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Editorial

CRISPR-Cas Based Methods for Detection of Nucleic Acids: PCR Empire now has a Vigorous Rival!

Roham Deyhimfar

Department of Microbiology, Faculty of Biology, College of Science, University of Tehran, Tehran, Iran

HIGHLIGHTS

- CRISPR-Cas systems are recently introduced as a diagnostic tool for detection of nucleic acids.
- SHERLOCK and DETECTR are two major CRISPR-Based methods having high sensitivity, specificity and ease of use.
- These systems can replace PCR for detection of nucleic acids in all fields of biology and medical sciences including urology for applications such as identification of various pathogens and mutations in cell free DNA.

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***Corresponding Author**:
Roham Deyhimfar
Email: Roham.deyhimfar@gmail.com
Address: Department of Microbiology, Faculty of Biology, College of Science, University of Tehran, Tehran, Iran

ABSTRACT

Rapid and accurate detection of nucleic acids is essential in many fields of modern biology and medical diagnosis of pathogens. PCR has some disadvantages for performing this task, and therefore demand for an alternative is highly appreciated. Recently, CRISPR-Cas systems have been introduced as a rapid, sensitive, and efficient method for nucleic acid detection. This editorial article reviews mechanisms of action and preclinical results of SHERLOCK and DETECTR as two significant forms of CRISPR-Cas systems for nucleic acid detection.

Keywords: CRISPR, SHERLOCK, DETECTR

Editorial: In 2020, Jennifer Doudna and Emmanuelle Charpentier were awarded Nobel Prize in chemistry for genome editing using the CRISPR-CAS system. Clustered Regular Interspaced Short Palindromic Repeats (CRISPR) and CRISPR associated protein (Cas) evolved as an adaptive immune system for prokaryotes against viruses and foreign nucleic acids. In 2012 Doudna, Charpentier, and colleagues reported that the CRISPR-Cas system could be harnessed as a cut and paste programmable tool for genome editing (1). Up to date, this system has revolutionized the field of genetic manipulation. Recently, novel applications for this system, such as nucleic acid detection, have been introduced. Quantitative PCR is the gold standard for nucleic acid detection and quantification but has disadvantages such as demand for equipment and reagents, trained personnel, and time

(2). CRISPR-Cas systems can provide fast, reliable, and highly accurate detection of DNA and RNA in diverse fields such as ecological studies, molecular research, and clinical identification of pathogens. CRISPR-Cas-based detection of nucleic acids is established using versions of Cas protein such as Cas13a and Cas12a instead of Cas9. These Cas proteins have an incredible ability called collateral activity (non-specific trans-cleavage of bystander quenched-fluorescent reporter RNA/DNA molecules upon activation through complementary matching of crRNA and RNA/DNA of interest). This Quenched-fluorescent Reporter molecule emits light after cleaving by Cas proteins, and this light can be measured and quantified as a criterion for detecting and quantifying nucleic acid of interest.

Specific high sensitivity enzymatic reporter unlocking (SHERLOCK) and DNA endonuclease targeted CRISPR trans reporter (DETECTR) are two main methods that have been used for the CRISPR-Cas detection of nucleic acids. SHERLOCK uses Cas13 (an endo-ribonuclease) and detect single-stranded RNA (it can also detect dsDNA through using RPA and T7 RNA-polymerase in pre-amplification phase) (3). DETECTR uses Cas12a (an endo-deoxyribonuclease) and detects single and double stranded DNA (4). Both approaches can be done after isothermal amplification of the initial specimen with Recombinase Polymerase Amplification (RPA).

Although CRISPR-Cas detection of nucleic acids has not been approved for diagnosis yet, promising pre-clinical research results were obtained. Doudna et al. demonstrated that DETECTR could diagnose human papillomavirus in the clinical specimen and discriminate between two similar subtypes HPV16 and HPV18 (5). In another study, Lee et al. Used SHERLOCK for ultrasensitive malaria diagnosis in symptomatic and asymptomatic patients. Their results demonstrated that SHERLOCK could detect the Plasmodium parasite and differentiate between four Plasmodium species causing malaria in about one hour (6).

Conclusions

These new methods of nucleic acid detection can shake up the medical field because of their high specificity, high sensitivity, high speed, cost-effectivity, and no need for highly trained personnel and advanced laboratories. In Urology, these methods can be used for various applications such as liquid biopsy and rapid detection of prostate or bladder cancer-specific mutations in cell-free DNAs.

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Data availability

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Abbreviations

CAS	CRISPR associated protein
CRISPR	Clustered regular interspaced short palindromic repeats
DETECTR	DNA endonuclease targeted CRISPR trans reporter
RPA	Recombinase polymerase amplification
SHERLOCK	Specific high sensitivity enzymatic reporter unlocking

References

1. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. Science. 2012;337(6096):816-21.
2. Mustafa MI, Makhawi AM, Kraft CS. SHERLOCK and DETECTR: CRISPR-Cas Systems as Potential Rapid Diagnostic Tools for Emerging Infectious Diseases. Journal of Clinical Microbiology. 2021;59(3):e00745-20.
3. Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F. SHERLOCK: nucleic acid detection with CRISPR nucleases. Nat Protoc. 2019;14(10):2986-3012.
4. Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, Dy AJ, Joung J, et al. Nucleic acid detection with CRISPR-Cas13a/C2c2. Science. 2017;356(6336):438-42.
5. Chen JS, Ma E, Harrington LB, Costa MD, Tian X, Palefsky JM, et al. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360(6387):436-9.
6. Lee RA, Puig HD, Nguyen PQ, Angenent-Mari NM, Donghia NM, McGee JP, et al. Ultrasensitive CRISPR-based diagnostic for field-applicable detection of Plasmodium species in symptomatic and asymptomatic malaria. Proceedings of the National Academy of Sciences. 2020;117(41):25722-31.

Author (s) biosketches

Deyhimfar R, MS, Department of Microbiology, Faculty of Biology, College of Science, University of Tehran, Tehran, Iran.
Email: Roham.deyhimfar@gmail.com

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