THE SCIENCE WORLD
A Monthly e Magazine
ISSN:2583-2212
May 2024 Vol.4(5), 2009-2013

Popular Article

Application and Importance of Polymerase Chain Reaction

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https://doi.org/10.5281/zenodo.11503212

Abstract

In molecular biology, the polymerase chain reaction (PCR) is a method that can amplify a single or a few copies of a DNA fragment over several orders of magnitude, producing thousands to millions of copies of a specific DNA sequence. Today, PCR is a widely used and frequently essential technique in biological and medical research labs for a wide range of purposes. The PCR method consists of three main steps: Denaturation, annealing, and extension. An increasing number of diseases can be investigated and diagnosed with the help of PCR. In forensics labs, PCR is also utilized. Genes linked to the emergence of cancer can be found through PCR. This article aims to review the fundamentals of PCR with respect to its usage, applications, and methods.

Keywords: Denaturation, DNA, Naturation, PCR, Primers.

Introduction

Polymerase chain reaction (PCR) is a new, popular molecular biology technique for enzymatically replicating DNA without using a living organism, such as E. coli or yeast. Through the use of this technique, a small amount of DNA can be amplified exponentially many times. The availability of more DNA makes analysis much simpler. In medical and biological research labs, polymerase chain reaction (PCR) is widely employed for a range of applications, including genetic fingerprint identification, infectious disease diagnosis, hereditary disease detection, gene cloning,

DNA computing, and paternity testing (www.wikipedia.org). Since Kary Mullis invented the method in 1983, PCR has become a widely used and significant technique with a wide range of applications in biological and medical research labs. These include the diagnosis of genetic diseases, the identification of genetic fingerprints (used in forensic sciences and paternity testing), the detection and diagnosis of infectious diseases, and DNA cloning for sequencing, DNA-based phylogeny, or functional analysis of genes. For his work on PCR, Mullis and Michael Smith shared the 1993 Chemistry Nobel Prize (Bartlett and Stirling 2003). The PCR is typically performed in a thermal cycler with small reaction tubes (0.2–0.5 ml volumes) and a reaction volume of 10-200 ml. To reach the temperatures needed for each stage of the reaction, the thermal cycler alternately heats and cools the reaction tubes.

Principle of PCR

The enzymatic replication of DNA is the foundation of the PCR technique. Using primer-mediated enzymes, a brief DNA segment is amplified during the PCR process. New DNA strands that are complementary to the template DNA are created by DNA polymerase. Only the pre-existing 3'-OH group can have a nucleotide added to it by the DNA polymerase. Consequently, a primer is needed. Consequently, the DNA polymerase's 3' prime end obtains more nucleotides.

Components of PCR

Components of PCR constitutes the following:

1. **DNA Template**– The DNA of interest from the sample.
2. **DNA Polymerase**– Taq Polymerase is used. It is thermostable and does not denature at very high temperatures.
3. **Oligonucleotide Primer**s- These are the short stretches of single-stranded DNA complementary to the 3’ ends of sense and anti-sense strands.
4. **Deoxyribonucleotide triphosphate**– These provide energy for polymerization and are the building blocks for the synthesis of DNA. These are single units of bases.
5. **Buffer System**– Magnesium and Potassium provide optimum conditions for DNA denaturation and renaturation. It is also important for fidelity, polymerase activity, and stability.

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Published 25.05.2024

Sr. No.	Components	Volume (µl)
1.	Template DNA (25 µg/µl)	2.0
2.	Forward Primer (10 mM)	1.0
3.	Reverse Primer (10 mM)	1.0
4.	Taq DNA Polymerase (5 U/µl)	0.3
5.	dNTPs (25 mM)	0.5
6.	10 x PCR buffer	5.0
7.	$MgCl_2$ (15 mM)	1.5
8.	Sterile double distilled water	38.7
	Total	50.0

Table. Components of Polymerase Chain Reaction

Types of PCR

PCR is of the following types:

- **Real-time PCR**

In this type, the DNA amplification is detected in real-time with the help of a fluorescent reporter. The signal strength of the fluorescent reporter is directly proportional to the number of amplified DNA molecules.

- **Nested PCR**

The purpose of this was to increase specificity and sensitivity. Because unexpected primer binding sites are amplified, they lessen the non-specific binding of products.

- **Multiplex PCR**

This is used to simultaneously amplify numerous distinct DNA sequences for the amplification of multiple targets in a single PCR experiment.

- **Quantitative PCR**

To identify, describe, and quantify a known sequence in a sample, it makes use of the linearity of DNA amplification.

- **Arbitrary Primed PCR**

It's a PCR-based DNA fingerprinting method that uses primers with randomly selected DNA sequences.

PCR (Polymerase chain reaction) Steps

The PCR involves three major cyclic reactions:

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Denaturation

Denaturation occurs when the reaction mixture is heated to 94℃ for about 0.5 to 2 minutes. This breaks the hydrogen bonds between the two strands of DNA and converts it into a single-stranded DNA. The single strands now act as a template for the production of new strands of DNA. The temperature should be provided for a longer time to ensure the separation of the two strands.

Annealing

It takes about 20–40 seconds to bring the reaction temperature down to 54–60°C. Here, the primers attach themselves to the template DNA's complementary sequences. Single-stranded sequences of DNA or RNA with a length of 20 to 30 bases are called primers. They act as the precursors to the synthesis of DNA. There are two primers—a forward primer and a reverse primer—because the two separated strands run in the opposite directions.

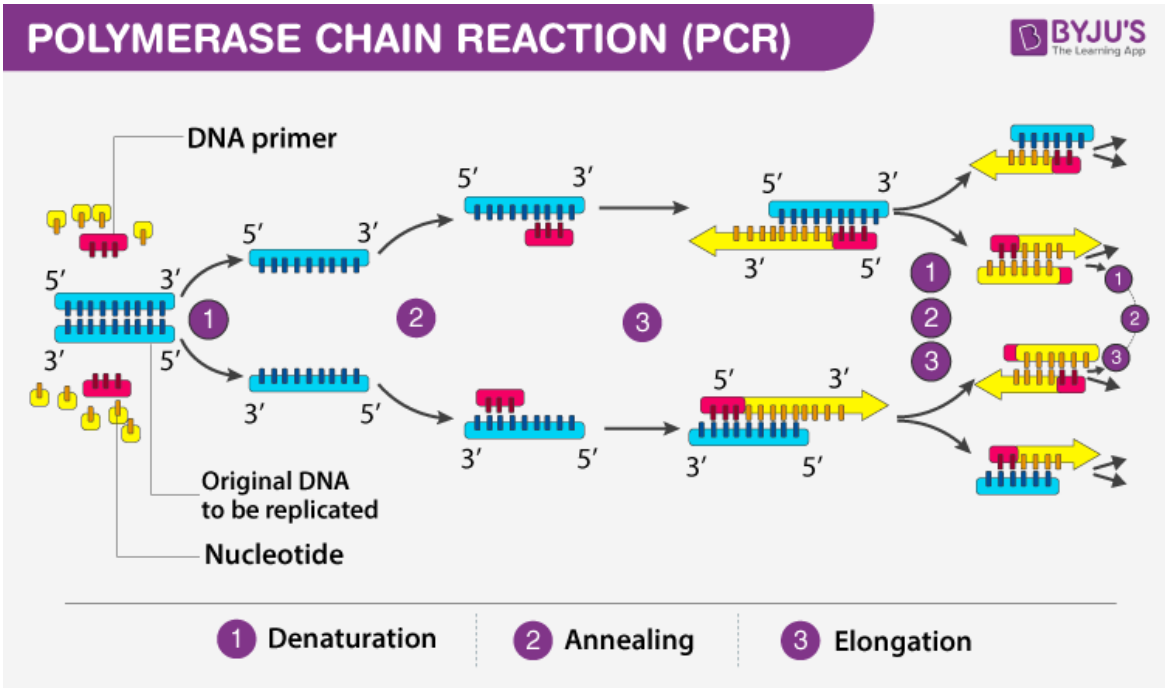

Figure No. 1. *A basic PCR protocol—DNA synthesis cycle.* (Shahzad *et al., 2020*)

Elongation

The temperature is now between 72 and 80 ℃. The Taq polymerase enzyme adds the bases to the 3' end of the primer. As a result, the DNA elongates from 5' to 3'. When conditions are ideal, the DNA polymerase adds roughly 1000 base pairs per minute. Taq Polymerase is heat-tolerant to an extreme degree. DNA bases are added to the single strand after it binds to the primer. Consequently,

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Published 25.05.2024

a molecule of double-stranded DNA is produced. In order to quickly obtain a number of sequences of interest from DNA, these three steps are repeated 20–40 times.

Applications of PCR

The following are the applications of PCR:

Medicine

- Testing of genetic disease mutations.
- Monitoring the gene in gene therapy.
- Detecting disease-causing genes in the parents.

Forensic Science

- Used as a tool in genetic fingerprinting.
- Identifying the criminal from millions of people.
- Paternity tests

Research and Genetics

- Compare the genome of two organisms in genomic studies.
- In the phylogenetic analysis of DNA from any source such as fossils.
- Analysis of gene expression.
- Gene Mapping

Conclusion

The PCR is continuously changing how we solve basic issues in many branches of biology, as well as in laboratory and clinical medicine. PCR will be used more frequently for routine laboratory diagnosis as well as for a range of issues in an ever-expanding range of fields, such as environmental pollution and monitoring of compliance with the International Biological Weapons Convention, as protocols, reagents, kits, and instrumentation continue to advance. In the fields of medicine and laboratory medicine, PCR has significantly increased the precision and speed of diagnosing a wide range of infectious diseases, as well as the ability to diagnose inherited diseases and match tissues for organ and tissue transplantation with greater success. The fundamentals of PCR should be incorporated into future clinical laboratory diagnostic research in practically all fields due to its cost-effectiveness. When it comes to diagnostic certainty, we are just entering a new era.

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