**Supplemental Figures 1-3 and Supplemental Tables 1-3**

**Detecting and characterizing microRNAs of diverse genomic origins via miRvial**

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**Supplemental Figure S1**. An example shows how miRvial finds miRNA-like hairpins in a secondary structure.

**Supplemental Figure S2**. Optimal and suboptimal secondary folding structures of miRNA precursors.

**Supplemental Figure S3**. A schematic example of miRtron.

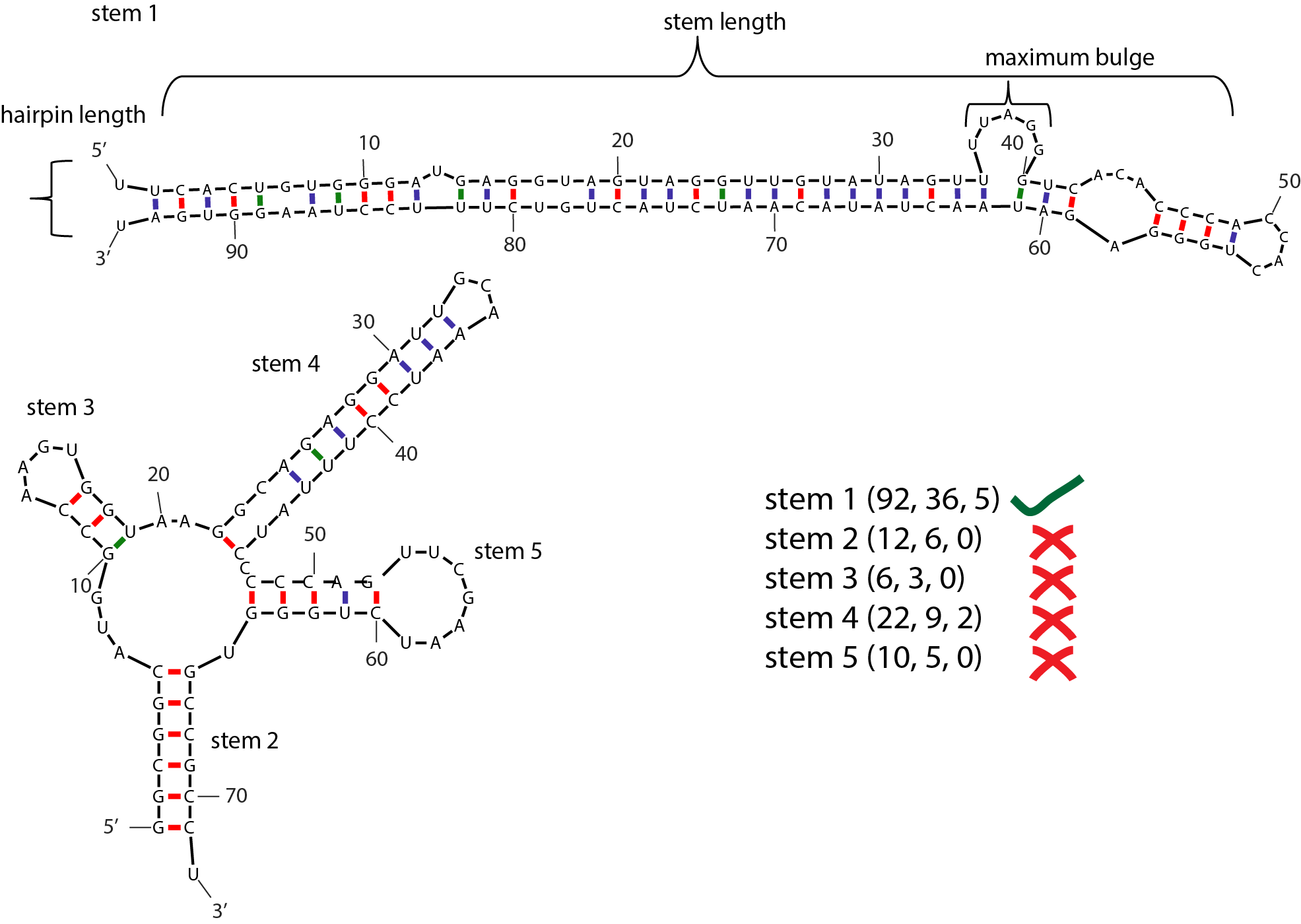
**Supplemental Table S1.** List of small RNA sequencing datasets used in the current study.

**Supplemental Table S2**. A comparison of features in various miRNA identification software packages.

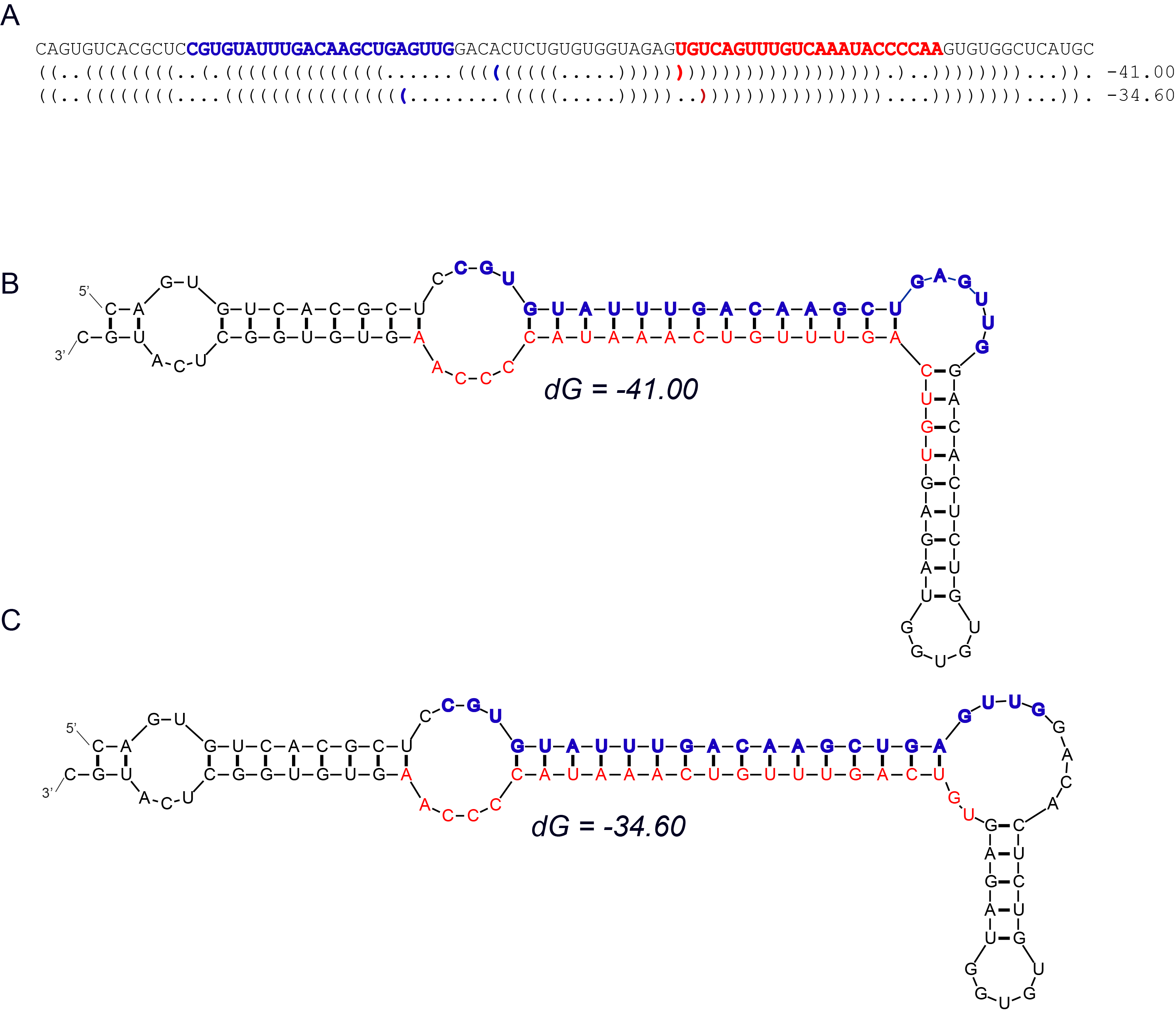
**Supplemental Table S3**. Sequences of primers used in validation of new miRNAs in C. reinhardtii.

**Supplemental Table S4**. The performance of miRvial after relaxing the criterion of presence of a duplex.

**Supplemental Figure S1**. An example shows how miRvial finds miRNA-like hairpins in a secondary structure. Three values, namely the length of the hairpin, the length of the stem and the size of the maximum bulge on the stem, are computed for a secondary structure. miRvial finds structures carrying miRNA-like hairpins if the values satisfy predefined criteria.



**Supplemental Figure S2**. Optimal and suboptimal secondary folding structures of miRNA precursors. (A) Predicted optimal and suboptimal structures for murine miR-223, where mature miR-223-5p and miR-223-3p are shown in blue and red, respectively. In the optimal secondary structure, the 5’ end of miR-223-3p (red parenthesis) pairs with another nucleotide on 5p-arm (blue parenthesis), which does not follow the rule of ~2nt 3’-overhang. In the suboptimal secondary structure, the 5’ end of miR-223-3p (red parenthesis) pairs with another nucleotide on 5p-arm (blue parenthesis). The two mature sequences follow the rule of ~2nt 3’-overhang. (B) The optimal secondary structure of miR-203, where mature miR-223-5p and miR-223-3p, respectively in blue and red, do not form a RNA-RNA duplex with 2nt 3’-overhangs. (C) The suboptimal secondary structure of miR-203, where mature miR-223-5p and miR-223-3p form a RNA-RNA duplex with 3’-overhangs. The sequence structures were predicted by the Mfold webserver.

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**Supplemental Figure S3**. A schematic example of miRtron. An example of miRtron indicated by the read alignment and the hairpin structure. 5’-ends of 5p-arm reads represent donor sites, while 3’-ends of 3p-arm reads represent acceptor sites. Both donor and acceptor sites indicate a miRtron derived from the locus.

