

Among the hundreds of genes encoding miRNAs in plants reported, much more were predicted by numerous computational methods. However, unlike protein-coding genes defined by start and stop codons, the ends of miRNA molecules do not have characteristics that can be used to define the mature miRNAs exactly, which made computational miRNA prediction methods often cannot predict the accurate location of the mature miRNA in a precursor with nucleotide-level precision. To our knowledge, there haven't been reports about comprehensive strategies determining the precise sequences, especially two termini, of these miRNAs.

In this study, we report an efficient method to determine the precise sequences of computationally predicted microRNAs (miRNAs) that combines miRNA-enriched library preparation, two specific 5' and 3' miRNA RACE (miR-RACE) PCR reactions, and sequence-directed cloning, in which the most challenging step is the two specific gene specific primers designed for the two RACE reactions. miRNA-mediated mRNA cleavage by RLM-5' RACE and sequencing were carried out to validate the miRNAs detected. Real-time PCR was used to analyze the expression of each miRNA.

The efficiency of this newly developed method was validated using nine trifoliate orange (*Poncirus trifoliata*) miRNAs predicted computationally. The miRNAs computationally identified were validated by miR-RACE and sequencing. Quantitative analysis showed that they have variable expression. Eight target genes have been experimentally verified by detection of the miRNA-mediated mRNA cleavage in *Poncirus trifoliata*.

The efficient and powerful approach developed herein can be successfully used to validate the sequences of miRNAs, especially the termini, which depict the complete miRNA sequence in the computationally predicted precursor.