18th National and 3rd International Conference of Iranian Biophysical chemistry
هجدهمین همایش ملی و سومین همایش بین المللی بیوشیمی فیزیک ایران
25-26 Des, 2024, University of Hormozgan
۶-۵ دی ماه ۱۴۰۳، دانشگاه هرمزگان

Principles of Droplet-based digital PCR (ddPCR) and its applications

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Abstract

As an innovative advancement in the realm of absolute quantitative polymerase chain reaction methodologies, the droplet-based digital PCR (ddPCR) technique offers a multitude of advantages including exceptionally high sensitivity, remarkable precision and outstanding reproducibility, which are critical parameters in the realm of molecular diagnostics. in light of the burgeoning demand for point-of-care (POC) detection and clinical diagnostics, a low-cost, portable, and user-friendly droplet-based digital PCR device has emerged as an intriguing focal point of research, attracting significant attention from the scientific community. Given its extraordinary potential for seamless integration and advanced miniaturization, microfluidic technology has been proficiently utilized across a diverse range of digital droplet polymerase chain reaction (ddPCR) methodologies, significantly augmenting both their operational efficiency and overall practicality in various applications.

Key words: PCR, Droplet-based digital PCR, qPCR, applications.

1. Introduction

Nucleic acids are crucial targets for analysis across diverse fields, including medicine, food safety, and environmental science. the polymerase chain reaction (PCR) and its derivatives have emerged as transformative tools in biological and diagnostic applications. The advent of Real-time PCR (qPCR) allowed for real-time visualization of amplification through fluorescent probes, establishing a quantitative relationship between fluorescence intensity and nucleic acid concentration [1]. Further advancements led to the development of digital PCR (dPCR).Digital PCR relies on partitioning samples into numerous micro-reaction chambers, performing single-

molecule amplification, and applying Poisson distribution for quantification [2]. This approach offers several advantages over qPCR, including increased sensitivity, accuracy, and reproducibility, as well as independence from standard calibration curves and reduced susceptibility to amplification efficiency and inhibitors. These attributes make dPCR particularly effective for detecting rare targets in complex sample matrices, such as in non-invasive diagnostics, early disease detection, and rare mutation analysis. dPCR methodologies are classified into chip-based (cdPCR) and droplet-based (ddPCR) systems[3]. The latter employs droplet microfluidics to generate microliter or nanoliter droplets as reaction compartments, providing flexibility and sensitivity due to scalable compartment sizes (Fig1.A). Droplet microfluidics, a subset of this technology, generates microdroplets that serve as isolated microreactors, enabling efficient, high-throughput analysis with minimal reagent consumption [4]. When combined with PCR technologies, microfluidic systems enhance automation, integration, and miniaturization, addressing challenges such as contamination, droplet fusion, and sample evaporation inherent in traditional ddPCR workflows. Innovations in integrated microfluidic chips have further streamlined ddPCR, incorporating nucleic acid extraction, amplification, and detection for point-of-care diagnostics [5].

2.The applications of ddPCR

The qPCR is a rapid test method and auxiliary examination in some infectious diseases, but it cannot be the diagnostic standards in some cases because of its low positive and sometimes false positive rate [3].In a comprehensive study, it was demonstrated that Droplet Digital PCR (ddPCR) exhibited a greater degree of sensitivity and accuracy in comparison to qPCR; thus, this advanced technique has undergone meticulous modifications to enhance its capability to detect nucleic acids present in low abundance, as substantiated by research findings referenced in, which suggests that ddPCR may prove to be more appropriate for applications in clinical diagnostics [6].

At present, the implementation of ddPCR has expanded significantly, as it is now routinely employed across numerous laboratories for the detection of nucleic acids that are present in low concentrations. Based on the previously cited original research studies, it is anticipated that with the continued advancement of ddPCR technology, it will emerge as a formidable instrument in the identification of pathogens responsible for communicable diseases [7]. Figure 1 shows several examples of ddPCR applications (Fig1.B).

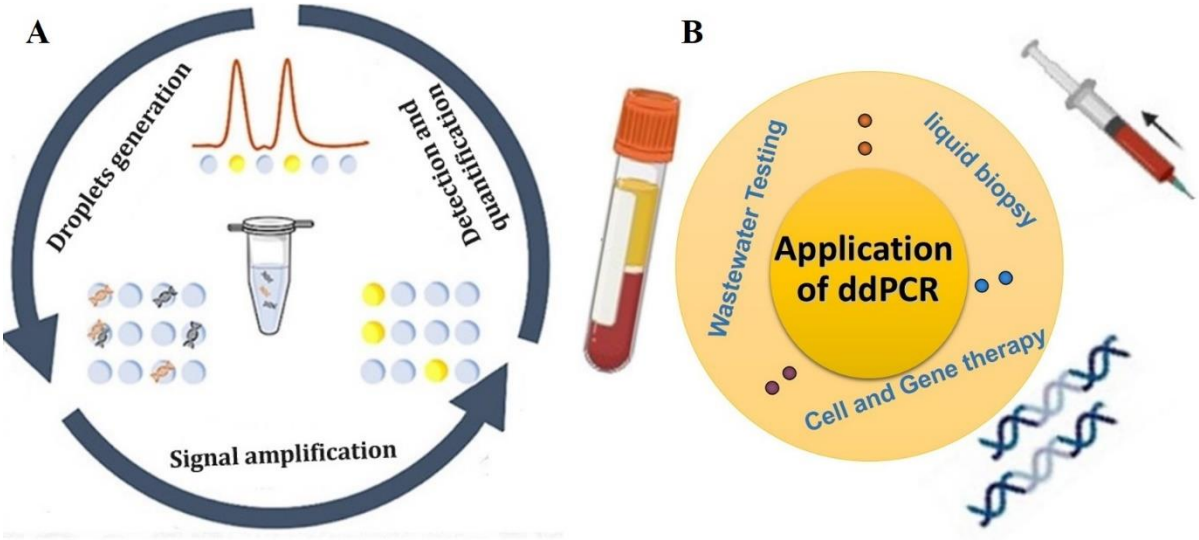

Figure 1 : Schematic Representation of Digital Droplet PCR (ddPCR): Workflow (A) and Applications (B).

3. Future Perspective

Digital PCR (dPCR), as a single-molecule amplification method, offers a high signal-to-noise ratio and exceptional sensitivity, making it particularly effective for detecting low-abundance DNA. Cost and throughput issues challenge chip-based dPCR (cdPCR), while droplet-based dPCR (ddPCR) struggles with instability in low-copy-number samples, requiring repeated runs to ensure data reliability [8]. Protocols for dPCR in complex diagnostic applications, such as detecting fetal chromosomal abnormalities or monogenic disorders, are not yet established, necessitating validation through large-scale analyses. ddPCR is undergoing continual advancements and is increasingly adopted in clinical settings, particularly in obstetrics, where its potential applications are being actively explored and refined [9].

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