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ABSTRACT

In this study, a rapid, sensitive, and specific assay for detecting miRNAs was developed based on a two-stage amplification reaction (TSAR) using molecular beacons (MBs) as turn-on probes. In the TSAR, different miRNAs can be converted to the same reporter oligonucleotides (Y), which can hybridize with the same MB. Therefore, in combination with specific templates, this method can be applied to multiplex miRNA detection by simply using the same MB. The loop region of the MB was screened by computer simulation methods. In particular, to improve the specificity of the MB in real sample analysis, the maximum similarity of the MB loop region to the human genome and human transcriptome is less than 70%. Two MBs were designed in this study. MB I, with nine flanking base pairs in its stem region, was used for real-time monitoring of the production of Y during the TSAR. MB II, with five flanking base pairs in its stem region, was used to detect the production of Y at the end of the TSAR. This assay exhibited high sensitivity with a limit of detection of 2.0×10^{-16} M and 6.7×10^{-16} M using MB I and MB II as turn-on probes, respectively. In addition, this assay can clearly discriminate single base differences in