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One-pot, Ultrasensitive, and Multiplex Detection of SARS-CoV-2 Genes Utilizing Self-Priming Hairpin-Mediated Isothermal Amplification

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Abstract

The global pandemic resulting from the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and its emerging variants highlights the need for convenient and accurate detection protocols to facilitate timely prevention and management of the disease. Herein, we propose a new self-priming hairpin-mediated isothermal amplification (SIAM) protocol enabling one-pot and ultrasensitive identification of SARS-CoV-2 in a multiplexed way. This approach works by targeting a specific RNA sequence with a self-priming hairpin (SP) probe and promoting continuously repeated extension and nicking reactions to produce numerous trigger molecules, which could specifically bind to molecular beacons (MBs) and produce fluorescent signals. Under an isothermal condition of 37 °C, this technique allowed for the simultaneous identification of the spike (S) and nucleocapsid (N) genes of SARS-CoV-2 down to single copy/μL levels. We further validated the practical diagnostic capabilities of the SIAM method by accurately testing 20 clinical samples with 100% sensitivity and specificity. The SIAM method has a lot of potential to be a reliable nucleic acid testing protocol to identify infections caused by a wide range of pathogens.

논문정보

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