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Polymerase Chain Reaction [PCR] methods

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Abstract
Polymerase Chain Reaction (PCR) stands as a prevalent method employed for the real-time detection of genes, as well as for the replication and amplification of DNA. The selection of a specific PCR method hinges on various factors, including sensitivity, processing time, cost, and other factors. The review discusses the similarities and differences among different types of PCR, focusing on real-time PCR, quantitative real-time PCR, reverse transcriptase PCR, multiplex PCR, nested PCR and other PCR methods.

Keywords: PCR, DNA, Amplification, Genes, qPCR, RTPCR

1. Introduction

PCR, or polymerase chain reaction, is a method used for the detection of genes in real-time during which we can copy and amplify DNA. It was first discovered in 1980s and, since then, it has deeply influenced biological science as a whole. It has been used for the diagnosis of different diseases, for cloning, gene sequencing and quantitative genome studies in a very rapid manner, but also a very sensitive one [1-4]. As PCR method plays a major role in discovering pathogens, the method is used widely in medical applications. The simplicity of the process enables using DNA as a source from many different organisms. The whole method is based on the ability of the DNA polymerase to synthesize the new strand of DNA which is the exact same as the template strand that is offered [5-7].

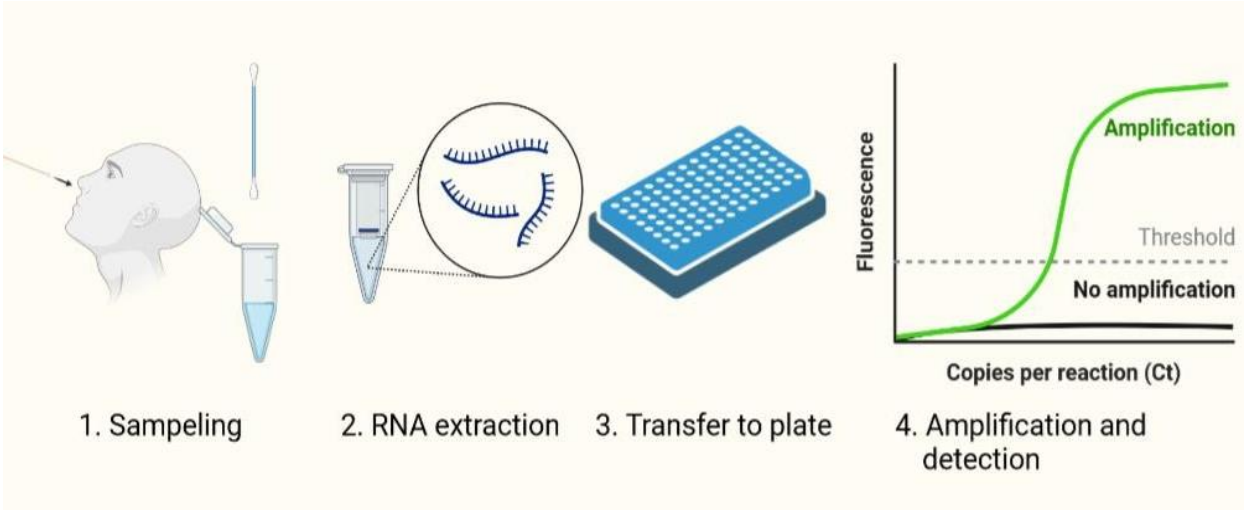

Figure 1: PCR procedure

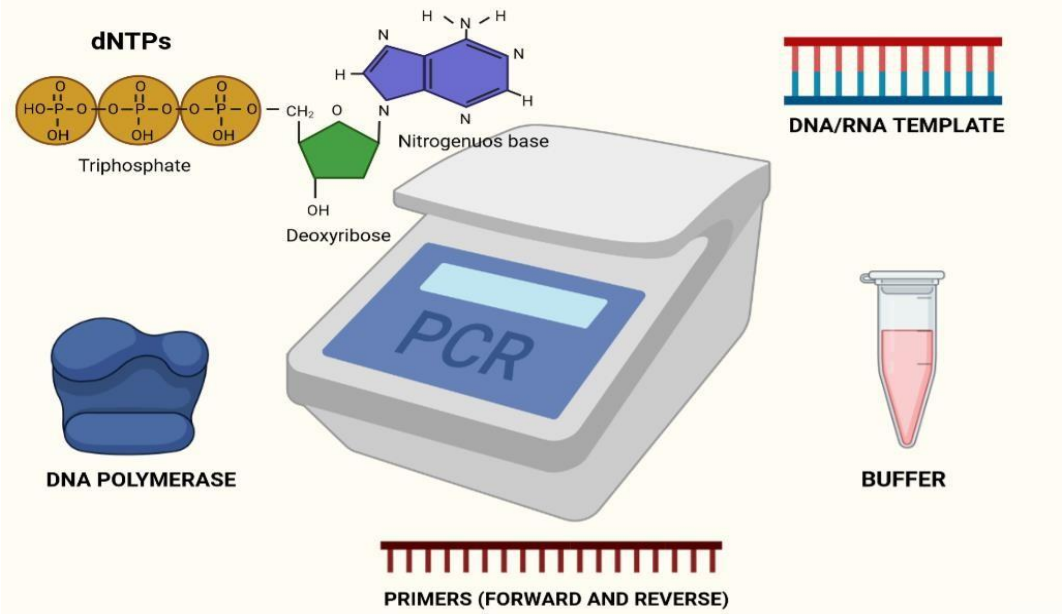

Figure 2: Five main components of PCR

2. PCR methods

Polymerase Chain Reaction (PCR) is a widely used molecular biology technique that amplifies DNA. There are several types of PCR methods, each designed for specific applications. In Figure 3 we present some common PCR variants.

2.1. Real-time PCR

While various types of PCR exist, the most widely utilized is the real-time PCR method. A crucial attribute of real-time PCR is its capability to measure the loads of viruses and bacteria, a functionality that has been in use since its development by Kary Mullis in the 1980s [8-9]. Real-time PCR represented a groundbreaking advancement in genetic research, providing researchers worldwide with the ability to perform procedures like cloning and unlocking numerous new and improved opportunities. Despite being commercially available as early as 1996, its widespread adoption and emergence as a focal point in various research endeavors did not commence until 2002 [7,10]. The real-time PCR is nothing but the amplification of the DNA which is monitored, using RNA as starting material. The real-time PCR method would not be possible without fluorogenic probes which show the amount of the DNA present in each stage of the PCR through lightning up [11-12]. It is important to bear in mind that all of the chemistries included in real-time PCR are fluorescent. Thus, the real time PCR is able to determine the fluorescent signal and, according to it, record the progress of the PCR process [10]. The primary objective achieved through the application of the real-time PCR method is the isolation and quantification of specific nucleic acids within a DNA sample, even when present in minute amounts. As previously outlined, the procedure entails the amplification of the nucleic acid sequence in the sample using real-time PCR, followed by the monitoring of the entire amplification process through fluorescent technology. Another critical aspect of real-time PCR is the temperature, which must be sufficiently high to enable the product of the amplification process to reach its melting point, determined by the nucleotide composition and length of the product. Subsequent to each cycle of DNA replication, the resulting "double-stranded" DNA must undergo melting and separation into single strands. Consequently, under optimal conditions, each PCR cycle should theoretically double the quantity of specific double-stranded DNA
[7-8]. However, this is an ideal situation which is not always possible in the real situation as the reactants get consumed after each regular PCR cycle and then the process reaches the stage known as “plateau effect” [7].

After this stage is reached, the amplification effect is not the same anymore and it is not possible to calculate the amount of the DNA in the starting sample through quantification of the product of the amplification process. The real-time PCR's advantage is that the amplification process is started in the early phase and is performed quickly and measures the products sooner than the reactants get consumed [8,11,12].

2.2. Quantitative real-time PCR (qPCR)

As explained above, the most interchangeable term with real-time PCR is quantitative PCR as real-time PCR is frequently employed for quantifying nucleic acids. On the other hand, the quantitative PCR method is different from the real-time PCR in that it allows for the quantification of the amount of DNA before the amplification process commences [13-14].

2.3. Reverse transcriptase PCR (RT-PCR)

There is also real-time reverse transcriptase PCR that is used as a quantification method for the cytokines from cells or from tissues. It is considered to be more advanced as it combines the amplification process with the detection in a single phase. Recognized as the latest innovation in PCR technology, this method is considered the optimal choice due to its high sensitivity and precision in detecting cytokine levels in cells or tissues [15,16]. A shared characteristic with real-time PCR is the utilization of the fluorescent signal during the amplification process and monitoring; however, the real-time reverse transcriptase PCR does not require additional processing post-PCR completion. Its superiority lies in its ability to directly measure the PCR product, a feature developed to expedite the accumulation of fluorescence emission. All PCR techniques share the commonality of being performed in a closed tube, measuring PCR product in real time during the amplification process. Nonetheless, distinctions arise in factors such as tube design, microplates, sample numbers, length, fluorescent reaction, fluorescent detection, cost, and various other considerations [17-18].

2.4. Multiplex PCR

The Multiplex PCR method consolidates the amplification of multiple sequences into a single reaction, resulting in significant savings in time, cost, and effort. It is a versatile tool commonly employed for the detection of viruses, bacteria, and various infectious agents. Recognized for its rapidity, Multiplex PCR enables the amplification of unique DNA segments concurrently [19]. Among PCR technologies, Multiplex PCR is considered the most mature, primarily due to its cost efficiency, allowing the amplification of multiple sequences within a single reaction. The primary objective of the Multiplex PCR method is to minimize unnecessary interactions present in other methods. It is crucial to note that using nested primers in the second round of amplification should be avoided due to the heightened risk of contamination. However, the inclusion of additives like glycerol, bovine serum, and betaine has proven highly beneficial in enhancing the method's reliability [20].

2.5. Nested PCR

Nested PCR is a very special method because it is used only when there is a need to increase the sensitivity and specificity of the process. It is different from other methods because it includes two one-after-another amplification reactions with different primers used in each [13, 21]. Numerous studies have shown that the nested PCR technique showed its great qualities in diagnosis of those diseases where the sensitivity and specificity were needed to be 100%, such as SARS-CoV-2, where researchers were able, through using this particular technique, to detect the SARS-CoV-2 RNA at concentrations even lower than 0.015 ng/μL [22]. The nested PCR technique is highly reliable, affordable, not difficult to use and it shows success in prevention and

controlling of the diseases. Although the technique was developed to be more sensitive, it is still important that exceptions can happen and the results might be mistaken in the case of the DNA contamination [23-24].

2.6. Long-range PCR

While the conventional PCR method facilitates amplification, the long-range PCR option proves to be a more efficient, adaptable, and expeditious choice, particularly when comprehensive coverage of whole genomic regions is required. The main advantage of this particular technique is that is can amplify almost 15 kb of genomic DNA or even longer [25]. A significant strength of this specific technique lies in its capability to amplify approximately 15 kb of genomic DNA or even longer [25]. Given the numerous benefits that many next-generation sequencing approaches can derive from long-range PCR, researchers must be cognizant of the distinct characteristics of enzymes and their impact on the process. Each enzyme introduces variations and reacts differently with others, emphasizing the importance of understanding these nuances. Notably, a key advantage of the long-range PCR technique is its commercial availability, enabling the amplification of lengthy genomic segments [26-27].

2.7. Single-cell PCR

Single-cell PCR is employed for the examination of various immunological and neurological issues. Optimal results are achieved when coupling single-cell PCR with ddPCR (Droplet Digital system) [14-15]. The analysis delves into heterogeneity within cell populations or cells in tissues. While some cells may exhibit the same phenotype, significant differences and variations emerge in the expression of genes. The primary purpose of single-cell PCR is to scrutinize these variations in both cells and tissues, shedding light on diverse cellular processes [16, 25]. The operational mechanism involves the segmentation of the sample into numerous nano-droplets, followed by amplification. The use of a fluorescent dye facilitates the identification of nano-droplets containing the target sequence, distinguishing between positive and negative outcomes. The single-cell PCR test boasts several advantages, including absolute quantification, reduced sensitivity to PCR inhibitors, and enhanced detection capabilities for low-abundance transcripts, among others [16-18].

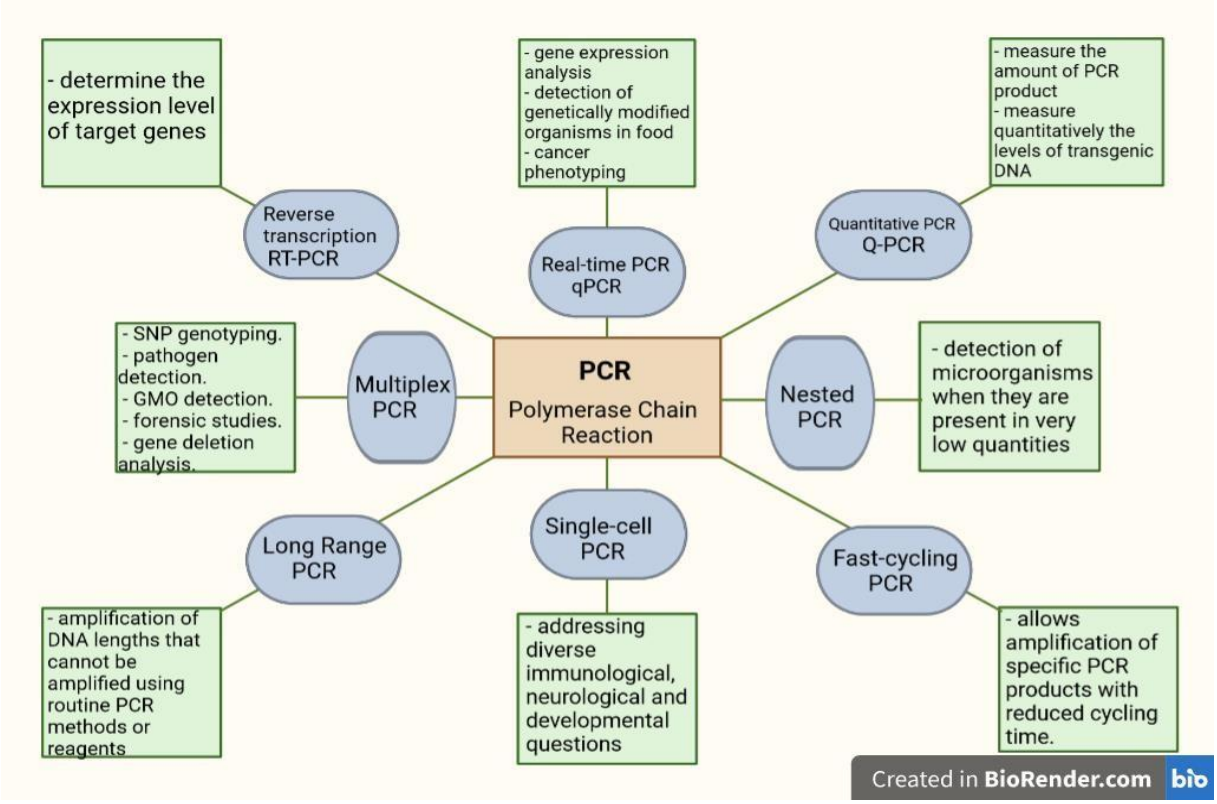

Figure 3: Most common PCR variants

3. Conclusion

PCR analysis stands out as a pivotal molecular diagnostic technique, renowned for its simplicity and efficiency. A primary advantage lies in its expeditious nature, swiftly amplifying DNA and generating numerous copies within minutes. Additionally, its heightened sensitivity renders it indispensable for sequencing and comprehensive analysis. Notably, PCR enables the absolute quantification of mRNA in individual cells, a crucial feature for studying gene expression variations in diverse diseases. Despite its myriad merits, PCR is not without limitations. While its exceptional sensitivity is advantageous, there exists a susceptibility to sample contamination, potentially yielding inaccurate results. Furthermore, PCR primers exhibit efficacy for sequences that are similar but not entirely identical. Looking ahead, the future holds promising advancements in PCR applications. Anticipated developments include microfluidic devices executing RT-PCR for exosomal RNA analysis, advanced Droplet-based qPCR methods, facilitating single-cell mRNA purification and gene expression analysis, miRNA quantitation assays, and chip-based digital RT-PCR for precise mRNA quantification in individual cells. Diversified assays and approaches are poised to contribute to an enhanced comprehension of gene combinations.

Declaration of competing interest

"The authors declare that they have no known financial or non-financial competing interests in any material discussed in this paper."

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Abbreviations

PCR- polymerase chain reaction
DNA- deoxyribonucleic acid
qPCR- quantitative polymerase chain reaction
mRNA- messenger RNA
RT-PCR- reverse transcription polymerase chain reaction
ddPCR -Droplet Digital PCR

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