Two novel alleles in C. elegans mir-1822 gene.
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Description
microRNAs are small noncoding RNAs of ~22 nucleotides in length that regulate gene expression by degrading target mRNAs or inhibiting their translation. To our knowledge mir-1822 currently lacks deletion alleles, impeding mir-1822 functional characterization. We generated two new deletions of the C. elegans mir-1822 locus, using the CRISPR-Cas9 genome editing technique. The following mir-1822 specific Alt-R crRNAs were ordered from IDT: gRNA1, 5’-AGTTTCTCTGGGAAAGCTAT-3’ and gRNA2: 5’-TGAGCCAAGAGTTTTTCTGA-3’. To create the deletions, Cas9 (Alt-R Cas9, IDT) was loaded with the two mir-1822 guide RNAs, dpy-10 guide RNA (Arribere et al, 2014) (IDT), and tracer RNA (IDT) and the mixture was injected into C. elegans. The resulting progeny were screened for CRISPR-Cas9 positive animals as previously described (Arribere et al, 2014). The following PCR primers were used to screen for deletions of interest: mir-1822.for1: 5’- CGGAAGGACACCTGCCACCAATG-3’ and mir-1822.rev1: 5’-GAGGGCAATCTTCTTCTGGTCGCC -3’.

Using PCR screening, we identified two independent deletions of approximately 40 nucleotides each, with the positions of each deletion schematized in Figure 1A. mir-1822(zen100) removes 43 base pairs (Fig. 1B), and mir-1822(zen101) deletion removes 41 base pairs from the mir-1822 precursor region (Fig. 1C). Each deletion was sequenced twice for confirmation. Both mir-1822 alleles are homozygous viable and appear to be superficially wild type, with no obvious phenotypes observed in either strain.

Reagents
UY265 mir-1822(zen100) and UY266 mir-1822(zen101) strains are available upon request.

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References

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