new CRISPR-generated alleles in the *C. elegans mir-1022* gene.. microPublication Biology. <https://doi.org/10.17912/micropub.biology.000233>