

POLYMERASE CHAIN REACTION: METHODS, PRINCIPLES AND APPLICATION

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Abstract

Polymerase chain reaction (PCR) is a molecular biology method that allows amplification of specific DNA fragments. PCR has found wide application in disease diagnostics, forensic medicine, as well as in genomics and biotechnology research. This technique has provided scientists with a powerful tool for analyzing genetic information, opening up new horizons in science and medicine.

Keywords: Polymerase chain reaction (PCR), DNA, matrix, primers, diagnostics.

INTRODUCTION

The polymerase chain reaction, like recombinant DNA technology, has had an enormous impact in both basic and diagnostic aspects of molecular biology because it can produce large amounts of a specific DNA fragment from small amounts of a complex template. Recombinant DNA techniques create molecular clones by conferring on a specific sequence the ability to replicate by inserting it into a vector and introducing the vector into a host cell. PCR represents a form of “in vitro cloning” that can generate, as well as modify, DNA fragments of defined length and sequence in a simple automated reaction. In addition to its many applications in basic molecular biological research, PCR promises to play a critical role in the identification of medically important sequences as well as an important diagnostic one in their detection.

Currently, one of the most modern and dynamically developing methods of molecular biology is the polymerase chain reaction (PCR). Elegance, simplicity of execution, unsurpassed sensitivity and specificity brought the new method unprecedented popularity in a short time, PCR analysis has spread throughout the world, quickly moving from the laboratories of scientific institutes into the sphere of practical clinical use. Polymerase chain reaction (PCR) is a method for amplifying specific fragments of DNA that has revolutionized molecular biology and genetic research. Developed in 1983 by Kary Mullis, PCR allows selected DNA sequences to be copied many times, making them possible to study and analyze in detail [1,4,6].

PCR is used in a variety of fields, including infectious disease diagnostics, forensics, genetic testing, and evolutionary studies. In this regard, further development of PCR technology and its modifications contributes to the progress of science and medicine.





With the development of technology, PCR is becoming increasingly accessible, which contributes to its widespread use in healthcare institutions and scientific laboratories. As a result, PCR has become the standard in molecular diagnostics, providing high accuracy and speed, especially in pandemics when timely diagnosis is critical.

The principle of PCR is based on the cyclic process of denaturation, annulation and elongation. In the first stage, when the temperature rises, the double-spiral structure of DNA disintegrates into individual chains. Then, when the temperature drops, special primers, short nucleotide sequences, bind to target areas of the matrix DNA. In the final stage, at the optimal temperature for DNA polymerase activity, new DNA strands are synthesized using the matrix and primers. By repeating this process cyclically, the number of copies of the target fragment grows exponentially, allowing significant concentrations to be achieved even in samples with low DNA content [5,7].

There are several types of PCR, each with its own specific applications and advantages.

1. Standard PCR is a classic method that allows DNA amplification with high accuracy. It is used in clinical diagnostics and scientific research.
 2. Quantitative PCR (qPCR) is a modification that allows for simultaneous amplification and quantitative assessment of the yield of target DNA. This makes qPCR indispensable in gene expression studies and infectious disease diagnostics.
 3. RT-PCR (reverse transcription PCR) is a method used to analyze RNA, where complementary DNA (cDNA) is first synthesized. It is especially important for virus and gene expression studies.
 4. Nested PCR is an improved version of standard PCR that allows for increased specificity and sensitivity, especially in cases where the source material is limited.
- These methods open up a wide range of possibilities for research, diagnostics and biotechnology, and continue to serve as the basis for many of the latest scientific discoveries.

In recent decades, PCR has undergone many improvements, including quantitative PCR (qPCR) and digital PCR (dPCR). Quantitative PCR allows not only to detect the presence of DNA, but also to quantitatively measure its quantity, which opens up new horizons in biomarker research and treatment monitoring. Digital PCR, in turn, provides higher accuracy and sensitivity, which makes it an indispensable tool in complex cases, for example, when detecting minor mutations in tumor DNA [3].

PCR also serves as the basis for other molecular techniques such as sequencing and cloning, expanding the possibilities of genetic research in addition, the development of "reverse" PCR (RT-PCR) allows the detection of expressed genes based on RNA, which is extremely important for the study of various diseases, including cancer and viral infections [2,6].

With the development of miniaturization and automation technologies, PCR equipment is becoming more compact and affordable. This simplifies the use of PCR in the field and allows diagnostics to be carried out directly in clinics, which significantly speeds up the process of obtaining results and facilitates access to quality medical care.

An equally important aspect is the continuous improvement of protocols and reaction mixtures for PCR, which increases its efficiency and reduces the risk of false positive and false negative results. These improvements contribute to the expansion of the application of PCR, including environmental studies, agronomy and forensics, confirming its status as a versatile tool in scientific research [1,3].



In addition, the integration of PCR with other high-tech methods, such as genome sequencing and machine learning, opens up new horizons for the diagnosis and monitoring of diseases. This allows not only to detect pathologies at early stages, but also to predict the likelihood of their development, providing a proactive approach to treatment. Taking all these factors into account, PCR continues to be a vital tool in the arsenal of modern specialists, providing many opportunities for progress in medicine and related fields.

Conclusion

The advancement of science has transformed our lives in ways that would have been unpredictable just a halfcentury ago. Molecular methods have shown a promise in this aspect. PCR and its applications hold scientific and medical promise. PCR has very quickly become an essential tool for improving human health and human life. PCR has completely revolutionized the detection of RNA and DNA viruses. PCR is valuable as a confirmatory test. PCR is a rapid technique with high sensitivity and specificity. PCR has also been credited to have been able to detect mixed infections with ease in many studies. PCR, a more sophisticated technique, requires infrastructural support, is expensive but nevertheless, one cannot discount its utilitarian advantages which are many compared to the existing conventional diagnostic methods.

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