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PCR Techniques and Applications

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***Abstract*:- Kary B Mullis (awarded Nobel chemistry in 1993) developed a revolutionary method name PCR (Polymerase Chain Reaction) in the year 1983. PCR is based on using the ability of DNA polymerase to synthesize new strand of DNA complementary to the template strand of DNA. This technique is having an impact on various fields like cloning, genetics, recombinant DNA research molecular biology, forensic analysis, evolutionary biology, and medical diagnostics.**

Keywords: PCR, Technique, Prime

INTRODUCTION

The Polymerase Chain Reaction (PCR) is a method of replicating DNA, it makes numerous copies of a specific segment of DNA quickly and accurately [1]. It is capable of taking a small amount of DNA or even single molecule and amplifying a specific region exponentially such that once the reaction is finished, there may exist up to 230 copies of each starting DNA molecule. Previously, the methods used to amplify, or generate copies of recombinant DNA fragments were time-consuming and labour-intensive. Now PCR reactions can complete many rounds of replication and produce billions of copies of a DNA fragment only in few hours [2-)

The Basics of PCR Cycling

There are three major steps in a PCR cycling reactions, which are repeated upto 20 to 40 cycles. It is always done on an automated thermo cycler, which has ability to heat and cool the reaction tubes in a very short period of time [3). (Figure 1)

1. Denaturation (95°C), 30 sec.
2. Annealing (55–60°C), 30 sec.
3. Extension (72°C), time depends on product size.

Denaturation *(95°C)*

During this stage, the double strand melts open to form single stranded DNA, and all enzymatic reactions stop [4].

Annealing (54°C)

In this stage,Hydrogen bonds are constantly formed and broken between the single stranded primer and the single stranded template. If the primers exactly fit the template, the hydrogen bonds formed are so strong that the primer stays attached [5]

Extension (72°C)

Here the Coupling of the bases (complementary to the template) to the primer on the 3' side takes place (the polymerase adds dNTP's from 5' to 3'side, reads the template from 3' to 5' side,and bases are added complementary to the template) [6]

The PCR technique is based on process, a cell uses to replicate a new DNA strand. The integral component is the template DNA (contains the region to be copied). Even single DNA molecule can serve as a template [8]. The fragment needed for this to be replicated is the sequence of two short regions of nucleotides at either end of the region of interest. The two short template sequences must be known hence two primers (short stretches of nucleotides) that correspond to the template sequences can be synthesised

The primers attachesl to the template at their complementary sites and act as the starting point for copying. DNA synthesis at one primer is directed toward the other thus resulting in replication of the desired sequence. Free nucleotides are used to build the new DNA strands and a DNA polymerase does the building by sequentially adding on free nucleotides according to the instructions of the template [10]. Every cycle results in a doubling of the number of DNA strands present. After starting stage of few cycles, most of the product DNA strands made are of same length as the distance between the primers. The result is amplification of DNA that exists between the primers [16]. The amount of amplification is 2 raised to the n power; n represents the number of cycles that are performed

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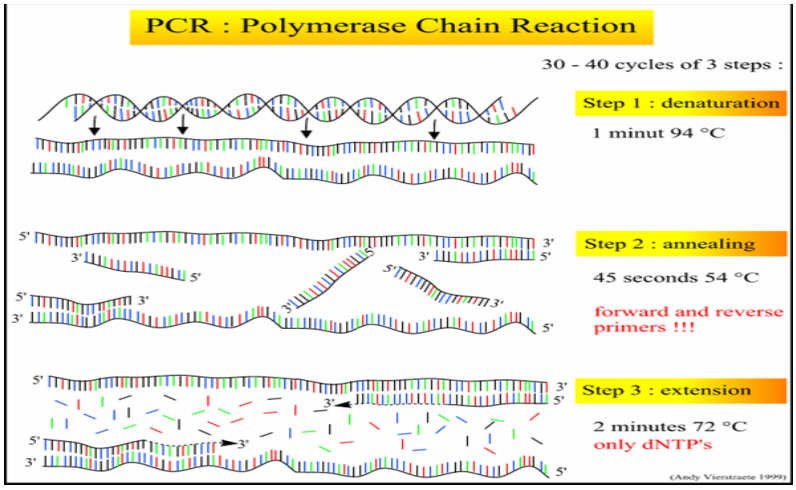

Fig:[1]

THERE ARE DIFFERENT TYPES OF PCR

Nested PCR
RT-PCR or Reverse Transcriptase PCR
Real Time PCR
Gradient PCR
Multiplex PCR
AFLP PCR
Allele-Specific PCR
Assembly PCR
Assymetric PCR
Colony PCR
Hot Start PCR
Inverse PCR
In Situ PCR
ISSR PCR
Late-PCR
Long PCR
Single Cell PCR
Standard PCR

Multiplex RT PCR is a method utilized for concurrent enhancement of more than one objective arrangement in a solitary response tube utilizing different groundwork sets. This RT PCR is sufficiently touchy however it has numerous objective arrangements [32]. Multiplex RT PCR is effective furthermore spares the bother of doing numerous PCR responses. Multiplex RT PCR is a strategy utilized for Gene Deletion and Mutation Detection [33]. This procedure increases genomic DNA tests utilizing different preliminaries and a temperature-interceded DNA polymerase in a warm cycler [45]. Multiplex-PCR was initially portrayed in 1988 as a technique to recognize erasures in the dystrophin gene. It has likewise been utilized with the steroid sulfatase gene [46]

Likewise Nested polymerase chain response includes two arrangements of preliminaries, utilized as a part of two progressive keeps running of polymerase chain response, the second set expected to intensify an auxiliary focus inside of the first run item [37].

Reverse interpretation PCR (RT-PCR) utilizes a couple of groundworks which are integral to a characterized grouping on each of the two strands of the cDNA [40]. These preliminaries are reached out by a DNA polymerase and a duplicate of the strand is made after every cycle, prompting exponential enhancement [39].

APPLICATIONS OF PCR

PCR can be utilized for hereditary testing, where an example of DNA is broke down for the vicinity of hereditary illness transformations [51].
PCR can be utilized for DNA fingerprinting can help in Parental testing (DNA sequencing) [56].

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PCR can be utilized for DNA cloning - It can concentrate fragments for insertion into a vector from a bigger genome, which may be accessible in little amounts [57].
Genetic finger impression is a standout amongst the most abused utilization of PCR (otherwise called DNA profiling).Profiles of particular extends of DNA are utilized as a part of hereditary fingerprinting (by and large 13 loci are looked at) which is contrast from individual to individual [80]. PCR additionally assumes a part in examination of genomic or mitochondrial DNA, in which examiners utilized examples from hair shafts and bones when different specimens are not open [81].
PCR has various applications in different fields. The Human Genome Project (HGP) for deciding the grouping of the 3 billion base combines in the human genome, depended vigorously on PCR. The qualities connected with an assortment of infections have been distinguished utilizing PCR. For instance, Duchenne strong dystrophy, which is created by the change of a quality, recognized by a PCR strategy called Multiplex PCR [74]. PCR can help to study DNA from different life forms, for example, infections or microscopic organisms. PCR has been utilized to distinguish and to investigate connections among species in the field of transformative science. In human studies, it is likewise used to comprehend the antiquated human movement ss designs.
In paleontology, it has been utilized to recognize the antiquated human race [75,865PCR normally utilized by Paleontologists to open up DNA from terminated species or cryopreserved fossils of millions years and accordingly can be further studied to elucidate on.
PCR innovation encourages the location of DNA or RNA of pathogenic living beings and, thusly, helps in clinical analytic tests for a scope of irresistible specialists like infections, microbes, protozoa [64] and so on. These PCR-based tests have various points of interest over routine counter acting agent based indicative systems that focus the body's resistant reaction to a pathogen [65].
Specifically, PCR-based tests are equipped to distinguish the vicinity of pathogenic operators ahead of time than serologically-based routines, as patients can take weeks to create antibodies against an infectious specialists [66].

PCR-based diagnostics tests are accessible for identifying and/or evaluating various pathogens,

1. HIV-1, which causes AIDS
2. Hepatitis B and C infections, may prompt liver malignancy
3. Human Papillomavirus, may bring about cervical growth
4. Chlamydia trachomatis, may prompt fruitlessness in ladies
5. Neisseria gonorrhoeae , may prompt pelvic provocative illness in ladies
6. Cytomegalovirus, may bring about existence debilitating illness in transplant patients and other immunocompromised individuals, including HIV-1/AIDS patients
7. Mycobacterium tuberculosis, which in its dynamic state causes tuberculosis and can prompt tissue harm of tainted organs [60-65].

PCR-based tests have been produced to specify the measure of infection in a man's blood ('viral burden') in this manner permitting doctors to check their patients' sickness movement and reaction to treatment. This has fantastic potential for enhancing the clinical administration of ailments created by popular disease, including AIDS [67] and hepatitis [68], appraisal of viral load all through and after treatment [69].

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