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Real-Time Quantitative Polymerase Chain Reaction Assay for the Detection of microRNAs Using Bi-Directional Extension Sequences

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Abstract : MicroRNAs (miRNA) are a class of endogenous, single-stranded, small, and non-protein coding RNA molecules typically 20-25 nucleotides long. They are thought to regulate the expression of other genes in a broad range by binding to 3'-untranslated regions (3'-UTRs) of specific mRNAs. The detection of miRNAs is very important for understanding of the function of these molecules and in the diagnosis of variety of human diseases. However, detection of miRNAs is very challenging because of their short length and high sequence similarities within miRNA families. So, a simple-to-use, low-cost, and highly sensitive method for the detection of miRNAs is desirable. In this study, we demonstrate a novel bi-directional extension (BDE) assay. In the first step, a specific linear RT primer is hybridized to 6-10 base pairs from the 3'-end of a target miRNA molecule and then reverse transcribed to generate a cDNA strand. After reverse transcription, the cDNA was hybridized to the 3'-end which is BDE sequence; it played role as the PCR template. The PCR template was amplified in an SYBR green-based quantitative real-time PCR. To prove the concept, we used human brain total RNA. It could be detected quantitatively in the range of seven orders of magnitude with excellent linearity and reproducibility. To evaluate the performance of BDE assay, we contrasted sensitivity and specificity of the BDE assay against a commercially available poly (A) tailing method using miRNAs for let-7e extracted from A549 human epithelial lung cancer cells. The BDE assay displayed good performance compared with a poly (A) tailing method in terms of specificity and sensitivity; the CT values differed by 2.5 and the melting curve showed a sharper than poly (A) tailing methods. We have demonstrated an innovative, cost-effective BDE assay that allows improved sensitivity and specificity in detection of miRNAs. Dynamic range of the SYBR green-based RT-qPCR for miR-145 could be represented quantitatively over a range of 7 orders of magnitude from 0.1 pg to 1.0 µg of human brain total RNA. Finally, the BDE assay for detection of miRNA species such as let-7e shows good performance compared with a poly (A) tailing method in terms of specificity and sensitivity. Thus BDE proves a simple, low cost, and highly sensitive assay for various miRNAs and should provide significant contributions in research on miRNA biology and application of disease diagnostics with miRNAs as targets.

Keywords : bi-directional extension (BDE), microRNA (miRNA), poly (A) tailing assay, reverse transcription, RT-qPCR
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