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Elution-free DNA detection using CRISPR/Cas9-mediated light-up aptamer transcription: Toward all-in-one DNA purification and detection tube

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Abstract

Accurate and efficient detection of DNA is crucial for disease diagnosis and health monitoring. The traditional methods for DNA analysis involve multiple steps, including sample preparation, lysis, extraction, amplification, and detection. In this study, we present a one-step elution-free DNA analysis method based on the combination of clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9)-mediated light-up aptamer transcription (CLAT) assay and a DNA-capturing poly(2-dimethylaminomethyl styrene) (pDMAMS)-coated tube. The sample solution and lysis buffer are added to the pDMAMS-coated tube, and the DNA is efficiently captured on the surface via electrostatic interaction and directly detected by CLAT assay. The ability of the CRISPR/Cas9 system to specifically

recognize DNA enables direct detection of DNA captured on the pDMAMS-coated tube. The combination of CLAT assay and pDMAMS-coated tube simplifies DNA detection in a single tube without the need for complicated extraction steps, improving sensitivity. Our platform demonstrated attomolar sensitivity in the detection of target DNA in cell lysate (0.92 aM), urine (7.7 aM), and plasma (94.6 aM) samples within 1 h. The practical applicability of this method was further demonstrated in experiments with tumor-bearing mice. We believe that this approach brings us closer to an all-in-one DNA purification and detection tube system and has potential applications in tissue and liquid biopsies, as well as various other DNA sensing applications.

논문정보

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