Two Deletion Alleles in the C. elegans mir-49 gene.
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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) (AGCAUAGGCGUUAUAUAAGGGCUAGUGCUGUAUAUAAGUGCCGCGGUGCGUUU).

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.fort1 (5’-AGGCACCACCACTTACCATTCAT-3’) and mir-49.rev1 (5’-GATGACTTACAGTCGCTTT-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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