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An Assay Based On CRISPR-Cas12a for One-Step Preamplification-Free Viral DNA Detection

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Abstract

It is difficult to achieve fM-level viral DNA detection without target amplification. Preamplifying low-abundant DNA targets is a traditional prerequisite that not only lengthens the detection time overall but also raises the risk of infectious material leakage and increases the likelihood of false positive detection. In order to improve the limit of detection (LOD) of this CRISPR-Cas12a detection system to the femtomolar level—three orders of magnitude lower than a standard protocol—we developed a combinatory approach.

Keywords: DNA; CRISPR; Preamplification

Introduction

By using multiplex crRNAs to simultaneously recognize multiple distinct sites of the same viral DNA target, by using a novel molecular reporter with a G-triplex structure that exhibits a significantly enhanced cleaving tendency by Cas12a, and by investigating the ideal molecular coexistence reaction environment for maintaining enzyme activity—which was crucial for sensing—the CRISPR-Cas12a system's detection sensitivity was specifically increased. Notably, we have found, for the first time, a low-K+ environment that promote the formation of sensitive G-triplex reporters without impairing CRISPR-Cas12a activity. We used this system to show that, in a physiologically-like setting, plasmids carrying the DNA sequences of the monkeypox virus could be detected with extreme sensitivity. More significantly, we discovered that human papillomavirus (HPV) subtypes could be detected and classified in a sensitive and specific manner [1-4].

Methodology

With our modern society's increased population density and mobility, the spread of infectious diseases is becoming a growing public health concern. Human papillomavirus (HPV), monkeypox (Mpox), and SARS-Cov-2 are a few of the main pathogens that raise concerns about the need for more sensitive, quick, and accurate nucleic acid detection techniques. The gold standard for nucleic acid detection is unquestionably real-time quantitative PCR, but its use in point-of-care testing is severely limited by its demanding requirements for large heat cycler equipment. Rapid thermocycler-free nucleic acid amplification can be accomplished using a number of isothermal amplification techniques, including rolling cycle amplification (RCA), loop-mediated isothermal amplification (LAMP), and recombinant polymerase amplification (RPA) which offer a good PCR substitute [5,6].

They might still experience PCR amplification's non-specific amplification and primer interference, though. Because of their accurate target recognition, quick reaction times, and instrument-free reaction processes, CRISPR-Cas systems have been used extensively in nucleic acid biosensing in recent years. For instance, by tracking the trans-cleavage of fluorescent reporters following target-specific activation, the CRISPR-Cas12a system, which possesses both cis-and trans-cleavage activity, may be able to detect target dsDNA. However, the CRISPR-Cas12a system by itself is relatively insensitive and can only narrowly achieve the detection of dsDNA down to the picomolar range without the addition of additional amplification steps. Consequently, in order to achieve real detection, nucleic acid targets still need to be amplified [7-9].

Four successful approaches to signal amplification have been recently implemented to address this challenge: adding cascade amplification, utilizing microfluidic droplet analysis for assistance, modifying signal output patterns, and optimizing critical CRISPR/Cas12a system parameters. Notably, the introduction of cascade steps, like nucleic acid cycling circuits and enzyme reactions, may result in issues akin to those caused by preamplification. Droplet analysis's use for the quick detection of pathogens in underdeveloped epidemic sites is limited by the need for sophisticated microfluidic chips and the high dependence of the signal output pattern on instrument-specific signal readout.

By creating multiplex crRNAs against the same target, investigating the reaction environment, and adding a G-triplex reporter, we were able to increase the quantity of activated Cas12a nucleases and the cleavage efficiency of reporters, ultimately leading to the development of a combinatory CRISPR-Cas12a system with femtomolar sensitivity. With the use of this combinatory CRISPR-Cas12a system, we were able to detect Mpox viral DNA in a physiologically realistic setting with high specificity and without the need for amplification or the addition of additional sophisticated equipment. More significantly, clinical samples from patients infected with HPV16 or HPV18 were accurately classified and successfully identified. All things considered, our approach has a lot of potential as a field-deployed, amplification-free assay for accurate viral DNA infection detection [10].

Conclusion

In comparison to a traditional Cas12a system, our combinatory CRISPR-Cas12a system can detect dsDNA targets with femtomolar sensitivity, three orders of magnitude lower. This new technique avoids the need for target preamplification while enabling point-of-care

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testing with just a fluorescence reader and no additional complicated equipment, which is necessary for the low-cost construction and scaling up of biosensing systems to allow for quick and easy application.

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