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Polymer Chain Reaction (PCR): Principle and Applications

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Abstract

The new, standard molecular biologic system for duplicating DNA enzymatically devoid of employing a living organism, like E. coli or yeast, represents polymerases chain reaction (PCR). This technology allows an exponential intensification of a minor quantity of DNA molecule several times. Analysis can be straightforward with more DNA available.

A thermal heat cycler performs a polymerization chain reaction that involves repeated cycles of heating and cooling the reactant tubes at the desired temperature for each reaction step. A heated deck is positioned on the upper reaction tube to avoid evaporating the reaction mixture (normally volumes range from 15 to 100 l per tube), or an oil layer can be placed on a reaction mixture surface.

The amplified DNA fragment is determined based on selecting primers in addition to the starting and end of the DNA fragment. The primers stand for short, artificial DNA stripes, no higher than fifty (typically 18-25bp) nucleotides have been based on a starting and ending of DNA fragment to be amplified. DNA-polymerase connects and starts a new DNA strand synthesis

The PCR products can be visualized by dual foremost methods: (1) staining of the product of DNA amplified by a chemical dye like bromide ethidium, or (2) marking of fluorescent dyes (fluorophores) PCR primers or nucleotides before amplification of PCRs.

PCR offers some benefits. First, it is a simple method of understanding and using and quick results. It has an extremely sensitive technology with the potential for sequencing, cloning, and analyzing millions or milliards of copies of a particular product.

keywords: PCR, Molecular techniques.

1.Introduction

The new, standard molecular biologic system for duplicating DNA enzymatically devoid of employing a living organism, like E. coli or yeast, represents polymerases chain reaction (PCR). This technology allows an exponential intensification of a minor quantity of DNA molecule several times. Analysis can be straightforward with more DNA available. For many tasks, for example, detecting hereditary disease, genetic fingerprint identification, gene cloning, genetic testing, and DNA computing, many PCR laboratories are commonly utilized in medical and biological research [1].

The technique was initiated by Kary Mullis in 1983. PCR has been nowadays an important and typical method for various applications in biological and medical research labs. These consist of sequencing DNA cloning, gene phylogenesis or gene functional analysis, identifying hereditary diseases, genetic fingerprints (useful in forensics and pathology) identification, and infectious disease detecting and diagnosis. In 1993, Mullis and Michael Smith, for his work on PCR, were awarded a Nobel Prize for Chemistry[2].

The PCR is usually performed in small reaction tubes in the thermal cycler in a volume of 10-200 ml. A thermal cycler heats and refrigerates the reacting tubes for achieving the necessary temperatures at any reaction stage. Numerous modern thermal cyclers based on the Peltier effect allow block-holding PCR tubes to be heated and cooled by simply retreating an electric current. Thin-walled reaction tubes enable good thermal conductivity for enabling speedy thermal balance. The majority of thermal cyclers possess heated pads in the reaction tube to prevent condensation. Older thermocyclers lacking a heated lid necessitate an oil layer on the upper reaction mix or wax ball in a tube.

2.PCR Process

Multiple basic components are required to PCR based on:

1- DNA or cDNA template containing the DNA fragment region to be enlarged

2- Dual primers that regulate the start and end of an area to be amplified (as in the next section about primers)

3- Taq polymerase that copies the amplified region

4- Nucleotides, the DNA-Polymerase for new DNA

5- Buffer that offers an appropriate DNA polymerase chemical surroundings.

A thermal cycler performs the PCR reaction. This machine heats and cools the reacting tubes at a required temperature for every reaction step. A heated deck is positioned on the upper reaction tube to avoid evaporating the reaction mixture (normally volumes range from 15 to 100 l per tube), or an oil layer can be placed on a reaction mixture surface.

PCR is an enzymatic test that can enhance a particular DNA fragment from a complex DNA body, a simple yet elegant one. Dr. Kary Mullis found that the PCR test "may let you determine the piece of DNA that you want and have based on your requirement" Three PCR can be done using various tissues, including peripheral blood, saliva, hair, skin, and microbes, as the DNA source. For generating sufficient copies for analysis by means of

conventional lab methods for PCR, just trace quantities of DNA are required. PCR is a sensitive test, therefore.

The DNA, primers, nucleotides, and DNA polymerases template must be available for every PCR assay. The main enzyme, which associates distinct nucleotides for forming the PCR product, stands for DNA polymerase. Nucleotides have four basic components (A, T, C, G) in DNA: adenine, thymine, cytosine, and guanine. These are the building blocks used to create of the resultant product of PCR by the DNA polymerase.

The primers identify a particular DNA product to be amplified in a reaction. The primers stand for small DNA fragments with an arrangement to complement and amplify the target DNA. The DNA polymerase is supplemented by these as an expanding point.

The components have been mixed in a 96-well tube and then positioned in the machine that enables repetitive DNA amplifying cycles to be carried out in 3 elementary steps. Most of the machine stands for a thermal cycler. It has a hollow thermal block. PCR reaction mixture testing tubes or plates have been added. Indistinct, accurate, and pre-programmed steps the machine increases and lowers block temperatures [4].

First of all, it is heated higher than a melting point for dual complementary DNA strands of a target DNA, enabling a denaturation process to be separated among the strands. Then the temperature is reduced to connect the specific primers with the target DNA segments, called a hybridization process. Hybridization among target DNA and primers occurs as they have been sequence-complementary (as in A binding to G).

Temperature can be elevated again, as the DNA polymerase has been capable of extending the primers through inserting nucleotides to a developing DNA strand, as depicted in **Figure 1**. An amount of copied DNA molecules doubles each time based on these three repeated steps.

3.Primers

The amplified DNA fragment is determined based on selecting primers in addition to the start and end of the DNA fragment. The primers stand for short, artificial DNA stripes, no higher than fifty (typically 18-25bp) nucleotides have been based on a starting and ending of DNA fragment to be amplified. DNA-polymerase connects and starts a new DNA strand synthesis, they are annealed (adhere) to a DNA template.

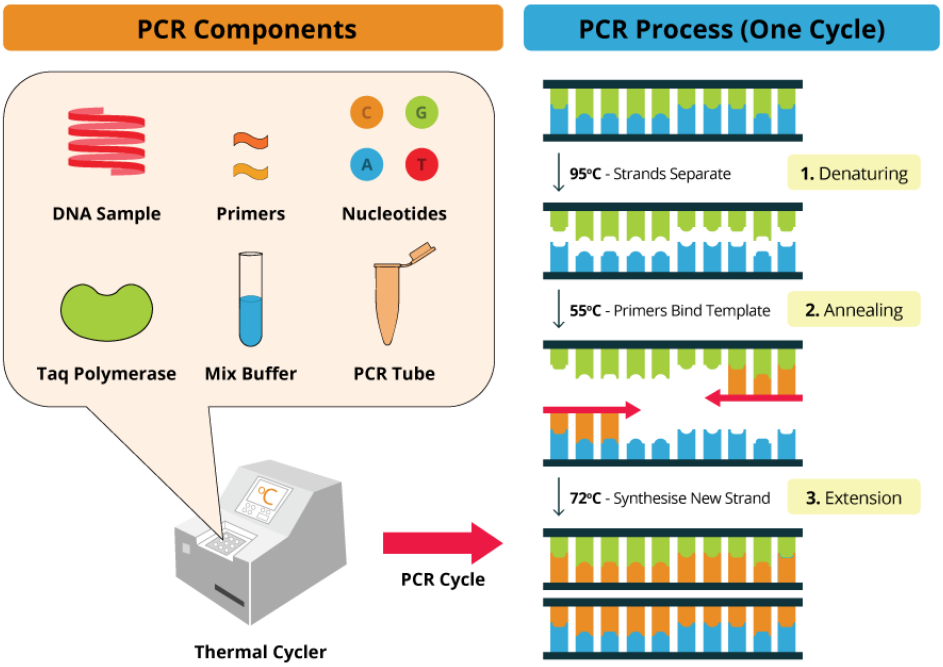

Figure 1: PCR Process and thermal cycle(Weier et al, 1988)

4.Analyzing PCR Product

The PCR products can be visualized by dual foremost methods: (1) staining of the product of DNA amplified by a chemical dye like bromide ethidium, that is intercalated among dual duplex strands or (2) marking of fluorescent dyes (fluorophores) PCR primers or nucleotides before amplification of PCRs.

This method permits the incorporation of the labels directly into the PCR product. An Agarose Gel Electrophoresis, which parts product of DNA according to their size and charging, is usually employed technique for investigating the PCR product. The easiest way to view and analyze the product of PCR is by using Agarose gel electrophoresis. It tolerates the existence and size of the product of PCR to be resolved as depicted (**Figure 2**).

A determined set of products of PCR with identified sizes have been run instantaneously on a gel as typical molecular indicators for determining the product size.

In general, PCR is referred to as qualitative PCR used to distinguish existence or lack of a particular DNA product.

PCR is an excellent method for cloning and/or identifying of a pathogen when PCR is performed.

For example, Merkel cell polyomavirus exists in the skin-scale cell carcinoma (SCC) in immunocompetent individuals has been detected through qualitative PCR.

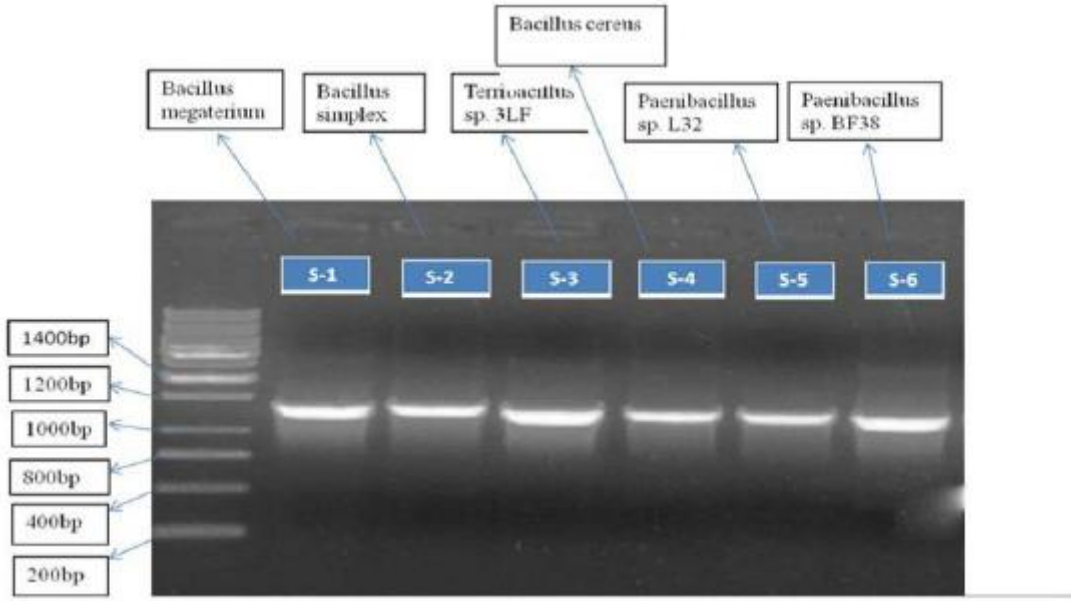

Figure 2: determining existence and PCR product size (Dworkin et al,2009)

In general, PCR is referred to as qualitative PCR to detect the existence or lack of a particular DNA product. PCR stands for an effective method for cloning and/or identifying a pathogen when PCR is performed. For instance, based on Dworkin et al. report, the existence of Merkel cell polyomavirus in skin squamous cell carcinoma (SCC) in immunocompetent persons was measured by qualitative PCR (Dworkin et al, 2009).

By means of isolated genomic DNA from excised SCCs from immunocompetent individuals and primers specific to virus genes. The investigators were capable of demonstrating an existence of a 351base pair (bp) viral gene in six out of sixteen tested samples, by the existence of a PCR-product band of 351 bp long, as noted in figure-3 on the 2% agarose gel with ethidium bromide depicted.

The experimentation also involved a plasmid-containing polyomavirus template DNA as a positive (P) and negative water management (W). A foremost (M) lane is a molecular marker employed to recognize the PCR product's size A presence of a PCR-detected viral particular gene has been characterized by (+); the viral gene is not present by (−).

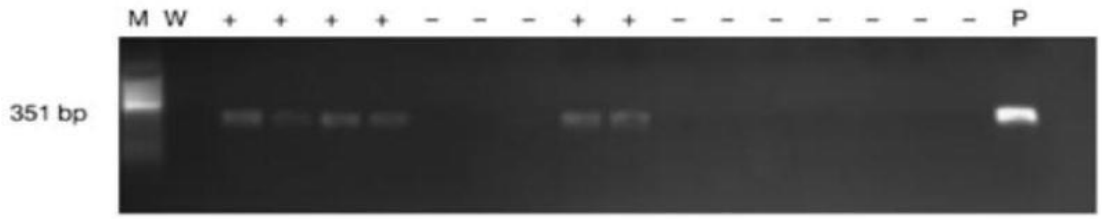

Figure-3.Qualitative PCR distinguishes Merkel cell polyomavirus existence in cutaneous squamous cell carcinoma (SCC) in immunocompetent individuals (Drowkin et al, 2009)

Based on Drowkin et al, 2009: MCPyV detecting. (a) An MCPyV existence in normal genomic DNA, SCCs, and adjacent skin DNA has been affected through PCR via VP1

primers. The representative consequence has been depicted with 6 of 16 tested samples viewing a PCR product at 351 bp. Each experimentation involved DNA from the MCPyV plasmid as a positive control (P) and negative water control (W). M, molecular weight indicator; +, positive for virus; −, negative for the virus.

5.PCR Uses

PCR may be used to diagnose a wide range of human diseases, experiments, and analyzes[6,7]

Below are some examples.

1. Diseases of VIH, CMV, Pneumonia, HIV, Syphilis (Mycoplasma), MSD, HIV, hepatitis, etc.

2. Cancer diagnosis mainly for leukemia and lymphoma

3. Paternity Test Genetic Fingerprinting. The PCR enables early diagnosis of malignant diseases as the most advanced diseases of cancer research currently in use, like leukemia and lymphomas.

To identify translocation-specific malignant cells, PCR tests may be done directly in genomic DNA samples at least 10,000 times greater than other techniques. PCR also, tolerates the identification of microorganisms that cannot be cultivated or slow-growing, like anaerobic bacteria, mycobacteria or tissues, and animal model viruses.

PCR diagnostics in microbiology are based on detecting infectious agents and discrimination against non-pathogenic agents by particular genes from pathogenic strains. For amplifying a short, well-defined portion of the DNA stream, PCR is used.

It can either be a single gene or a gene part. The process of PCR can only copy short DNA fragments, typically fit for 10 kb as opposed to living organisms (kb represents kilo base pairs). Some approaches may copy fragments fit for 40 kb, much smaller than a eucaryotic cell's chromosomal DNA – for example a human cell has approximately 3 billion basic pairs.

6.Application of PCR and diseases diagnosis:

Detecting Mycobacterium tuberculosis from the DOT corner of Mymensingh Medical College Hospital in 100 suspected cases of TB. In all strains of mycobacterium tuberculosis, PCR has been carried out with primers mtb1 and mttb2 based on IS6110 sequences of the PCR sensitivity and was found to be 94,7% and 100%, respectively, for sputum samples [6].

One of the studies revealed that Klebsiella pneumonia carbapenems genes, which have high analytical sensitivity and specificity of multidrug Resistance, have been accurately detected in real time PCR.

According to SYBR Green I, a real-time PCR assay has been designed to amplify a 106bp product for the blaKPC gene from 159 clinical gram -ve isolates resistant to numerous kinds of lactam antibiotics. A real-time PCR detecting limit has been approximately 0.8cfu using clinical isolates [8].

PCR diagnosis has also, investigated in detecting and differentiating para typhi, Salmonella typhi, Brucellosis, Malaria parasite detection, Dengue infection, and H.pylori in various studies all over the world. A target gene made amplicons at 429bp, 330 bp, and 600bp showing 100% sensitivity for detecting typhi , salmonella species, and paratyphi

Regarding detecting Plasmodium vivax, the + ve predictive magnitudes of thick blood microscopy, nested PCR, and real-time PCR have been 47.8%, 56.5%, and 60.9%. A real time PCR has 75% specificity, and 100% sensitivity, whereas nested PCR has shown 81.2 and 97.7% , respectively [10]

For diagnosing brucellosis, the most common zoonotic disease, PCR detection using primers B4/B5 amplifies gene encoding a 31kda immunogenic outer membrane protein (bcsp31) showing 51.3% exact sensitivity and specificity of 100 and 10, respectively. Tests of Histopathological and urease in biopsy samples occasionally generate incorrect negative consequences. For comparison, PCR assays have been implemented in each positive control for H pylori showing sensitivity and specificity of 64% and 80% using optimal combination of 16rRNA+urea[11]

A major tropical threat is Chagas or Treypanosoma cruzi. Priorities are mainly on accurately diagnosing and parasitological response to treatment.

PCR detection was investigated usingsequential dilutions of purified DNA stocks including human blood spiked with parasite cells (SetB), Trypanosoma cruzi discrete typing units I, IV, VI (set A), and Guinadine Hydrochroloride EDTA blood samples from thirty-two seropositive and ten seronegative controls (Set C) run by diverse PCR approaches based on primers 121/122.

The consequence has given 83.3%-94.4% sensitivity and specificity of 85-95%. This characterizes global substantiation for PCR techniques for detecting Trepanosoma cruzi in human blood samples [12].

For diagnosing scrub typhus, a study studied conventional PCR (C-PCR) performances, nested PCR(N-PCR), real-time quantitative PCR(Q-PCR) aiming O.tsutsugamushi specific 47kDa gene.

Dual template plasmid DNA and genomic DNA from buffy coat sample for one patient has been employed. N-PCR, C-PCR, and Q PCR sensitivities have hown with blood samples taken from patients with four weeks for onset of fever have been 7.3%,85.4%, and 82.9%(95%CI)[13]

Newly diagnosing PCR was investigated in [14] in on samples from 150 healthy volunteers and hospitalized adults. Employing culture as gold standard, the PCR sensitivity and specificity percentages have been 100% and 99.2% respectively.[14]

Also, PCR can detect viral DNA. The primers employed must be targeted in the virus DNA sequences, and PCR is feasibly used or diagnostic analysis or viral genome DNA sequences. PCR's high sensitivity enables the virus to be detected immediately after and before the infection. Such early detection may lead to significant treatment leads for doctors. PCR-based DNA quantitation techniques can also quantify the quantity of viral load in a patient." Genetic printing is the technique of forensics employed to categorize a person by

comparing the blood in a crime script with the blood in a suspect's DNA to a given sample [15,16,17].

7.PCR Advantages

PCR is feasibly employed for diagnosing numerous human diseases in various experiments and analyses. "PCR stands for very imperative confirmatory diagnostic aid in infectious disease as in tuberculosis, Syphilis, HIV, CMV, Mycoplasma, fungous and protozoal diseases, Hepatitis, cancers particularly lymphoma Malaria and leukemia, Tuberculosis, Staphylococcal bacteremia, Toxoplasma Gondoic [18, 19, 20].

"For the fingers and paternity test, PCR is also necessary. High sensitivity and specificity of PCR (95-100 percent) (100 percent) [21,22]

The disadvantage of PCR *, it involves pricey instruments such as a thermal cycling device, a diagnostic tray of gar gels, a DNA separation kit, and other chemical agents that are unable to buy in all laboratories. * Adequate air conditioning space, dehumidifier, laminar flow equipment, *Maximum scope of disease diagnosis, *Expensive, not everyone, *False positive and false negative results could reduce specificities and sensitivities.

8.Benefits and drawbacks of PCR

PCR offers some benefits. First, it is a simple method of understanding and using quick results (Bolognia et al., 2008). It has an extremely sensitive technology with the potential for sequencing, cloning, and analyzing millions or milliards of copies of a particular product. This also applies to qRT-PCR. The quantification of the made product has the advantage of qRT-PCR. It can thus be used to analyze changes in tumor, microbe, or other conditions of gene expression. PCR is a valuable technique, but it is limited. Due to the high sensitivity of PCR, any form of sample contamination can produce misleading results with even trace quantities of DNA [9,11].

Furthermore, certain previous sequence data are necessary for designing primers for PCR. The existence or nonexistence of an identified pathogen or gene can therefore only be identified by PCR. A further restriction is represented by employed primers for PCR that cannot especially be annealed to not identical sequences to the target DNA but they are similar. Furthermore, DNA polymerase can include improper nucleotides into the PCR sequence, albeit at a highly low rate.

9.Conclusions

PCR is a simple and widely used process in which minute amounts of DNA can be amplified into multiple copies Also, a specific sequence can be shown quantitatively with a short time and accurate results for the speed with which this technique works.

It is considered an accurate diagnostic method for some diseases and is characterized by high sensitivity and specificity ; polymerase chain reaction (PCR) is a very common and widely accepted method now all over the world

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