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Polymerase chain reaction: An efficient tool for genotypic studies

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Abstract

Since its discovery, the polymerase chain reaction (PCR) has fundamentally changed biological science. It made it possible to produce enormous amounts of DNA and detect it specifically for the first time. Large-scale scientific initiatives like the Human Genome Project have been fuelled by PCR-based techniques. Nowadays, the method is widely utilised by researchers and clinicians to quickly and sensitively perform complex genomic and quantitative studies, clone and sequence genes, and diagnose disorders. Pathogen detection is one of the most significant medical uses for the traditional PCR technique. Furthermore, forensic medicine uses the PCR assay to identify criminals. This article focuses on the PCR process and its applications.

Keywords: Denaturation; Annealing; DNA amplification; Polymerase chain reaction; STR amplification

1. Introduction

- Primer-based amplification of targeted genomic as well cloned DNA sequences by application of an unusual enzyme is known as Polymerase Chain Reaction (PCR).
- To synthesize DNA utilizing deoxynucleotide substrates upon a single-stranded DNA template, polymerase chain reaction (PCR) makes utilization of the enzyme DNA polymerase.
- During annealing to a lengthier template DNA, DNA polymerase inserts nucleotides onto the 3' ends of a customized oligonucleotide.
- For this reason, DNA polymerase is capable of using a synthetic oligonucleotide like a primer for extending its 3'end as well as produces a next-ended stretch of double-stranded DNA by annealing it with a single-stranded template which possesses a portion complementary with the oligonucleotide.[1-6]

In molecular biology, PCR is used to amplify, or create multiple copies of, tiny regions of DNA or a gene. Because of this, a relatively modest initial amount of a certain DNA segment can be copied millions of times.

The American biologist Kary Mullis invented the polymerase chain reaction (PCR) in 1983. His groundbreaking work earned him the 1993 Chemistry Nobel Prize.

PCR was employed in the Covid-19 pandemic testing process to verify if an individual carried the virus.[4,6]

A method called polymerase chain reaction (PCR) is used in molecular biology and medicine to duplicate a particular gene or region of DNA dozens or even millions of times.

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It can be applied in a variety of contexts, such as the initial phases of DNA processing for sequencing or the creation of forensic DNA profiles from minuscule DNA samples.

It can also be used to determine whether a gene is present or not, which aids in the identification of pathogens during infection. For instance, PCR testing were utilised during the Covid-19 pandemic to determine whether a person's swab sample contained any genetic material from the virus, hence providing either a positive or negative disease result.[7-10]

The five main "ingredients" of a PCR are:

- The blue print for copying DNA.
- Primers are brief segments of 20–30 bases long, either end of the desired DNA segment, that bind to indicate the beginning of the PCR process.
- DNA nucleotide bases, or dNTPs for short. The fundamental components of DNA are A, T, C, and G, and they are required to create an additional strand of DNA.
- An enzyme known as Taq polymerase that complements the duplicated sequence with bases.
- A buffer to guarantee the reaction's ideal circumstances.

1.1. Procedure-[8-12]

There are three primary steps of PCR, all of which require heating and cooling:

- Step 1: Denaturing: Heat is applied to the double-stranded template DNA, causing it to split into two single strands.
- Step 2: Annealing: In order to allow the DNA primers to bind to the template DNA, the temperature is decreased.
- Step 3: Extending: The Taq polymerase enzyme creates a new strand of DNA when the temperature is increased once more.

Twenty to forty times through, these three phases are repeated, multiplying the amount of DNA copies every single time. This is referred to as machine-assisted thermal cycling.

It is operated by a device known as a heat cycler. PCR can be finished in less than an hour or in several hours, depending on the machine's speed.

The size and quantity of DNA fragments generated during PCR can be verified using an apparatus known as electrophoresis.

1.2. First Step Denaturing

For a period of fifteen to thirty seconds, the reaction mixture becomes heated to 94 to 95 degrees Celsius.

Two distinct strands containing template DNA split apart due to the high temperature breaking the hydrogen bonds that hold the bases together.

Two identical strands of DNA are produced as a result, and these will serve as templates for the new replicas from every strand of DNA.

It is crucial to keep the temperature at this level for a sufficient amount of time to guarantee that all of the DNA strands completely fully separated.

1.3. Step 2 The Annealing Process

In order for the primers to form hydrogen bonds with a specific region on the single strand of template DNA, the reaction must be cooled.

Although it varies depending on the primer's properties, the temperature is often between 50 and 65°C.

The complementary, opposing directions of the two split DNA strands travel from one end, or the5' end, to the other, or the 3' end. Consequently, two primers are available: a forward primer and a reverse primer.

This stage is crucial because the primers provide the polymerase enzyme with a brief segment of double-stranded DNA to deal with, which acts as the beginning point for DNA synthesis. The polymerase enzyme cannot bind and begin creating the new symmetrical strand of DNA derived from loose DNA bases during the extending phase until the primer has bound.

The annealing process typically takes ten to thirty seconds.

1.4. Step 3 Extending

To allow a unique Taq enzyme for DNA polymerase to create new DNA by adding DNA bases, the temperature is raised to 72 °C.

Scientists isolate the Taq DNA polymerase enzyme using the bacterium Thermus aquaticus ('Taq').

Although this bacterium can withstand temperatures exceeding 80 °C because it typically inhabits hot springs, its ideal temperature is 72 °C.

The temperature required to split DNA strands during the denaturing phase of PCR cannot be withstood by the bacteria since its DNA polymerase exhibits remarkable stability at elevated temperatures.

The DNA polymerase seen in most other creatures would melt at these temperatures. For instance, 37 ˚C being the ideal temperature to enable human polymerase to function.

The complementary strand is produced by the Taq polymerase starting at 72 °C. One by one, it attaches DNA bases to every single strand in the 5' to 3' direction after attaching to the primer.

Two molecules of DNA- one double-stranded and the other new- are produced as a result.

The length of the amplified DNA sequence determines how long this step takes. Typically, copying 1,000 DNA bases takes around a minute.

1.5. Replicate the procedure

The target DNA sequence is produced in large quantities by repeating these three heat cycling procedures 20-40 times.

Moreover, the newly created DNA fragments from PCR act as templates for the DNA polymerase enzyme, which binds to them to begin synthesizing new DNA.

As a result, plenty of replicas of the targeted DNA segment are created in a comparatively short amount of time.

1.6. PCR primers (13-16)

1.6.1. What distinguishes one PCR from another is mostly the PCR primers

Target sequences that are going to be amplified have ends and beginnings that are complementary to the DNA segments known as PCR primers. The exact sequence of DNA that is copied during a PCR is determined by the primers. Knowing the part of the genome or DNA sequence the fact that will be the target sequence for your PCR is a prerequisite for designing primers. A person would search like the beta- globin gene sequence, for instance, to see if they carry the mutation linked to sickle cell disease. Millions of genes spanning thousands of distinct species have known sequences compiled in public databases like RefSeq; these databases are constantly expanding as scientists acquire and contribute additional sequencing data.

1.6.2. Two primers, commonly referred to as the "forward" and "reverse" primers, are used in a typical PCR. On the opposing strands of the DNA are the forward and reverse primers positioned

The primers will attach to the DNA during a PCR run, connecting the sequence you want to amplify. After that, DNA polymerase amplifies your desired sequence by copying the portion of your desired sequence that lies between the primers. The dimensions of your "amplicon," or PCR result, are determined by the primer spacing. Fragments ranging in length from a handful of base pairs through a few thousand base pairs can be amplified using PCR. However, the majority of PCRs, particularly those used for diagnosis, seek to amplify fragments with several hundred base pairs. This spectrum of amplicons is easily manipulable, observable, and distinguishable in the post-PCR experimental procedures

(e.g., gel electrophoresis). The secret to a successful primer design is to ensure that both primers have similar qualities and fall within a few fundamental criteria.

1.7. Applications of PCR:(12-15)

PCR assays can be performed directly on genomic DNA samples to detect translocation-specific malignant cells at a sensitivity that is at least 10,000-fold higher than that of other methods. PCR can be used for a wide range of experiments and analyses, some of which are discussed below. PCR can be used for the diagnosis of many human diseases, including:

- Diagnosis of infectious diseases, such as HIV, CMV, Mycoplasma, Pneumonia, Cancer, Syphilis, fungal & protozoal disease, hepatitis, etc.
- Diagnosis of cancer, particularly leukaemia and lymphomas.
- Genetic fingerprinting, paternity testPCR permits early diagnosis of malignant diseases, such as leukaemia and lymphomas, which is currently the most advanced and already being used routinely. Through tissue culture tests and animal models, PCR also makes it possible to identify slow-growing or non-cultivable microbes such viruses, anaerobic bacteria, or mycobacteria.
- PCR diagnostic applications in microbiology are based on the identification of infectious agents and the use of particular genes to distinguish between pathogenic and non-pathogenic strains. PCR is used to amplify a specific, brief segment of a DNA strand. This may be a single gene or perhaps a portion of a gene. Unlike live creatures, the PCR technique can only replicate small DNA fragments, typically up to 10 kb (kilo base pairs)

2. Conclusion

These days, PCR is a very popular and widely accepted approach for accurate diagnosis of several diseases with better sensitivity and specificity. It is also becoming more and more popular in Bangladesh. PCR is a common lab diagnostic and research technique used by numerous advanced medical centres, contemporary diagnostic labs, and medical institutes. PCR can be useful in the diagnosis of diseases with a variety of atypical clinical presentations. It can also result in an early and conclusive diagnosis, which aids the physician in initiating treatment early, managing a better treatment plan, and providing patient follow-up. As a result, the patient and their family experience less financial and social hardship.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflicts of interest in any matters related to this article

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