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Ligation-free isothermal nucleic acid amplification

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Abstract

In this study, we uncover a ligation-free DNA extension method in two adjacent fragmented probes, which are hybridized to target RNA, for developing a ligation-free nucleic acid amplification reaction. In this reaction, DNA elongation occurs from a forward probe to a phosphorothioated-hairpin probe in the presence of target RNA regardless of ligation. The second DNA elongation then occurs simultaneously at the nick site of the phosphorothioated probe and the self-priming region. Therefore, the binding site of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) 12a is repeatedly amplified, inducing a fluorescence signal in the presence of CRISPR-Cas12a. This ligation-free isothermal gene amplification method enables the detection of target RNA with 49.2 fM sensitivity. Moreover, two types of mRNA detection are feasible, thus, demonstrating the potential of this method for cancer companion diagnostics. Notably, the proposed method also demonstrates efficacy when applied for the detection of mRNA extracted from human cells and tumor-bearing mouse tissue and urine samples. Hence, this newly developed ligation-free isothermal nucleic acid amplification system is expected to be widely used in a variety of gene detection platforms.

논문정보

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