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Increase in Specificity of MicroRNA Detection by RT-qPCR Assay Using a Specific Extension Sequence

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Abstract : We describe an innovative method for highly specific detection of miRNAs using a specially modified method of poly(A) adaptor RT-qPCR. We use uniquely designed specific extension sequence, which plays important role in providing an opportunity to affect high specificity of miRNA detection. This method involves two steps of reactions as like previously reported and which are poly(A) tailing and reverse-transcription followed by real-time PCR. Firstly, miRNAs are extended by a poly(A) tailing reaction and then converted into cDNA. Here, we remarkably reduced the reaction time by the application of short length of poly(T) adaptor. Next, cDNA is hybridized to the 3'-end of a specific extension sequence which contains miRNA sequence and results in producing a novel PCR template. Thereafter, the SYBR Green-based RT-qPCR progresses with a universal poly(T) adaptor forward primer and a universal reverse primer. The target miRNA, miR-106b in human brain total RNA, could be detected quantitatively in the range of seven orders of magnitude, which demonstrate that the assay displays a dynamic range of at least 7 logs. In addition, the better specificity of this novel extension-based assay against well known poly(A) tailing method for miRNA detection was confirmed by melt curve analysis of real-time PCR product, clear gel electrophoresis and sequence chromatogram images of amplified DNAs.

Keywords : microRNA(miRNA), specific extension sequence, RT-qPCR, poly(A) tailing assay, reverse transcription
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