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PCR Technique with its Application

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Review Article

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ABSTRACT

PCR (Polymerase Chain Reaction) is a revolutionary method developed by Kary B Mullis (awarded Nobel Prize for chemistry in 1993) in the 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 many areas of molecular cloning, genetics, recombinant DNA research molecular biology, forensic analysis, evolutionary biology, and medical diagnostics.

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. Before the development of PCR, the methods used to amplify, or generate copies of recombinant DNA fragments were time-consuming and labour-intensive. But PCR reactions can complete many rounds of replication and produce billions of copies of a DNA fragment only in few hours [2-4].

The Basics of PCR Cycling

The 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 [5,6]. (Figure 1)

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

Denaturation (94 °C)

During this stage, the double strand melts open to form single stranded DNA, all enzymatic reactions stop [7].

Annealing (54°C)

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 [8].

Extension (72°C)

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The bases (complementary to the template) are coupled to the primer on the 3' side (the polymerase adds dNTP's from 5' to 3'side, reading the template from 3' to 5' side, bases are added complementary to the template) [9-11].

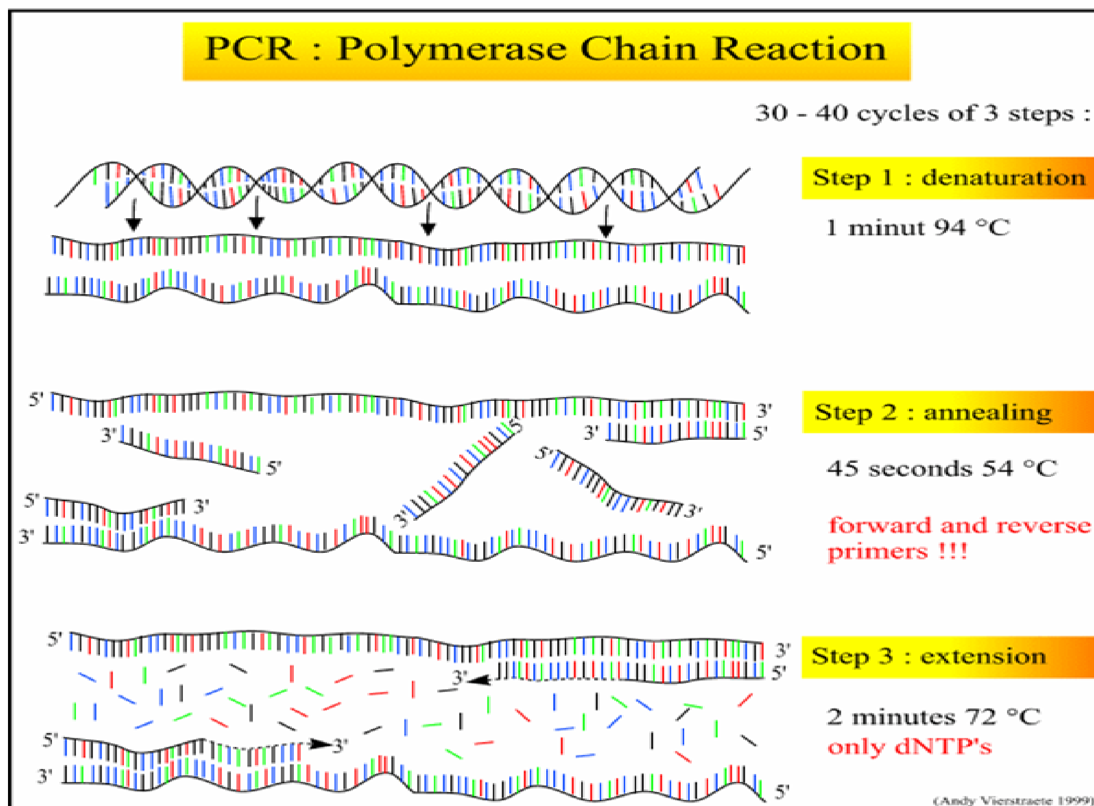

Figure 1: Basics of PCR Cycling.

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 [12]. 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 synthesized. The primers anneal to the template at their complementary sites and act as the starting point for copying [13]. DNA synthesis at one primer is directed toward the other thus resulting in replication of the desired sequence. Also needed are free nucleotides 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 [14-15].

Every cycle results in a doubling of the number of strands DNA present. After starting stage of few cycles, most of the product DNA strands made are the 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. After 20 cycles, this would give approximately 1 million fold amplification. After 40 cycles the amplification would be 1x1012 [17,18]. (Figure 2)

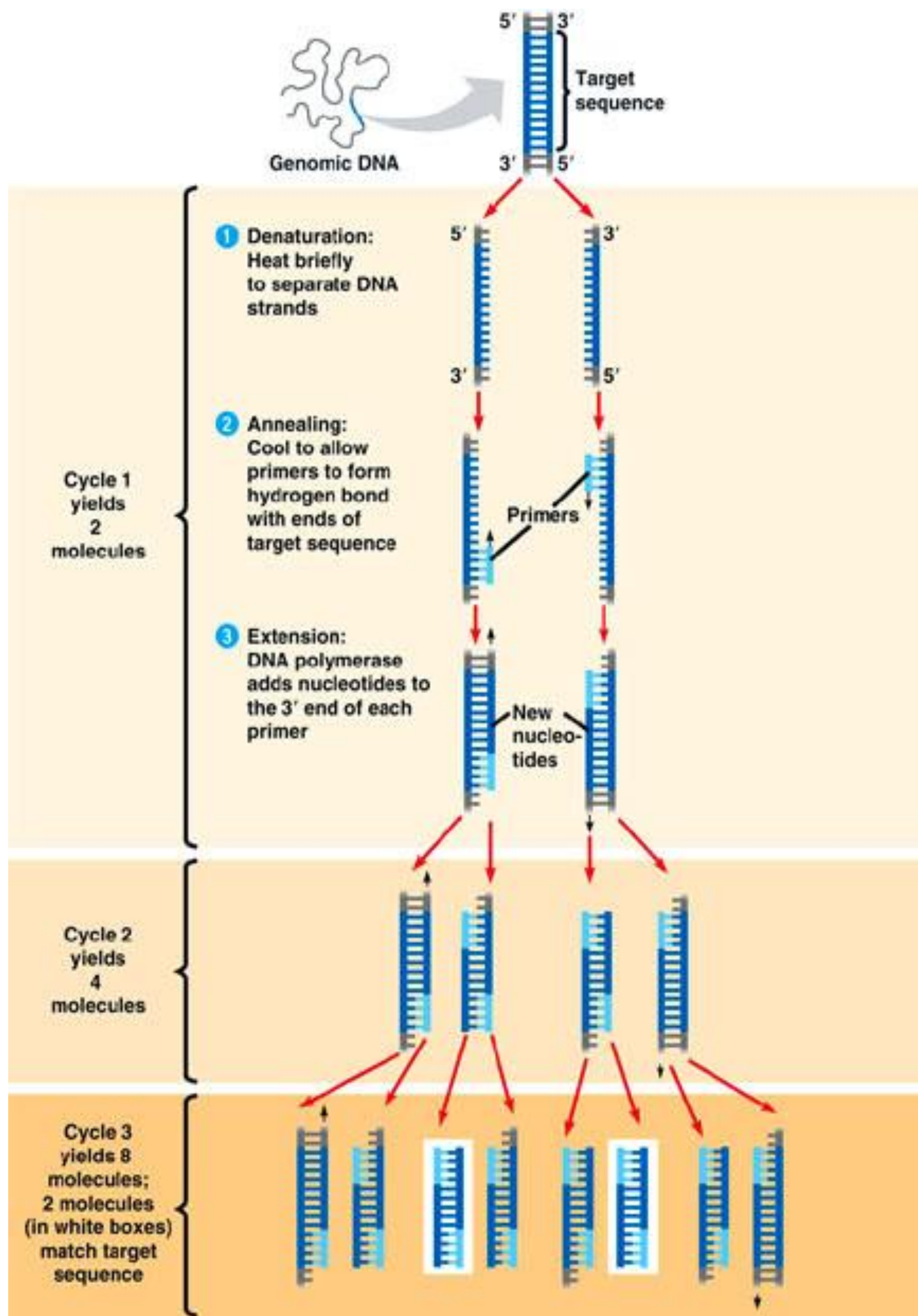

Figure 2: The amount of amplification after 3 cycles.

PREPARATION OF 18 MICRO LITRES REACTION MIXTURE

• 1mM Nucleotides (dNTPs)	2.5 micro litres
• Taq DNA polymerase	0.4 micro litre
• 10X Taq Buffer	2.0 micro litres
• Sterile water	13.1 micro litres

For each tube

- 18 micro litres of reaction mixture

- 1 micro litre of DNA sample
- 1 micro litre of primer

Altogether each tube contains 20 micro litres of sample,This tube is subjected to pcr procedure by following steps of pcr cycling ,the target DNA is amplied. This procedure takes 3 to 4 hrs to complete 40 cycles [19-23].

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 [24-28]

Capacity of Few Different Types of PCR

- Inverse PCR otherwise called IPCR and was initially depicted by Ochman et al. in 1988. The primary constraint of standard PCR is that 5' and 3' flanking areas of DNA part of interest must be known however in converse PCR permits you to lead PCR when just data of one interior succession is given [29].
- Reverse translation polymerase chain response (RT-PCR) is the most delicate procedure utilized for mRNA discovery and quantitation at present. Contrasted with the Northern blotch examination and RNase security measure which are fundamentally utilized for evaluating mRNA levels RT-PCR can likewise be utilized to evaluate mRNA levels from littler examples [30]. RT PCR is so touchy and effective that it can measure mRNA from a solitary cell. RT-PCR is thought to be most quick, effective and profoundly touchy to infection confinement and is very prescribed as an essential instrument for infection recognition [31].
- 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].
- Nested polymerase chain response is an adjustment of polymerase bind response expected to decrease the defilement in items because of the intensification of surprising groundwork tying locales [34].
- Polymerase chain response itself is the procedure used to intensify DNA tests, by means of a temperature-interceded DNA polymerase. The items can be utilized for sequencing or

examination, and this procedure is a key piece of numerous hereditary qualities research labs, alongside utilizations in DNA fingerprinting for criminology and other human hereditary cases [35]. Routine PCR obliges groundworks correlative to the ends of the objective DNA. A regularly happening issue is preliminaries tying to inaccurate districts of the DNA, giving unforeseen items [36].

- 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].
  Process
  The objective DNA experiences the first keep running of polymerase chain response with the first arrangement of groundworks. The choice of option and comparable groundwork tying locales gives a determination of items, stand out containing the planned succession. The item from the first response experiences a second keep running with the second arrangement of preliminaries. It is impossible that any of the undesirable PCR items contain tying destinations for both the new preliminaries, guaranteeing the item from the second PCR has little sullying from undesirable results of groundwork dimers and option preliminary target groupings [38-40].
- 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 [41]. These preliminaries are reached out by a DNA polymerase and a duplicate of the strand is made after every cycle, prompting exponential enhancement [42].
- RT-PCR incorporates three noteworthy steps. The main step is reverse interpretation (RT), in which RNA is opposite translated to cDNA utilizing converse transcriptase. This stride is imperative keeping in mind the end goal to perform PCR since DNA polymerase can act just on DNA layouts [43]. The RT step can be performed either in the same tube with PCR (one-stage PCR) or in a different one (two-stage PCR) utilizing a temperature somewhere around 40°C and 50°C, contingent upon the properties of the opposite transcriptase utilized .All the remaining strides in pcr amplication is same [44].
- Multiplex polymerase chain response (Multiplex PCR) is a change of polymerase anchor response with a specific end goal to quickly recognize cancellations or duplications in a substantial quality. 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].

APPLICATIONS OF PCR

- The polymerase chain response is utilized by a wide range of researchers in a continually expanding scope of experimental orders. In microbiology and atomic science, for instance, PCR is utilized as a part of exploration labs in DNA cloning techniques, Southern smudging, DNA sequencing, recombinant DNA innovation, to give some examples. In clinical microbiology labs PCR is priceless for the analysis of microbial diseases and epidemiological studies. In nourishment science PCR has turn out to be progressively essential to the horticultural and sustenance businesses as an important distinct option for customary identification strategies [47]. PCR is additionally utilized as a part of crime scene investigation labs and is particularly valuable on the grounds that just a little measure of unique DNA is needed, for instance, adequate DNA can be gotten from a bead of blood or a solitary hair [48].
- Constant PCR (or qPCR) is right now utilized as a part of all applications set up of customary, legacy PCR. Constant PCR has applications in all branches of organic science. Applications

incorporate agrarian and nourishment commercial ventures, quality expression examination, the conclusion of irresistible ailment and human hereditary testing [49]. Because of their capacity in fluorimetry the continuous machines are additionally good with option enhancement routines [50], for example, NASBA gave a fluorescence end-point is accessible. A portion of the use of PCR are as per the following

- 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 as a major aspect of a touchy test for tissue writing, key to organ transplantation [52].
- PCR can be utilized for HIV test (vicinity of the HIV infection that causes AIDS can be resolved utilizing PCR on platelets. PCR tests have been created with the goal that it can recognize one viral genome among the DNA of more than 50,000 host cells [53].
- PCR can be utilized for Genetic fingerprinting (scientific science) can remarkably separate one individual from the whole populace of the world [54]. Moment tests of DNA can be detached from a wrongdoing scene and contrasted with that from suspects or from a DNA database of prior confirmation or convicts [55].
- PCR can be utilized for DNA fingerprinting can help in Parental testing (DNA sequencing) [56].
- 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].
- PCR can be utilized for the investigation of examples of quality expression. Tissues or individual cells can be examined at diverse stages to see which qualities have get to be dynamic or which have been exchanged off [58].
- PCR can at the same time increase a few loci from individual sperm has significantly upgraded hereditary mapping by considering chromosomal hybrids after meiosis [59].
- PCR can be utilized to build up connections among species in human studies and developmental science [60].
- PCR can be utilized to help recognize antiquated human stays in paleohistory.
- PCR can be utilized PCR to intensify DNA from terminated bugs saved in golden for 20 million years [61].
- PCR can be utilized to identify the vicinity of a quality moved into a living being (transgene) [62].
- PCR can be utilized to focus the sex of developing lives. Accordingly sex of in vitro treated steers incipient organisms could be resolved utilizing Y chromosome particular preliminaries before their implantation in the uterus [63].
- 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 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].
- PCR-based diagnostics tests are accessible for identifying and/or evaluating various pathogens, including:

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 [70-75].

- The utilization of PCR in diagnosing hereditary maladies, whether because of intrinsic hereditary changes or as a consequence of a characteristic hereditary transformations, is turning out to be more normal. Irregularity can be analyzed even preceding conception. Single-strand conformity polymorphism (SSCP), or single-strand chain polymorphism, is characterized as conformational contrast of single-stranded nucleotide groupings of indistinguishable length as actuated by contrasts in the arrangements under certain trial conditions [76]. Nowadays, SSCP is most pertinent as an analytic instrument in sub-atomic science. It can be utilized as a part of genotyping to recognize homozygous people of diverse allelic states, and also heterozygous people who acquire hereditary abnormalities [77].
- Genetic advising is ruined the folks to check the record of hereditary malady in advance to settle on a choice on having kids [78]. This is obviously administered by national laws and rules. Identification of hereditary malady before implantation of an incipient organism in IVF (In vitro preparation) otherwise called preimplantation analysis should likewise be possible abusing PCR based strategy. Further to analyze acquired or an unconstrained sickness, either symptomatic or asymptomatic (as a result of family history like Duchene solid dystrophy) PCR based technique is exceptionally valuable [79].
- 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 is a fundamental method in cloning technique which permits era of a lot of unadulterated DNA from minor measure of format strand and further investigation of a specific quality. A few changes to the PCR convention can create transformations (general or site-coordinated) in a grouping either by an embedded part or base change [82]. PCR is utilized for s equence-labeled destinations (STSs) as a pointer that a specific section of a genome is available in a specific clone. A typical utilization of Real-time PCR is the investigation of expression examples of qualities amid diverse formative stages. PCR can likewise explore 'ON or OFF" of specific qualities at diverse stages in tissues (or even in individual cells) [83].
- 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 [84]. PCR can help to study for 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

designs. In paleontology, it has been utilized to recognize the antiquated human race [85,86]. PCR 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.

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