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

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.

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

**Request a detailed protocol**

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’-GCACCACGAGAAGCTGCAGA-3’), dpy-10 targeting guide RNA (IDT, custom) (5’-GCACCACGAGAAGCTGCAGA-3’), dpy-10 targeting guide RNA (IDT, custom) (5’-GCACCACGAGAAGCTGCAGA-3’), dpy-10 targeting guide RNA (IDT, custom) (5’-GCACCACGAGAAGCTGCAGA-3’). 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 (~10,000 rpm). 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′-AGGCACCCACCTACCACTCAT-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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