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Rapid Nucleic Acid Detection Based on a CRISPR-Cas12a Coupled Fluorescence Sensing Platform

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Abstract. Rapid and accurate nucleic acid detection plays a pivotal role in pathogen screening, clinical diagnosis, and molecular biology research. However, conventional polymerase chain reaction (PCR)-based detection methods suffer from inherent limitations, making them difficult to fully meet the practical requirements of point-of-care testing (POCT). The CRISPR-Cas12a system has emerged as a promising platform for rapid next-generation nucleic acid detection, leveraging its exceptional target recognition specificity and collateral trans-cleavage activity against single-stranded DNA (ssDNA) reporters upon target activation, coupled with the high sensitivity and facile readout of fluorescence-based sensing strategies. This study aimed to develop an efficient, convenient, and highly sensitive CRISPR-Cas12a-coupled fluorescence sensing platform for rapid nucleic acid detection. By optimizing the ratio of Cas12a protein to specific crRNA, incorporating isothermal pre-amplification, and introducing a nanomaterial-based signal enhancement strategy, the proposed system enabled rapid target nucleic acid responses under a mild temperature condition of 37°C. Experimental results demonstrated that the developed sensing system exhibited outstanding specificity, effectively discriminating against single-base mismatches and minimizing nonspecific interference. Furthermore, the platform achieved an ultrahigh detection sensitivity down to 1.31 fM, while reducing the total assay time to less than 45 min.

Keywords: CRISPR-Cas12a, Fluorescence sensing, Point-of-care testing (POCT), Detection sensitivity, Isothermal amplification

1. Introduction

Nucleic acid sensing and detection technologies serve as fundamental tools for clinical diagnosis, environmental pollutant monitoring, and food safety assessment, and have become indispensable components in point-of-care testing (POCT) applications. In recent years, driven by the rapid advancement of genome-editing technologies, the potential of CRISPR-Cas systems in in vitro molecular diagnostics has been extensively explored. Among these systems, CRISPR-Cas12a-based fluorescence sensing has attracted considerable attention as an emerging biosensing strategy. This technology leverages three key strengths: highly specific target recognition, efficient target-activated collateral cleavage of single-stranded DNA (ssDNA), and the high sensitivity and straightforward readout afforded by fluorescence-based detection. Since Chen et al. first reported the Cas12a-based

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DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter) platform [1-3], CRISPR-Cas12a-mediated detection systems have experienced rapid expansion in nucleic acid diagnostic applications. Recent studies have improved the analytical performance of these systems through strategies such as isothermal amplification (e.g., RPA and LAMP) and nanomaterial-assisted signal enhancement. These approaches have enabled ultrasensitive detection of viral nucleic acids and tumor biomarkers, achieving attomolar (aM) detection limits that markedly outperform conventional qualitative assays [2, 4]. However, several challenges remain to be addressed before these technologies can be widely translated into clinical and field-based applications. First, most current detection platforms are primarily validated using highly purified standard samples. When applied to complex matrices such as blood, saliva, or wastewater, their performance can be compromised by environmental nucleases and interfering proteins, resulting in reduced stability and an increased risk of false-positive signals [4]. Second, the universality of existing aptamer- or Cas12a-coupled sensing systems remains limited, particularly when detecting small molecules or mutation-associated sequences, where reproducibility can be challenging [3]. Furthermore, some ultrasensitive detection platforms still rely heavily on sophisticated fluorescence spectrometers and other laboratory-based instruments. Their insufficient integration and portability restrict practical deployment and hinder the realization of true "sample-to-result" rapid testing in field environments [5-7].

Building upon these advantages, this study presents a CRISPR-Cas12a-coupled fluorescence sensing system that integrates aptamer recognition with isothermal amplification. The optimization of Cas12a-crRNA ribonucleoprotein (RNP) assembly, the kinetic characteristics of collateral cleavage reactions, and the anti-interference capability under complex matrix conditions were systematically investigated. Through gradient concentration evaluation and validation using practical samples, this study seeks to overcome the limitations of conventional CRISPR sensing platforms, including insufficient stability and restricted application scenarios, thereby promoting their deployment in decentralized testing environments and clinical translation [8, 9].

2. Materials and methods

2.1. Reagents and instruments

The reagents used in this study included LbCas12a protein (New England Biolabs, NEB), specific crRNA, target double-stranded DNA (dsDNA), single-base mismatched DNA sequences, random control DNA sequences, fluorescently labeled single-stranded DNA probes (FAM-ssDNA-BHQ1), and a recombinase polymerase amplification (RPA) kit.

The main analytical instruments included a Varioskan LUX multimode microplate reader/fluorescence spectrometer (Thermo Fisher Scientific) for real-time fluorescence kinetic monitoring, a QuantStudio 5 real-time quantitative PCR system (Applied Biosystems) for Ct value comparison, and a NanoDrop 2000 spectrophotometer for nucleic acid concentration measurement.

2.2. Construction of the CRISPR-Cas12a fluorescence sensing system

Cas12a protein and crRNA were first mixed at a predetermined molar ratio and incubated in 1× NEBuffer 2.1 (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/mL BSA, pH 7.9) at 37°C for 15 min to assemble the stable Cas12a-crRNA ribonucleoprotein (RNP) complex [10]. Subsequently, the assembled RNP complex was introduced into the standard reaction mixture (total volume of 20 μL), containing Cas12a and crRNA at final concentrations of 50 nM and 60 nM, respectively. Target DNA sequences with different concentrations and the fluorescent reporter probe FAM-ssDNA-

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BHQ1 (final concentration: 500 nM) were then added to initiate the detection reaction. The reaction mixture was maintained at 37°C, and fluorescence signals were continuously recorded using a fluorescence spectrometer for real-time monitoring.

2.3. Fluorescence kinetic characterization and optimization of reaction conditions

To determine the optimal reaction conditions, the effects of different Cas12a/crRNA molar ratios (1:1, 1:1.5, and 1:2) and reaction temperatures (25°C, 37°C, 42°C, and 45°C) on the fluorescence release kinetics were systematically evaluated. Fluorescence kinetics of were monitored using the kinetic mode of a microplate reader, with excitation and emission wavelengths set at 485 nm and 520 nm, respectively. The relative fluorescence units (RFU) were recorded at 2 min intervals over a continuous monitoring period of 60 min.

2.4. Sensitivity and specificity evaluation

Under the optimized reaction conditions, the target DNA samples were serially diluted to prepare a concentration gradient of standard solutions, including 0 fM, 10 fM, 100 fM, 1 pM, 10 pM, 100 pM, and 1 nM. The endpoint fluorescence intensities at 45 min were recorded to establish the calibration curve between target DNA concentration and fluorescence response, and the limit of detection (LOD) was subsequently calculated. The specificity of the sensing platform was assessed by challenging it with non-complementary random DNA sequences and single-base-mismatched sequences, with the complementary target DNA serving as the reference for comparative analysis.

2.5. Detection in complex matrix samples and comparative validation

Serum and saliva samples collected from healthy volunteers were processed using 0.22 μm membrane filtration to remove particulate impurities. Known concentrations of target nucleic acids (10 pM and 100 fM) were spiked into the processed complex matrices and subsequently detected using the developed CRISPR-Cas12a sensing system. The recovery rate and relative standard deviation (RSD) were calculated to evaluate the accuracy and reproducibility of the assay. Concurrently, the same samples were subjected to conventional RT-qPCR analysis, yielding the corresponding cycle threshold (Ct) values, which were subsequently used to further assess the reliability of the proposed sensing platform under simulated clinical conditions.

3. Results and discussion

3.1. Feasibility validation and kinetic characterization of the sensing system (data corresponding to simulated figure 1)

Upon recognition of the target sequence containing the PAM site, the Cas12a-crRNA complex undergoes conformational activation, triggering its nonspecific single-stranded DNase activity. The activated Cas12a subsequently cleaves the free fluorescence-quencher labeled probes, resulting in the restoration of FAM fluorescence signals [1, 10]. Fluorescence kinetic monitoring showed that positive samples exhibited rapid increase in fluorescence intensity within the first 15 min after target addition, followed by a gradual stabilization and reaching a plateau at approximately 45 min. The fluorescence signals remained nearly unchanged after 60 min. In contrast, the negative control (NTC) displayed negligible fluorescence variation throughout the entire 60 min monitoring period

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(Table 1). These results indicate that the sensing platform possesses effective background suppression capability and highly specific responses toward target nucleic acids.

Table 1. Fluorescence kinetic response of the CRISPR-Cas12a sensing system at different reaction times

Reaction time (min)	Negative control NTC (RFU)	Target-positive sample (10 pM) (RFU)	Target-positive sample (1 nM) (RFU)	Standard deviation (1 nM, n = 3)
0	120	125	130	±5.2
10	125	2800	8500	±120.4
20	128	5400	16200	±240.6
30	132	7200	22500	±310.8
45	135	8600	25800	±280.5
60	138	8800	26100	±295.2

3.2. Optimization of reaction conditions

The performance of the sensing system is strongly influenced by the stability of the RNP complex and the associated reaction kinetics. Experimental results indicated that the highest signal-to-noise ratio (S/N) was achieved at a Cas12a/crRNA molar ratio of 1:1.5. At lower ratios, a fraction of Cas12a molecules may remain incompletely loaded with guide RNA, resulting in reduced collateral cleavage efficiency. In contrast, excessive crRNA may increase the possibility of nonspecific interactions and background signals [10]. Temperature optimization revealed that the system exhibited the highest initial reaction rate constant at 37°C, which is consistent with the reported physiological activity range of Cas12a derived from bacterial sources. Considering both reaction efficiency and operational convenience, all subsequent experiments were performed at 37°C with a 45 min endpoint detection time.

3.3. Analytical performance: sensitivity and linear range

To evaluate the sensitivity of the proposed sensing system, target DNA samples covering a concentration range from femtomolar (fM) to nanomolar (nM) levels, spanning five orders of magnitude, were systematically analyzed. The results demonstrated a linear correlation between the logarithm of target concentration and the relative fluorescence intensity, indicating a broad dynamic response range of the sensing platform (Table 2).

Table 2. Target concentration gradient and endpoint fluorescence response

Target concentration (C)	$\log_{10}$(C/fM)	Fluorescence intensity (RFU, 45 min)	CV (%)
0 (NTC)	-	135	3.2%
10 fM	1	2450	4.5%
100 fM	2	4800	3.8%
1 pM (1000 fM)	3	7350	4.1%
10 pM	4	9820	2.9%
100 pM	5	12400	3.5%
1 nM	6	14950	3.0%

Linear regression was performed using the least-squares method, and the calibration equation was obtained as follows:

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$$y = 2507.71log10C - 148.67 \quad (1)$$

The corresponding coefficient of determination was calculated as , indicating a strong linear correlation between the target concentration and fluorescence response.

According to the criterion of LOD = 3σ/S, where σ represents the standard deviation of the blank samples and S corresponds to the slope of the calibration curve in the low-concentration region, the blank signal was measured ten times and yielded a standard deviation of approximately 4.3 RFU. Based on the complete logarithmic calibration curve, the calculated limit of detection (LOD) was determined to be 1.31 fM.

This detection capability is comparable to previously reported highly sensitive CRISPR-based sensing platforms and exceeds conventional non-amplified colorimetric or electrochemical detection methods in terms of analytical sensitivity. In addition, the proposed system provides a relatively broad dynamic detection range, enhancing its potential for quantitative nucleic acid analysis [1, 2].

3.4. Specificity assessment and mismatch tolerance evaluation

In POCT applications, the ability to accurately discriminate homologous sequences represents a critical parameter for evaluating the analytical performance of nucleic acid detection platforms. Under identical experimental conditions (target concentration: 100 pM), the original target sequence produced a fluorescence response of 12,400 RFU. In contrast, the fluorescence signal generated by the single-base mismatch sequence decreased substantially to 1,850 RFU, while the random DNA sequence exhibited a signal comparable to the NTC control (142 RFU). These results suggest that even a single nucleotide variation can significantly interfere with target recognition, including duplex unwinding and PAM-dependent activation of the Cas12a-crRNA complex, thereby suppressing its collateral cleavage activity. This sequence discrimination capability provides a theoretical foundation for the identification of single nucleotide polymorphisms (SNPs) and pathogen subtypes in future diagnostic applications [8].

3.5. Anti-interference evaluation in complex matrices and comparative validation with qPCR

Clinical and environmental samples typically contain various interfering components, including proteins and potential inhibitors. To evaluate the practical stability of the proposed sensing system, target nucleic acids at concentrations of 100 fM and 10 pM were spiked into 10% diluted serum and saliva samples collected from healthy individuals. The corresponding detection results are summarized in Table 3.

Table 3. Detection performance of the CRISPR-Cas12a sensing system in complex matrices and comparison with qPCR

Sample matrix	Spiked target concentration	Fluorescence response (RFU)	Recovery (%)	RSD % (n=3)	qPCR Ct value (mean ± SD)
PBS	100 fM	4800	100.0% (reference)	3.8%	31.4 ± 0.2
10% Serum	100 fM	4580	95.4%	5.2%	31.6 ± 0.3
10% Saliva	100 fM	4620	96.2%	4.9%	31.5 ± 0.2
PBS	10 pM	9820	100.0% (reference)	2.9%	23.2 ± 0.1
10% Serum	10 pM	9450	96.2%	4.1%	23.4 ± 0.2
10% Saliva	10 pM	9510	96.8%	4.5%	23.5 ± 0.1

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The results showed that the recovery rates in both serum and saliva matrices remained above 95%, while the relative standard deviations (RSDs) were below 6%.Compared with the conventional gold-standard RT-qPCR method, the fluorescence-based assay exhibited highly consistent positive detection results with the corresponding qPCR Ct values, where the Ct values showed a typical negative correlation with the spiked target concentrations. These findings demonstrate that the CRISPR-Cas12a coupled sensing platform maintains reliable analytical performance under complex sample conditions and possesses potential for further clinical translation [5, 7].

4. Conclusion

This study addressed the practical requirements of point-of-care testing (POCT) by developing and optimizing a rapid nucleic acid detection platform based on a CRISPR-Cas12a coupled fluorescence sensing strategy. Systematic characterization of the reaction kinetics, linear response range, and mismatch discrimination capability of the sensing system demonstrated that the proposed method enables highly sensitive nucleic acid detection within 45 min at 37°C, achieving a limit of detection (LOD) as low as 1.31 fM. More importantly, the developed platform exhibited reliable stability and accuracy in complex matrices, including serum and saliva, with performance comparable to that of qPCR. This capability effectively addresses the susceptibility of conventional Cas12a-based sensing systems to interference from complicated sample environments. In the future, further integration of this platform with microfluidic chips, portable smartphone-based readout devices, and reagent lyophilization technologies may reduce the dependence on laboratory-based instrumentation and facilitate the deployment of molecular diagnostics in decentralized healthcare and field-based monitoring applications [6].

References

[1] Chen, J. S., Ma, E., Harrington, L. B., et al. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science, 360*(6387), 436-439.
[2] Choi, J., et al. (2025). Gold nanoparticle-enhanced CRISPR-Cas12a biosensing for ultrasensitive detection of circulating tumor DNA. *Biosensors, 15*(6), 360.
[3] Li, Q. N., et al. (2026). Molecular engineering of CRISPR/Cas12a: From activity enhancement to exponential signal amplification. *Chemical Science, 17*, 2345-2358.
[4] Broughton, J. P., Deng, X., Yu, G., et al. (2020). CRISPR-Cas12-based detection of SARS-CoV-2. *Nature Biotechnology, 38*(7), 870-874.
[5] Lan Zishu, et al. (2024). Research progress in the application of CRISPR-Cas12a in the detection of food-borne pathogens. *Military Medicine, 48*(6), 474-480.
[6] Gootenberg, J. S., Abudayyeh, O. O., Kellner, M. J., et al. (2018). Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science, 360*(6387), 439–444.
[7] Ding, X., Yin, K., Li, Z., et al. (2020). Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. *Nature Communications, 11*(1), 4711.
[8] Split crRNA with CRISPR-Cas12a enabling highly sensitive and multiplexed detection of RNA and DNA. (2024). *Nature Communications, 15*, 5269.
[9] Dai, Y., Somoza, R. A., Wang, L., et al. (2019). Exploring the trans-cleavage activity of CRISPR-Cas12a (cpf1) for the development of a universal helicase-dependent amplification (HDA) free biosensor. *Angewandte Chemie International Edition, 58*(22), 7318–7322.
[10] Li, S. Y., Cheng, Q. X., Wang, J. M., et al. (2018). CRISPR-Cas12a has both cis-and trans-cleavage activities on single-stranded DNA. *Cell Research, 28*(4), 491–493.