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Multifunctional self-priming hairpin probe-based isothermal nucleic acid amplification and its applications for COVID-19 diagnosis

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Abstract

We herein present a multifunctional self-priming hairpin probe-based isothermal amplification, termed MSH, enabling one-pot detection of target nucleic acids. The sophisticatedly designed multifunctional self-priming hairpin (MSH) probe recognizes the target and rearranges to prime itself, triggering the amplification reaction powered by the continuously repeated extension, nicking, and target recycling. As a consequence, a large number of double-stranded DNA (dsDNA) amplicons are produced that could be monitored in real-time using a dsDNA-intercalating dye. Based on this unique design approach, the nucleocapsid (N) and the open reading frame 1 ab (ORF1ab) genes of SARS-CoV-2 were successfully detected down to 1.664 fM and 0.770 fM, respectively. The practical applicability of our method was validated by accurately diagnosing 60 clinical samples with 93.33% sensitivity and 96.67% specificity. This isothermal one-pot MSH technique holds great promise as a point-of-care testing protocol for the reliable detection of a wide spectrum of pathogens, particularly in resource-limited settings.

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목록


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