Two Deletion Alleles in the \textit{C. elegans} mir-49 gene.

Cassandra Delich\textsuperscript{1,*}, Annabelle Dillon\textsuperscript{1,*}, Noah Winans\textsuperscript{1,*}, Shilpa Hebbar\textsuperscript{1,*}, Dustin Haskell\textsuperscript{1} and Anna Zinovyeva\textsuperscript{1§}

\textsuperscript{1}Division of Biology, Kansas State University, Manhattan, KS

\textsuperscript{*}These authors contributed equally.

\textsuperscript{§}To whom correspondence should be addressed: zinovyeva@ksu.edu

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

Using CRISPR-Cas9 genome editing, we generated two deletion alleles, \textit{zen99} and \textit{zen102}, that disrupt the \textit{C. elegans} \textit{mir-49} gene (Fig 1A). \textit{mir-49(zen99)} and \textit{mir-49(zen102)} delete 56 base pairs and 58 base pairs from the \textit{mir-49} locus, respectively (Fig 1B and Fig 1C). Each deletion nearly completely removes both strands generated by the \textit{mir-49} locus, \textit{mir-49-3p} and \textit{mir-49-5p}. Both \textit{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 \textit{mir-49} deletions.

**Methods**

Request a detailed protocol

To generate the \textit{mir-49} deletion alleles, N2 animals were injected with the CRISPR-Cas9 components as an RNA-protein complex (Paix \textit{et al.} 2015). The following components were used: Alt-R Cas9 (IDT, cat# 1081058) loaded with \textit{mir-49} crRNAs (IDT, custom) (\textit{mir-49} crRNA1 sequence: 5’-GAGCACATCACAACAAACTG-3’, \textit{mir-49} crRNA2 sequence: 5’-GCACCACGAGAAGCTGCAGA-3’), \textit{dpy-10} targeting guide RNA (IDT, custom) (5’-GCUACCAUGGCCAAGCTGAG-3’, Arribere \textit{et al.} 2014) and tracer RNA (IDT, cat# 1072532) (AGCAUAGCAAGUUAAAAUAAGGCUCAGGCUUUUAACAUUGAAAAGUGGACCAGGUCGGGUUUU).

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 \textit{mir-49} crRNA1 (0.4µg/µL), 0.8µL of \textit{mir-49} crRNA2 (0.4µg/µL), 1.3 µL of \textit{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 \textit{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’-AGGCACCACCACCTACCATCAT-3’) and mir-49.rev1 (5’-GATGACTTACAGTCGCGTCTT-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.

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**References**


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