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Laconic Concept on Polymerase Chain Reaction (PCR)

Yusuf Yahaya Miya
Department of Molecular Genetics & Infectious Diseases, Abubakar Tafawa Balewa University Bauchi, Nigeria
Suleiman Yusuf Alhaji
Department of Molecular Genetics & Infectious Diseases, Abubakar Tafawa Balewa University Bauchi, Nigeria
Hamisu Sule
Department of Human Anatomy, Abubakar Tafawa University Bauchi, Nigeria

Abstract:

The objective of this paper is to provide a laconic concept pertaining PCR. Polymerase Chain Reaction (PCR) is a molecular technique used to amplify specific DNA segments, revolutionizing genetic testing, forensic science, and biomedical research. Developed by Kary Mullis in 1983, PCR involves denaturation, annealing, and extension steps, utilizing Taq polymerase and nucleotides. PCR enables isolation of DNA fragments, generates hybridization probes, and facilitates DNA sequencing, cloning, and genetic fingerprinting. It's widely used in disease diagnosis, detecting pathogens like HIV, COVID-19, and cancer cells. PCR also aids forensic analysis, oncology, food safety, veterinary medicine, pharmacy, and environmental science. Types of PCR include Conventional PCR, Real-time PCR, Reverse Transcriptase PCR, Nested PCR, and Multiple PCR. The process involves mixing DNA with primers, nucleotides, Taq polymerase, and MgCL2, followed by denaturation, annealing, and extension cycles. PCR's applications are diverse, driving advancements in genetics, disease diagnosis, and various scientific fields. Its sensitivity and specificity make it a valuable tool for detecting genetic changes, identifying pathogens, and analyzing DNA samples.

Keywords: *Pathogens, PCR, laboratory, Taq polymerase, DNA samples*

INTRODUCTION

The polymerase chain reaction PCR is a popular method utilized in laboratory for amplifying DNA with the use of Taq polymerase (thermostable DNA polymerase) obtained from Thermus aquaticus to make more DNA after thermal denaturation of the template, and annealing. PCR is fundamentally applicable in many methods in genetic testing such as analysis of DNA samples, forensic science, and biomedical researches (Suchman, 2016). The objective of this paper is to provide a laconic concept pertaining PCR.

Some Applications of PCR

PCR help in allowing isolation of DNA fragments from genomic DNA; by encouraging selective amplification of given regions of DNA. Pure DNA through PCR is supplied to generating hybridization probes, for southern or northern hybridization, and as well DNA cloning. DNA sequencing for determining unknown PCR-amplified sequences is another application. One of the amplification primers is applied in Sanger sequencing. Bacterial colonies can be speedily screened using PCR for having correct DNA vector construct (Joshu & Deshpande, 2010; Postollec et al., 2011; Saroj et al., 2015). PCR can be utilized in methods of genetic fingerprinting, a forensic approach to determine a person or organism by comparing different experimental DNAs by PCR-inclined intervention. Verily, the powerful nature of some PCR methods on fingerprint techniques allowed the assessment of relationship, between individuals such as siblings, and in paternity testing. Health experts utilized PCR in determining small amounts of genetic changed in cells, as well as cancer cells. PCR tests the presence of disease causing pathogens such as HIV, Ebola, influenza, tuberculosis, hepatitis, COVID-19, etc (Joshu & Deshpande, 2010; Suchman, 2016).

Simple tips in the laboratory for PCR

A laboratory researcher takes sample from the person to investigate. Then a special machine is use to heat the sample. Hereby, allowing the separation of DNA into two pieces of strands, and the reaction is cooled to encourage primers to adhere to the template DNA sequences. Then heat is applied again to allow Taq polymerase add bases to the templates; thereby, duplicating the original DNA to make two strands. The process is carried out by machines in automation. A positive result indicates the presence of pathogen, while negative the absence of disease pathogen (Rajalakshmi, 2017).

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a molecular photo typing technique applied so that small targeted segments of DNA are amplified to make millions of copies of given the fragment (Rajalakshmi, 2017). Historically, PCR was coined in 1983 by the effort of Kary Mulls, who was later in 1993 awarded with a Nobel Prize for this gigantic innovation. Commonly, the thermocyclers, buffers, nucleotides, are typically required in PCR. Each PCR cycle has major significant steps including denaturation, alignment of specific primers (or annealing) and the terminal extension process.

Principle of PCR

The principle of PCR entails that a double stranded DNA molecule, upon heating to high temperature, tend to separate and give out two single-stranded DNA molecules, that consequently each molecule strand is copied using DNA polymerase and available nucleotides; that consequently spur duplication of the initial (original) DNA molecules. These processes (events) are repeated to generate multiple copies of DNA molecule (Giasuddin, 1995; Houghton & Cockerill, 2006).

Types of PCR

Indeed, due to wide range applications PCR is of many types, therefore, this paper will show some examples as follows:

Conventional PCR

Conventional PCR is otherwise denoted as endpoint PCR and it is a kind of PCR that consists of DNA denaturation, primer annealing, and DNA elongation as well

Realtime PCR- Realtime PCR is denoted as quantitative approach as it's facilitates the measurement of DNA quantity in the course of amplification.

Reverse transcriptase PCR- Reverse transcriptase PCR is a kind of PCR utilized in amplifying RNA molecules wherever the need arises. Therefore, the RNA is reversely transcripts to make complementary DNA (cDNA) with the action of reverse transcriptase. Thereafter, the cDNA has to undergo the conventional amplification.

Nested PCR- in Nested PCR there are two rounds of PCR amplifications. Therein, an external primer is utilized to amplify a DNA region, thereafter; the part of the first product is utilized to make another round of PCR (Laura & Rukmanidevi, 2023).

Multiple PCR- Multiple PCR methods involved a technique whereby different pathogens are detected in single sample at a time. This method involved a base pair length that are having different band from different DNA genes targeted. It is useful in reducing cost of using single PCR for determination of pathogen at a time (Rajalakshmi, 2017).

Procedural outline of PCR

The procedure involved in PCR can be outlined here. The DNA (to be amplified) is mixed with surplus of the two primer molecules, all the 4 categories of nucleotides, Taq polymerase, and

MgCL2 in a suitable medium are assembled (Persing, 1991). Thus, the application of the three major steps is ensured as follows:

Denaturation - Denaturation consists of heating the mixture to a high temperature (such as 94-96ºC) therewith, the double helix DNA separates into 2 DNA strands. Each of the strands is a target for further action.

Annealing - Annealing consists of Cooling the mixture at low temperature (such as 55-65ºC) therefore the 2 primers annealed to each of the strands template.

Extension - In extension step, the temperature is modified to facilitate the Taq polymerase activation to start new synthesis of a new brand of DNA between the primers, Mg^{2+}, and dNTPs. Optimization is achieved at 72ºC. Therein, as soon as one cycle halts, the next cycle begins. Thus, first DNA strand spur the 2nd and progressive turns occur (Giasuddin, 1995).

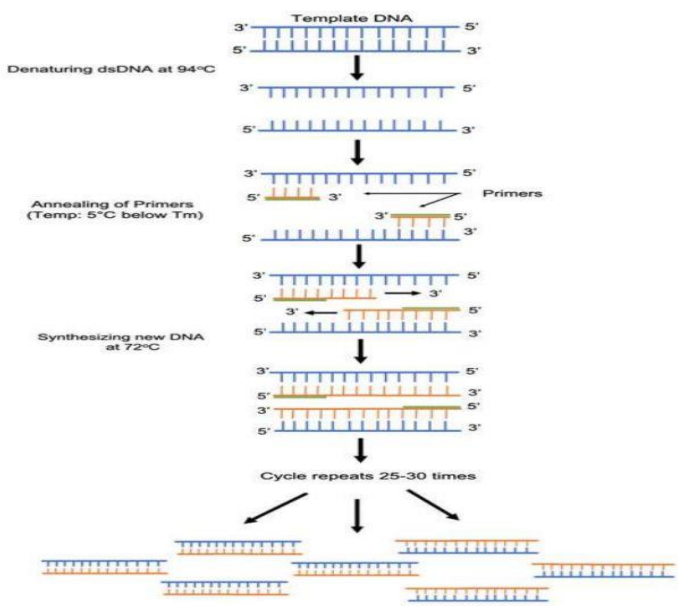

Figure 1: Steps involved in PCR; Source: Laura & Rukmanidevi, (2023)

PCR in various fields

PCR is widely utilized in diverse array of scientific fields some of which are listed below:

Genetics - PCR has been widely utilized I'm genetics for the study of DNA sequences, by allowing specific target genes to be amplified. It is employed in genomics, population genetics, and gene expression studies.

Disease diagnosis - Medical diagnosis take advantage of advancements in PCR to detect diseases. PCR facilitates the detection and diagnosis of infectious agents such as viruses, bacteria, and parasites. Through PCR amplifications of DNA segments, diseases such as HIV, influenza, cystic fibrosis, Lyme disease, COVID-19, etc can be easily diagnosed.

Forensic analysis - PCR is applicable in the identification purposes by generating a portion of DNA from the sample, the possible suspects of crimes can be determined.

Oncology - PCR is utilized to study cancer and in making diagnosis of cancer cells. Mutations in DNA, and different types of cancer can be determined through PCR techniques (Lilit & Nidhi, 2013).

Food safety - PCR methods are applied in food industries to detect the different pathogens and allergies in food materials thereby aiding the quality control interventions.

Veterinary medicine (VM)- VM applied PCR to detect and diagnose pathogens that cause diseases or cancer in animals.

Pharmacy - PCR is utilized in pharmacy to elucidate infection, interaction between different individuals and differences in response to drug products.

Environmental science - Environmental studies that aimed to monitor toxicants in samples such as air, water, soil, use PCR to detect different pathogens through analysis of their genetic materials (Suchman, 2016; Laura & Rukmanidevi, 2023).

Conclusion

In conclusion, Polymerase Chain Reaction (PCR) is a revolutionary molecular technique that has transformed the field of genetics, disease diagnosis, and biomedical research. Its ability to amplify specific DNA segments has made it an indispensable tool in various scientific disciplines. PCR's applications range from genetic testing and forensic analysis to disease diagnosis, oncology, food safety, veterinary medicine, pharmacy, and environmental science. The technique's sensitivity, specificity, and versatility have made it a cornerstone in modern molecular biology. As PCR continues to evolve with advancements in technology and methodology, its impact is expected to grow, driving further breakthroughs in scientific research and healthcare. The advent of Polymerase Chain Reaction (PCR) has revolutionized the field of molecular biology, enabling the amplification of specific DNA segments with unprecedented precision and efficiency. This technique has far-reaching implications in various scientific disciplines, including genetics, disease diagnosis, forensic analysis, oncology, food safety, veterinary medicine, pharmacy, and environmental science. The versatility of PCR is evident in its diverse applications, ranging from detecting genetic mutations and infectious agents to identifying pathogens and analyzing DNA samples. As PCR technology continues to evolve, its impact is expected to grow, driving breakthroughs in scientific research, healthcare, and beyond. With its sensitivity, specificity, and adaptability, PCR remains an indispensable tool in modern molecular biology, poised to shape the future of scientific inquiry and discovery.

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