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REVIEW

Research and Application Progress of Digital Nucleic Acid Amplification Detection Techniques

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Abstract: Recently, micro/nano-scaled fluidic control technologies have been developed to be the alternative to traditional analysis approaches due to the capability of realizing miniaturized multiphase and multistep reactions. The perfect combination of these techniques and nucleic acid amplification methods effectively promotes the establishment and development of digital nucleic acid detection (dNAD) techniques. As a single-molecule analysis approach, dNAD plays an essential role in molecular diagnosis. In this paper, the research and application progress of dNAD techniques are reviewed, including the development history, principle, superiority, and the future prospect of dNAD.

Key Words: Micro/nano-scaled fluidic control technologies; Microfluidics; Digital nucleic acid amplification detection; Digital polymerase chain reaction; Fluidic control; Review

1 Introduction

It has been confirmed by modern biomedical researches that the analysis of biomarkers associated with nucleic acids is beneficial for effectively monitoring and evaluating the occurrence, development, and prognosis of related diseases[1–3]. The analysis of nucleic acids can be classified into qualitative and quantitative analysis, and the latter is essential for unveiling the molecular mechanism of disease' occurrence and development. As the future development trend, single-molecule-level digital nucleic acid detection (dNAD) techniques become mature and arouse great concern in the fields of modern biology, medical research and molecular diagnosis.

Presently, the conventional or "gold standard" nucleic acid analysis method is polymerase chain reaction (PCR) technique. Since firstly introduced in 1983, PCR and its expanded technologies have greatly promoted the development of various research fields in life sciences. Throughout the whole history, PCR has experienced three generations[4]. The first generation PCR entirely depends on agarose or polyacrylamide gel electrophoresis to confirm the amplification products, but the process is cumbersome, easily causes cross-contamination and only allows for qualitative analysis. As the next generation technique, real-time quantitative PCR (qPCR), which launched by the Applied Biosystems in USA, makes nucleic acids analysis step into quantitation determination. According to different strategies, the quantification analysis by qPCR can be divided into relative quantification and absolute quantification analyses. The former uses a housekeeping gene as a reference to normalize the expression levels of gene, thereby usually being applied to gene expression analysis. The latter accomplishes the quantitation of target molecules based on an established standard curve by using known number of nucleic acid standards. However, the influence of bias or inhibitors during the PCR makes it difficult to keep the amplification efficiency constant in various samples, which eventually causes obvious deviations of cycle threshold (C_T) value and is unable to detect the nucleic acid number in the original samples. Therefore,

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qPCR still confronts the defects such as limited sensitivity, insufficient accuracy and lack of good reproducibility. These defects give qPCR embarrassment on determining the rare gene expression, the nucleic acids with ultralow copy number, and so on. Given this, the third generation of PCR technique, digital PCR (dPCR) emerges at a historic moment. By contrast, the accuracy and sensitivity of dPCR are remarkably improved[5]. So dPCR is the real sense of absolute nucleic acid quantitative analysis technique[6]. In this paper, the research and application progress of dNAD are reviewed, including the development history, principle, superiorities, and future prospect of development.

2 Development history of dNAD

As a ground-breaking technique for quantitative analysis, dPCR has raised PCR into a new height, making the quantitative analysis step into a new stage in which the "endpoint signal on or off" is highly considered[7]. Although the embryonic dPCR has been formed before the maturity of qPCR, the clear concept of dPCR was firstly proposed by Vogelstein and Kinzler from USA in 1999[8]. In essence, dPCR is to pick the weak positive signal from massive background signal via large-scale parallel qPCRs[7]. Consequently, how to get enough number of parallel qPCRs becomes the key problem to achieve dPCR. Fortunately, the development and application of micro- or nano-scaled fluidic controls and the related devices provide a high cost-effective scheme to address this problem. As of now, a variety of dPCR devices have been launched, such as integrated fluidic circuit (IFC) chip-based device[9], spinning disk-based device[10], SlipChip-based device[11], droplet-based device[12], self-priming compartmentalization (SPC)-based device[13], and so on. In addition, commercial dPCR devices are also available, mainly including Bio-mark™ high throughput genetic analysis system from Fluidigm, QuantStudio™ 3D dPCR system from Life technologies (ThermoFisher), QX200™ droplet-based dPCR system from Bio-rad, RainDrop™ dPCR system from RainDance Technologies, Constellation™ dPCR system from Formulataix technologies (Fig.1).

Following dPCR, digital isothermal nucleic acid amplification (dINAA) was also developed, which now has become the indispensable part of dNAD. Differing from dPCR, dINAA just needs a constant temperature instead of depending on thermal cycling. Therefore, the emergence of dINAA enables dNAD to be developed into a point-of-care (POCT) technique. In principle, dINAA actually resembles dPCR, except for the replacement of PCR with INAA. Among the currently proposed dINAAs, digital loop-mediated isothermal amplification (dLAMP) arouses great concern due to its simplicity, rapidity, high specificity and high sensitivity[14–17]. Apart from dLAMP, other dINAAs also have been reported, including digital multiple strand displacement amplification (dMDA)[18], digital isothermal hyperbranched rolling circle amplification (dHRCA)[19], digital isothermal multiple-self-matching-initiated amplification (dIMSA)[20], digital recombinant polymerase amplification (dRPA)[21], and digital isothermal rolling circle amplification (dRCA)[22].

3 Principle and superiority of dNAD

Basically speaking, dNAD is the same as conventional NAD since both of them amplify the target molecules to a level which can be detected by the detection approaches, but there is also a big difference between them. Firstly, hundreds of thousands of tiny units with sample dispersed into undertake large-scale parallel nucleic acid amplification reaction, which makes dNAD be capable of analyzing single nucleic acid molecule. Secondly, after amplification, the quantitative analysis of the nucleic acid molecules by dNAD is entirely based on the number of units with positive signal or without. Thirdly, dNAD can directly indicate the nucleic acid molecules from the original samples or indirectly with the aid of Poisson distribution, avoiding the establishment of standard curve.

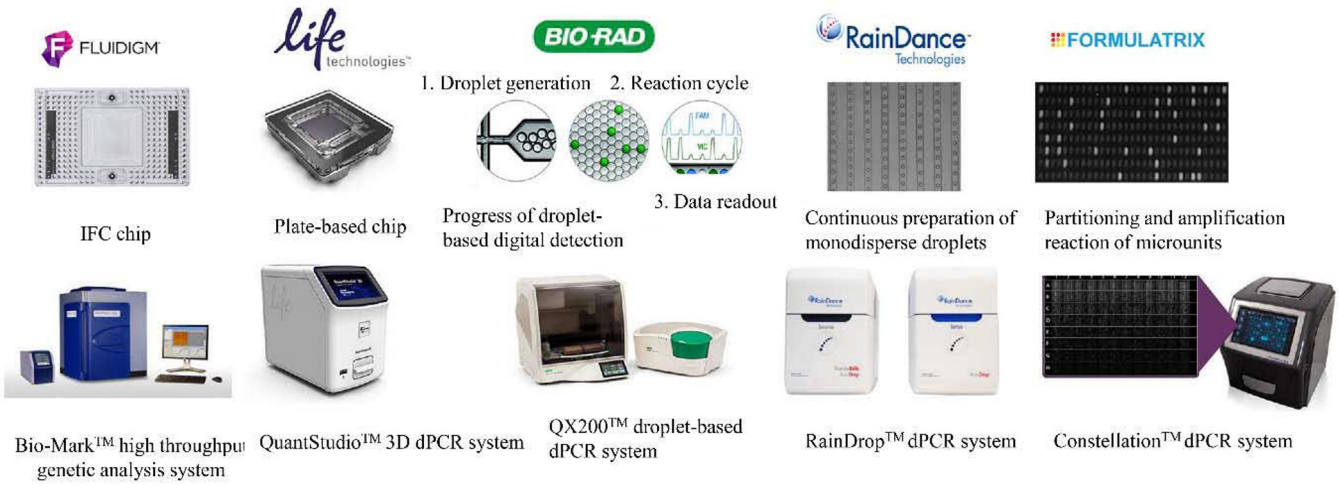

Fig.1 Common commercial digital nucleic acid detection (dNAD) platforms