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Polymerised Chain Reaction – A Review

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ABSTRACT:

PCR is a molecular biology technique that amplifies a single or few copies of DNA, generating thousands to millions of copies. It is widely used in medical and biological research labs for various applications. It consists of three major steps which includes denaturation, annealing, and extension. PCR is useful in investigating and diagnosing various diseases and detects human genes, as well as bacterial and viral genes. It requires only a tiny amount of original DNA, making it valuable in forensic science. PCR identifies genes implicated in cancer development and has also benefited many molecular cloning techniques. This review provides a solid foundation for understanding the basics of PCR, its history, applications, and impact on various fields.

Keywords: DNA cloning, protein, molecular biology, genetics

INTRODUCTION:

PCR is a molecular biology technique that amplifies a single or few copies of DNA, generating thousands to millions of copies. It was developed by Kary Mullis in 1984, with the basic principle described by Gobind Khorana in 1971.Kary Mullis has received the Nobel Prize and Japan Prize in 1993(1) for this innovation. PCR is widely used in medical and biological research labs for various applications. It is quick, inexpensive, simple, and amplifies specific DNA fragments from minute quantities of source DNA material. PCR has rapidly become one of the most widely used techniques in molecular biology. It is used in medical diagnosis, including analysis of tiny amounts of genetic material. It also is used in courts of law for analysis of genetic material and also in field studies of animal behavior and ecology. Progress was limited by primer synthesis and polymerase purification issues. (2)

PRINCIPLE:

PCR is a continuous doubling process, where one DNA molecule produces two copies, then four, eight, and so on. Specific protein enzymes string together the DNA building blocks nucleotides, to form long molecular strands. To do their job polymerases require a supply of DNA building blocks, i.e. the nucleotides consisting of the four bases adenine (A), thymine (T), cytosine (C) and guanine (G). (3) They also need a small fragment of DNA, known as the primer, to which they attach the building blocks as well as a longer DNA molecule to serve as a template for constructing the new strand. If these three ingredients are supplied, the enzymes will construct exact copies of the templates. A means of selectively amplifying a particular segment of DNA, allowing for the acquisition of many copies of a specific strand. The segment may represent a small part of a large and complex mixture of DNAs e.g. a specific exon of a human gene. PCR can amplify a usable amount of DNA in ~2 hours, even from impure template DNA. (4) In Mullis's original PCR process, the enzyme was used in vitro. The double-stranded DNA was separated into two single strands of DNA by heating it to 96°C. At this temperature, however, the E. Coli DNA polymerase was destroyed, so that the enzyme had to be replenished with new fresh enzyme after the heating stage of each cycle. Mullis's original PCR process was very inefficient since it required a great deal of time, vast amounts of DNA-Polymerase, and continual attention throughout the PCR process. (5)

STEPS OF PCR:

To amplify a segment of DNA using PCR, the sample is first heated so the DNA denatures, unwound or separates into two pieces of single-stranded DNA at a higher temperature of 90-97°C. This step is known as denaturation. Next happens the annealing phase where the primers (short DNA sequences) bind to their complementary sequences on the single-stranded DNA template, providing a starting point for DNA synthesis. The temperature is lowered to 50-60°C to allow primers to bind to their complementary template strands. This step is crucial for forensic chemistry, as it enables the creation of identical copies of the original template strand. (6) The newly-formed DNA strand of primer attached to template is then used to create identical copies off the original template strands desired. DNA polymerase enzymes add nucleotides to the annealed primers, synthesizing a comp This process is the extension which occurs at approximately 72°C for 2-5 minutes. (6)

The beauty of the PCR cycle and process is that it is very fast compared to other techniques and each cycle doubles the number of copies of the desired DNA strand. After 25-30 cycles, whoever is performing the PCR process on a sample of DNA will have plenty of copies of the original DNA sample to conduct experimentation. Assuming the maximum amount of time for each step, 30 cycles would only take 6 hours to complete. As the process of denaturation, annealing, and polymerase extension is continued the primers repeatedly bind to both the original DNA template and complementary sites in the newly synthesized strands and are extended to produce new copies of DNA. The end result is an exponential increase in the total number of DNA fragments. (6)

The introduction of Taq DNA polymerase has been a game-changer in PCR technology, (7) enabling:

- Repeated heating and cooling cycles without enzyme degradation
- Improved yield and specificity of PCR products
- Automation of the PCR process
- Increased utility of PCR in various applications

The introduction of Taq DNA polymerase, isolated from Thermus aquaticus, revolutionized PCR by allowing the enzyme to withstand higher temperatures up to 94°C, eliminating the need for new enzyme addition after each cycle. (8) The thermostable properties of Taq DNA polymerase have greatly contributed to the yield, specificity, automation, and utility of PCR. After the last cycle, samples are incubated at 72°C for 5 minutes to fill in the protruding ends of newly synthesized PCR products. Care should be taken in preparing the

reaction mixture and setting up cycling conditions to ensure success. Increasing the cycle number above ~35 has little positive effect due to reagent depletion and accumulation of non-specific products. The specificity of amplification depends on the extent to which primers can recognize and bind to sequences other than the intended target DNA sequences. (9)

METHODS:

Various techniques were introduced to enhance the versatility and accuracy of PCR, enabling researchers to tackle various molecular biology applications.

1. Real-Time PCR (Quantitative PCR)

. Amplifies and quantifies targeted DNA molecules.

. Analyses products in real-time using fluorescent dyes.

. Facilitates quantitation of DNA.

2. Digital PCR (dPCR)

. Refinement of conventional PCR for direct quantification and clonal amplification.:

. Measures nucleic acid amounts more precisely than PCR.

. Separates samples into partitions for individual reactions.

3. Inverse PCR

. Amplifies DNA with only one known sequence.

. Overcomes limitation of conventional PCR requiring primers for both termini.

4. Nested PCR

. Reduces contamination in products.

. Amplifies specific sequences using multiple primer sets.

5. Touchdown PCR

. Avoids amplifying nonspecific sequences.

. Uses high annealing temperatures initially, decreasing in increments.

ADVANTAGES:

1. Speed: PCR is remarkably fast, with each cycle doubling the number of copies of the desired DNA strand.

2. Detection of mixed infections: PCR can detect mixed infections with ease, which is a significant advantage.

3. High sensitivity and specificity: PCR offers superior accuracy compared to conventional diagnostic methods.

4. Rapid results: PCR provides quick results, enabling timely medical interventions.

5. Utilitarian advantages: Despite requiring infrastructural support and being expensive, PCR's benefits outweigh its costs. Increases exponentially, providing ample material for experimentation.

6. Confirmatory test: PCR serves as a valuable confirmatory test due to its high sensitivity and specificity.

7. Time-efficient: Assuming maximum time for each step, 30 cycles can be completed in just 6 hours.

8. Primer binding and extension: The primers bind to both the original DNA template and complementary sites in the newly synthesized strands, leading to an exponential increase in DNA fragments. (9)

APPLICATIONS:

PCR has become an indispensable tool in modern medicine, offering unparalleled sensitivity, specificity, and speed. (10) Its applications continue to expand, transforming our understanding and diagnosis of various diseases.

Overall, PCR has revolutionized various fields by providing a powerful tool for DNA amplification, detection, and analysis.

Medical Applications

1. Disease diagnosis: PCR helps diagnose diseases, including infectious diseases, genetic disorders, and cancer.

2. Viral detection: PCR detects viral genomes, such as HIV, herpes simplex, and human papillomavirus.

3. Cancer research: PCR identifies genes involved in cancer development and progression.

4. AIDS diagnosis: PCR detects the AIDS virus sooner than standard tests.

Research Applications

1. Molecular biology: PCR amplifies specific DNA sequences, enabling research in genetics, genomics, and molecular biology. (10)

2. Gene expression analysis: PCR quantifies gene expression levels, helping researchers understand gene regulation.

3. DNA sequencing: PCR prepares DNA samples for sequencing, enabling genome assembly and analysis.

Forensic Applications

1. DNA fingerprinting: PCR amplifies specific DNA sequences, helping identify individuals and solve crimes.

2. Forensic analysis: PCR analyses DNA evidence, such as bloodstains and hair samples.

Other Applications

1. Environmental monitoring: PCR detects microorganisms in environmental samples, monitoring water and air quality.

2. Food safety: PCR detects foodborne pathogens, ensuring food safety.

3. Molecular cloning: PCR enables direct cloning of DNA fragments, facilitating genetic engineering.

CONCLUSION

The advancement of science has transformed our lives in ways that would have been unpredictable just a half-century ago. PCR and its applications hold scientific and medical promise. PCR has very quickly become an essential tool for improving human health and human life and completely revolutionized the detection of RNA and DNA viruses. PCR has also been credited to have been able to detect mixed infections with ease in many studies. It also helps in the investigations and diagnosis in a number of growing diseases. (11)

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