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A Review of PCR, Principle, and Its Applications

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ABSTRACT: PCR (Polymerase Chain Reaction) is a laboratory method for laboriously creating millions to billions of copies of a certain DNA region. In PCR, a section of the genome to be amplified is chosen using short synthetic DNA fragments called primers, and the segment is subsequently amplified by numerous rounds of DNA synthesis. PCR was first developed in the middle of the 1980s, about at the same time as the Human Genome Project was being discussed and then launched. Since then, PCR has been incredibly essential to a lot of biology and biomedical research. It was a key technological advancement in the early stages of the Human Genome Project. And it continues to play a significant role in biological and medical research today. This review article discusses the main uses of PCR technology in the areas of disease diagnosis, medical research, food and drug regulations, microbiologic properties, forensic science, epidemiology, evolutionary genetics, anthropology, etc. It also discusses the types of conventional PCR, such as RT-PCR (Real-time - polymerase chain reaction), qPCR (Quantitative polymerase chain reaction), and combined RT-PCR/qPCR.

KEYWORDS: Real-Time PCR, Molecular biology, PCR, Applications.

I. INTRODUCTION:

A technique for amplifying DNA in vitro is the polymerase chain reaction (PCR). Since its invention in 1985, the PCR has found widespread use in a variety of applications in clinical and criminal investigations as well as scientific research[1].The discovery of the molecular structure of DNA over half a century ago marks the introduction to the modern era of biotechnology. Research at those times uncovered numerous facts about the molecular processes that take place inside the living cell. At present using the principles of the PCR diagnosis and treatment for human disease or carried out. The most significant application of modern-erabiotechnology is gene amplification. This is a process DNA sequences are continuously replicated in vitro, at the same time producing millions of copies. There are many different methods that are used to amplify gene sequences. The first developed and most widely used is polymerase chain reaction (PCR). First introduced in 1985. The applications of this technique have increased over the past several years to the point where it may well revolutionize diagnostic medicine [2].

In the history of biological and medical sciences, the development of the polymerase chain reaction (PCR) has been a turning point. In addition to completely revolutionizing research in the fields of molecular genetics, animal and plant biotechnology, and PCR applications, these techniques have also demonstrated their applicability and relevance in a number of other fields, including forensic sciences, molecular systematics, molecular epidemiology, archaeology, anthropology, and evolutionary genetics. The development of RT-PCR, qPCR, and combination RT-PCR/q-PCR was facilitated by conventional PCR.

[3] By enabling the amplification and sequencing of human genes, PCR has also made it feasible to successfully complete the "human genome project," which has created the groundwork for genetic engineering and now even makes it possible to make meaningful alterations to an organism's genome. Most of the most recent advancements in modern sciences have effectively utilized PCR variations.

[4] The study on the diagnosis of respiratory tract disease concluded that RT-PCR assays gave a significant improvement in the diagnosis since the time taken is less and can work efficiently in managing and controlling of the emergence these important neglected diseases.

[5]High throughput screening, improved precision, and the integration of robotic setup technologies with real-time quantitative PCR (qPCR) are revolutionizing the field of

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biotechnology. Applications of qPCR in biotechnology are reviewed, with a focus on genetic stability testing and biosafety in particular: the biodistribution of gene therapy vectors in animals, determination of the amount of leftover DNA in therapeutic products, the discovery of bacterial and viral nucleic acids in compromised cell banks and finished goods, measurement of the degree of virus elimination in process validation viral clearance tests, very sensitive and specific detection of retroviral RT activity in vaccines, and measurement of gene copy number for tracking genetic stability during manufacturing.

[6]Nowadays, most illness diagnoses are based on lab testing. Recent developments in medical science and molecular biology have made it possible to diagnose the majority of rare, complex, or unique illness presentations, with molecular diagnostics remaining one of the most effective diagnostic modalities. Today, a variety of molecular methods are in use all around the world, including PCR, flow cytometry, tissue microarray, various blots, and genetic diagnostics. One of these diagnostic methods with the highest specificity and sensitivity for accurate diagnosis is PCR, which is the most well-acknowledged and often utilized.

[7]The common cystic fibrosis mutation (AF) was discovered in an Irish family using allele-specific PCR. Based on the resolution of allelic amplifications on an agarose gel, it was determined that each member's genomic status was either heterozygous (carrier) or homozygous (affected) for the mutation. Five staphylococci were treated to PCR intended to amplify a DNA fragment from the S. aureus protein A gene (spa). These examples have been chosen to highlight the adaptability of this method and some of its possible uses.

[8]With the invention of the polymerase chain reaction (PCR), molecular scientists may now amplify and analyze minuscule amounts of uncommon genetic material. This capability has been extensively investigated, particularly in prenatal diagnostics, since it has opened the possibility of pre-implantation genetic analysis and the use of enriched fetal cells from the blood of pregnant women for the evaluation of single-gene Mendelian illnesses. Single-cell PCR has been demonstrated to be of great value to basic scientists, addressing many immunological, neurological, and developmental problems aside from diagnostic applications. where the expression patterns of messenger RNA and the genome were studied. Additionally, recent developments including single-cell complementary DNA, efficient whole genome amplification (WGA) techniques, and even Single-cell comparative genomic hybridization will make it possible for the genetic study of individual cells to become standard practice, creating new opportunities for research and diagnosis.

[9]Prenatal diagnosis is now a common practice in obstetric care. Prenatal genetic diagnoses are made by taking fetal genetic samples using invasive techniques such as amniocentesis or chorionic villus sampling, which carry a small risk of fetal loss. Consequently, intriguing opportunities for noninvasive prenatal diagnostics have emerged as a result of the recent discovery of fetal DNA in maternal plasma. Since then, many applications for the study of circulating fetal DNA have been reported. The proper pre-analytical processing of maternal blood samples, a successful fetal DNA extraction protocol, and a sensitive and precise detection technology are all necessary for accurate fetal DNA assessment from maternal plasma.

[10]It is becoming increasingly clear that answering scientific problems in molecular toxicology, molecular epidemiology, and human risk assessment requires a quantitative study of changes in molecular targets. Real-time and quantitative (RTAQ) polymerase chain reaction is one of the cutting-edge methods being employed to examine these molecular targets (PCR).

[11]The use of polymerase chain reaction (PCR), a nucleic acid amplification technique, has completely changed how we detect, treat, and study diseases. Real-time PCR, a recent advancement in PCR technique, has grown in prominence due to its many clinical and basic research applications. Real-time PCR combines the chemistry of PCR with the capacity to identify and continually track the buildup of reaction products (amplicons) following each amplification cycle. Real-time PCR has the capacity to identify whether there are any sequence alterations as well as the presence and quantity of nucleic acid sequences (target nucleic acids). By detecting amplified PCR products using fluorescent-tagged oligonucleotide probes, real-time PCR can be made more precise, useful, and specific. In the same procedure, nucleic acid amplification and detection are performed closed vessel, which offers rapid, continuous data collection and reduced time requirements for findings while minimizing the likelihood of cross-contamination (amplified product carryover) between tests.

[12]More and more scientists are using molecular techniques to identify, count, and

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analyze microbial communities in food or during food processing. PCR-based approaches have received a lot of attention among these techniques, and ISO guidelines have been developed for the identification of food-borne infections. More specifically, real-time quantitative PCR (qPCR) is regarded as the preferred technique for the identification and measurement of microorganisms. Its ability to be quicker than traditional culture-based procedures is one of its main advantages. Additionally, it allows for the simultaneous identification of many microbes and is highly sensitive and specific. Contrary to the usage of qPCR, reverse transcription-qPCR (RT-qPCR) is just now being applied to research population dynamics and activities by quantification of gene expression in food. As long as the studies contain sufficient controls, Gene and gene expression can be quantified using qPCR and RT-qPCR, which appear to be very accurate and reliable methods. This review discusses several crucial technical issues to consider while employing these strategies. The most recent uses of qPCR and RT-qPCR for food microbiology are presented. There have been reports of some intriguing uses, including risk assessments and research into how industrial processes affect microbial activity and gene expression.

[13]The polymerase chain reaction (PCR), a potent method with a large and expanding variety of uses, has emerged as a crucial research and diagnostic tool. DNA extraction from biological materials, which should produce the substrate for the amplification reaction assuming a minimal level of quality and quantity is met, is the foundation of PCR. For this goal, a number of quick techniques that mostly depend on the sample and microorganisms to be studied have been proposed in recent years. As the temperature increases to 95 °C for 4-5 minutes during the first denaturation stage, cells in the colony that is added immediately to the reaction mix are disturbed. This approach has been demonstrated to be effective for isolated colonies of yeast, certain Lactobacillus species, and gram-negative bacteria. Cell lysis with lytic enzymes and detergents has been utilized for refractory microorganisms because this procedure is frequently ineffective. This is particularly true for gram-positive bacteria, for which colony extraction requires digestion with mutanolysin, cell disruption with Triton X-100, and boiling. The entire procedure takes around 45 minutes. Therefore. The lengthy process of extracting and preparing the DNA significantly slows down the PCR process and delays the results.

[14,15] The most well-characterized amplification technology is still PCR. There has been a lot of work put into increasing amplification fidelity,specificity, and addressing the carryover contamination issue. The creation of novel thermophilic DNA polymerases was revealed by researchers at Roche Molecular Systems. The UITma DNA Polymerase, which has proofreading activity and allowed for the cloning of high-fidelity amplification products, was one of the most intriguing enzymes ever described.The variety of stated new technologies has the prospect of significantly improving PCR specificity. The inherent challenges in identifying low target copy numbers in diseases like HIV-1 have sparked this research. The so-called "hot start," which involves injecting a specific enzyme at an ideal temperature, is promoted by Applied Biosystems. This straightforward method directs polymerase activity toward primer-dimer synthesis and non-specific product amplification. Hot starts can be performed either manually or automatically using wax beads that have been carefully prepared to melt at the ideal PCR temperature and physically release the enzyme.

[16,17] The wax approach is replaced by several novel reagents that offer chemical hot-start options. A method used by Life Technologies and PerkinElmer involves the enzyme uracyl-N-glycosylase (UNG) with PCR primers that have 3'-terminal deoxyuridine residues. The dU residues are preferentially integrated into the PCR product from dUTP, therefore removing nonspecific products. Additionally, specificity advantages can be obtained from single-stranded DNA-binding proteins.Eastman Kodak developed one of the best hot-start strategies. The business developed a monoclonal antibody that inhibits Taq DNA polymerase to offer hot-start advantages. The antibody denatures when the reaction mixture gets hot, which permits amplification to start.

II. PRINCIPLE:

[18-21]The principle of PCR (Polymerase Chain Reaction) is a widely used molecular biology technique that amplifies a specific segment of DNA (deoxyribonucleic acid) in vitro. It was developed by Kary B. Mullis in the 1980s and has since become an essential tool in various areas of biological research, diagnostics, and forensic analysis.

The PCR process involves a series of temperature cycles that enable the replication of the target DNA sequence exponentially. The primary PCR stages are listed below:

Denaturation: To divide the double-stranded DNA sample carrying the target sequence into two single strands, it is heated to a high temperature (usually approximately 95°C). As a result of this phase, DNA becomes denaturized because the hydrogen connections between the complementary base pairs are broken.

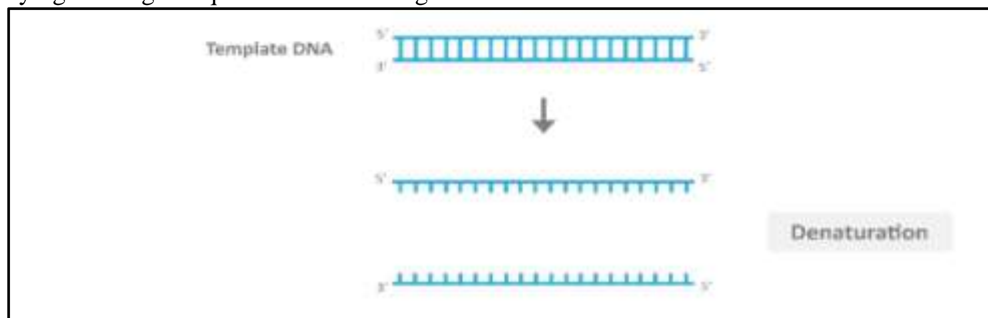

Figure 1: Step 1- Denaturation

Annealing: The temperature is lowered (typically around 50-60°C) to allow short DNA primers to bind to the complementary regions flanking the target DNA sequence. These primers are designed to be specific to the target sequence and serve as starting points for DNA synthesis.

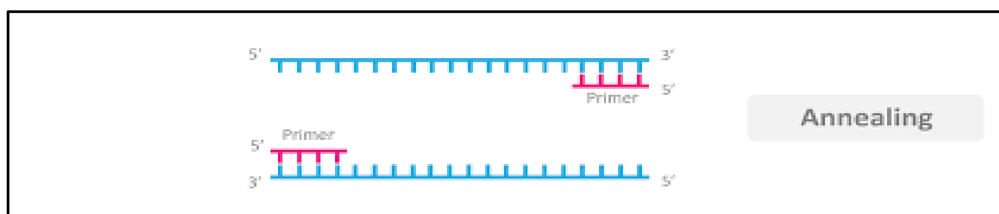

Figure 2: Step 2- Annealing

Extension: The temperature is raised (typically around 72°C), and a heat-stable DNA polymerase enzyme (e.g., Taq polymerase) synthesizes new DNA strands using the primers as a starting point. The DNA polymerase extends the primers by adding nucleotides complementary to the template DNA strand.

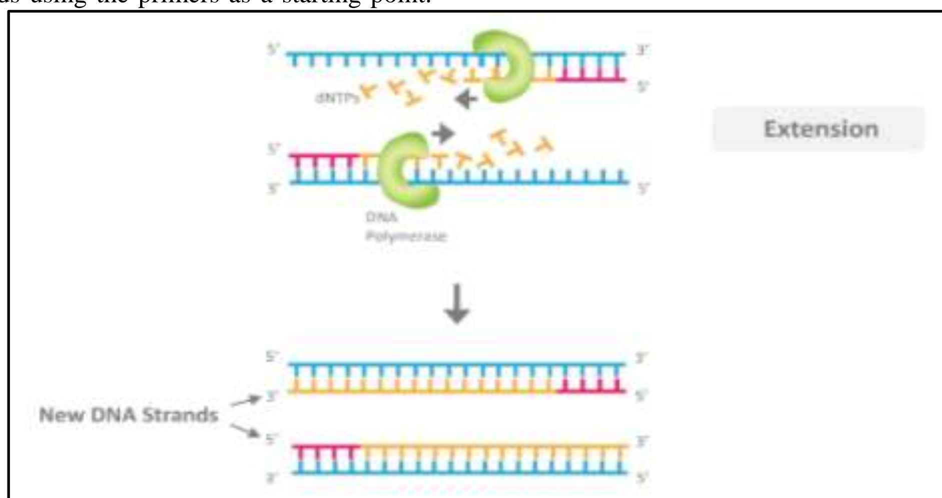

Figure 3: Step 3- Extension

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Repeat cycles: Steps 1-3 are repeated multiple times (typically 20-40 cycles) to achieve exponential amplification of the target DNA sequence. Each cycle doubles the amount of DNA, resulting in a substantial increase in the number of target DNA molecules.

By the end of the PCR process, the desired DNA segment has been amplified to a detectable level. The amplified DNA can then be analyzed using various methods, such as gel electrophoresis or DNA sequencing, depending on the specific application.

PCR has revolutionized many areas of biology and has numerous applications, including genetic research, medical diagnostics, forensic analysis, paternity testing, and infectious disease detection.

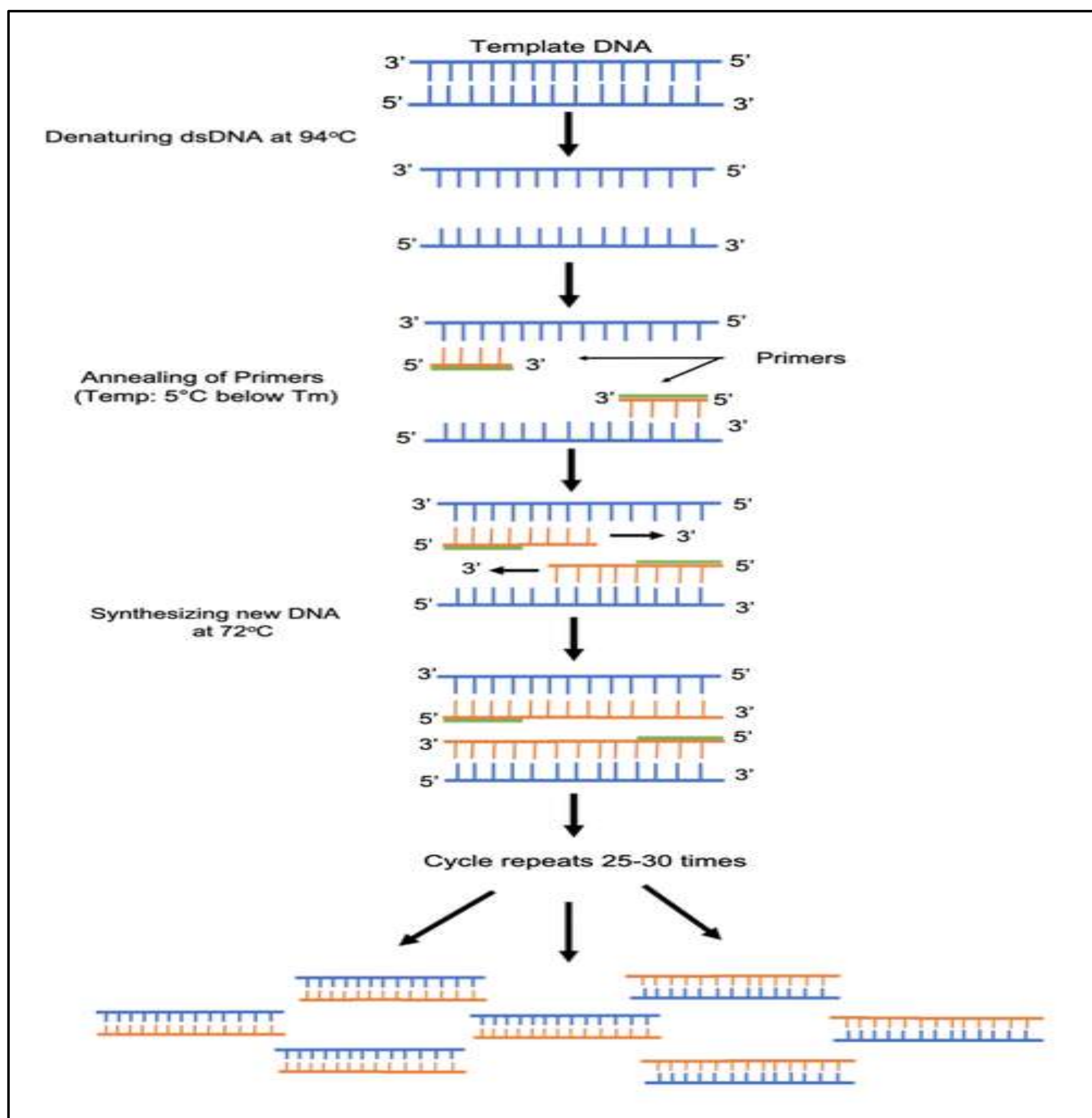

Figure 4- Steps in PCR

TYPES OF PCR:

PCR (Polymerase Chain Reaction) is a widely used technique in molecular biology and genetics for amplifying specific DNA sequences. There are several types of PCR that have been developed for various applications.

1) Conventional PCR: Also known as endpoint PCR, it is the original PCR method developed

by Kary Mullis in 1983. It involves cycles of DNA denaturation, primer annealing, and DNA extension using a heat-stable DNA polymerase such as Taq polymerase. It amplifies a specific DNA target region, and the results are analyzed after the PCR is complete.

2) Real-time PCR: Also called quantitative PCR (qPCR), this technique allows for the quantification of DNA during the amplification process. It uses fluorescent DNA-binding dyes or specific probes (such as TaqMan probes) that emit a signal when bound to the amplified DNA. Real-time PCR can measure the DNA amount in real-time, providing information on the initial quantity of the target DNA.
3) Reverse Transcription PCR (RT-PCR): This technique is used to amplify RNA molecules. It involves the reverse transcription of RNA into complementary DNA (cDNA) using reverse transcriptase enzyme. The cDNA is then amplified using conventional PCR or real-time PCR. RT-PCR is commonly used for gene expression analysis and studying RNA viruses.
4) Nested PCR: This PCR method involves two rounds of amplification. In the first round, external primers bind to the target DNA region and amplify a larger fragment. Then, a portion of the product from the first round is used as a template in the second round with a different set of internal primers. Nested PCR increases specificity and sensitivity by reducing nonspecific amplification.
5) Multiplex PCR: This PCR technique allows for the amplification of multiple target DNA sequences in a single reaction. It involves the use of multiple primer pairs that target different DNA regions. Multiplex PCR is useful for detecting multiple pathogens simultaneously and for genotyping studies.
6) Hot Start PCR: This method includes modifications to the PCR reaction mix to prevent nonspecific amplification. It involves using modified DNA polymerases that are inactive at lower temperatures, and they become active only after an initial heat activation step. Hot Start PCR improves specificity and sensitivity by reducing background amplification.
7) Multiplex Ligation-dependent Probe Amplification (MLPA): MLPA is a PCR-based technique used for detecting copy number variations (CNVs) in genomic DNA. It involves the hybridization of multiple probes to the target DNA regions of interest. The ligated probes are then amplified using PCR, and the resulting products are analyzed to determine the copy number status of specific DNA sequences.
8) Digital PCR (dPCR): Digital PCR is a method that enables absolute quantification of DNA targets. It involves partitioning the PCR reaction into thousands or millions of individual reactions, each containing a few target DNA molecules or none at all. The amplification is then carried out in each partition, and the presence or absence of the target DNA is determined. Digital PCR provides higher precision and sensitivity for detecting rare targets or determining precise target concentrations.
9) Inverse PCR (iPCR): In iPCR, the DNA fragment of interest is ligated to adaptors, and subsequent PCR amplification is performed using primers that anneal to the adaptors rather than the target sequence. This technique is useful for amplifying DNA sequences flanking known regions or for identifying unknown sequences adjacent to known DNA sequences.
10) Assembly PCR: Assembly PCR is employed to generate large DNA fragments by assembling smaller overlapping DNA fragments. Each fragment contains complementary ends that can anneal to adjacent fragments. PCR amplification is then performed using primers that hybridize to the outermost ends of the assembly. Assembly PCR is commonly used in cloning and gene synthesis.
11) Nested Reverse Transcription PCR (Nested RT-PCR): This technique combines the principles of nested PCR and reverse transcription PCR. It is used for the detection and amplification of specific RNA sequences. The first round involves reverse transcription of RNA into cDNA, followed by PCR amplification using external primers. A portion of the first-round PCR product is then used as a template for a second round of PCR with internal primers, increasing specificity.
12) Methylation-Specific PCR (MSP): MSP is designed to detect DNA methylation patterns at specific CpG sites within a DNA region. It involves bisulfite treatment of DNA to convert unmethylated cytosines to uracils, followed by PCR amplification using primers specific to methylated or unmethylated DNA sequences. MSP is widely used in epigenetic studies and

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can provide information on DNA methylation patterns in various biological samples.

III. APPLICATIONS OF PCR:

PCR (Polymerase Chain Reaction) is a widely used molecular biology technique that allows for the amplification of specific DNA sequences. It has numerous applications in various fields.

- **Genetic research:**

PCR is extensively used in genetic research to study DNA sequences. It enables the amplification of specific DNA regions of interest, which can then be analyzed for genetic variations, mutations, or polymorphisms. PCR is employed in fields like genomics, gene expression analysis, and population genetics.

- **Disease diagnosis:**

PCR plays a crucial role in medical diagnostics. It allows for the detection and identification of infectious agents such as bacteria, viruses, and parasites. By amplifying and detecting the specific DNA or RNA sequences of pathogens, PCR-based tests can diagnose various diseases, including viral infections (e.g., HIV, influenza), bacterial infections (e.g., tuberculosis, Lyme disease), and genetic disorders (e.g., cystic fibrosis).

- **Forensic analysis:**

PCR is utilized in forensic science for DNA profiling and identification purposes. The technique allows for the amplification of small DNA samples found at crime scenes, enabling investigators to generate enough DNA material for analysis. PCR-based methods like short tandem repeat (STR) analysis and DNA fingerprinting are commonly used in forensic investigations.

- **Paternity testing:**

PCR-based DNA testing is employed in paternity testing to determine biological parentage. By comparing specific DNA regions between a child and potential parents,PCR can establish familial relationships. It is a reliable method used in legal cases, immigration proceedings, and personal identification scenarios.

- **Archaeology and paleontology:**

PCR has revolutionized the fields of archaeology and paleontology by enabling DNA analysis of ancient or extinct organisms. By amplifying and studying DNA from preserved remains, such as bones or teeth, scientists can gain insights into evolutionary relationships, genetic diversity, and ancient populations.

- **Environmental monitoring:**

PCR is employed in environmental science to detect and monitor organisms present in various environmental samples. For example, it can be used to identify microbial species in soil, water, or air samples. PCR-based techniques like quantitative PCR (qPCR) allow for the quantification of target organisms, providing valuable information for environmental studies and monitoring.

- **Oncology:**

PCR is utilized in cancer research and diagnosis. It can detect genetic mutations or alterations associated with cancer, enabling the identification of specific types of cancer and guiding treatment decisions. PCR-based methods like allele-specific PCR and quantitative PCR are used in cancer studies and monitoring.

- **Pharmacogenomics:**

PCR plays a role in pharmacogenomic studies, which investigate the relationship between an individual's genetic makeup and their response to drugs. By amplifying and analyzing specific genetic markers, PCR can help predict an individual's likelihood of responding to certain medications or experiencing adverse drug reactions.

- **Food safety and quality control:**

PCR-based techniques are employed in the food industry to detect and identify pathogens, allergens, and genetically modified organisms (GMOs) in food products. PCR allows for rapid and accurate screening of food samples, ensuring food safety and adherence to regulatory standards.

- **Veterinary medicine:**

PCR is used in veterinary diagnostics for the detection and identification of pathogens that affect animals. It enables the diagnosis of infectious diseases in livestock, pets, and wildlife, facilitating appropriate treatment and control measures.

- **Ancient DNA studies:**

PCR has been instrumental in the field of ancient DNA research. By amplifying and sequencing DNA from ancient samples, including

fossils and preserved remains, scientists can reconstruct the genomes of extinct species, study evolutionary history, and explore genetic relationships between ancient and modern organisms.

- **Microbial ecology:**

PCR-based techniques like metagenomics and microbial community profiling allow for the study of microbial diversity and dynamics in various environments, includingsoil,oceans, and the human microbiome. PCR amplification of specific genetic markers helps identify and characterize microbial communities.

- **Cloning and gene expression:**

PCR is an essential component of cloning techniques, enabling the amplification of DNA fragments for insertion into vectors. It is also used in quantitative PCR (qPCR) or reverse transcription PCR (RT-PCR) to measure gene expression levels and analyze gene function.

- **Prenatal testing:**

PCR-based methods, such as amniocentesis and chorionic villus sampling, are used for prenatal diagnosis of genetic disorders. PCR amplification and analysis of fetal DNA obtained from these procedures can identify genetic abnormalities or chromosomal disorders in developing embryos.

APPLICATIONS OF PCR IN TOXICOLOGY[20,21]:

- **Molecular Genetics:**

Two distinct Taqman probes can be used in conjunction with Taq DNA polymerase's 5' nuclease activity to produce allele-specific amplification detection (allelic discrimination), which can then be used to quickly genotype individual subjects. While real-time PCR analysis of these assays helps in their creation and can aid in the assessment of the allele specific amplification, these allelic discrimination assays simply need a terminal fluorescence analysis. Aldehyde dehydrogenase, N-acetyltransferase, thiopurine methyltransferase, glutathione S transferases (GSTM1, GSTT1, GSTP1), cytochromes P450 (CYP2C, CYP2D), and other enzymes contributing to human vulnerability have all been examined for polymorphisms using Taqman-based allelic discrimination.

- **Exposure Monitoring:**

The measurement of DNA-based viral loads in clinical pathology first made extensive use of real-time PCR for quantitative detection of DNA copy number. Studies evaluating the tissue distribution of DNA-based medications, such as DNA-based vaccinations, have more recently used quantitative analysis of DNA. The monitoring of genetically modified organisms (GMOs) in food is also applicable to this.

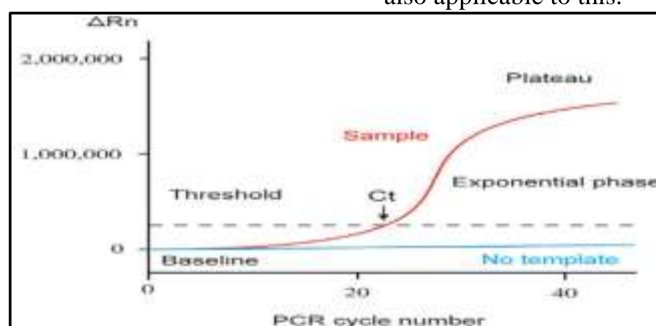

Figure 5: Exposure monitoring

- **Molecular Toxicology:**

The majority of quantitative PCR applications, or gene expression profiling, involve checking the levels of gene expression. In addition to helping with toxicologic disease, gene expression profiling has been used to examine changes in immune function and cytokine expression caused by toxicants. Real-time methods have occasionally been used as a stand-in for more traditional RNA detection techniques like Northern blot analysis. Because they required more development than other expression analysis tools, real-time assays, particularly those using a specific probe, have certain very specific applications: (1) A quantitative examination of dose-response.

(2) Molecular epidemiological gene expression biomarker research, as an illustration.

(3) Quantitative evaluations of tiny tissues or samples, such as those acquired using laser capture microdissection (LCM).

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➢ **Toxicogenomic:**

Genome-wide patterns of gene expression identified by microarray analysis are one of the cutting-edge techniques that could revolutionize the assessment of a compound's potential toxicity. Microarray data on differential gene expression, however, is frequently only semi-quantitative in nature. To expand initial findings to larger data sets and account for interindividual biological variability, a quantitative technology like RTAQ-RT-PCR can be utilised to confirm the results of microarray investigations in a high throughput and quantitative manner. The ability to create molecular signatures for classes of toxicants that are defined by changes in certain gene sets is one of the goals of a toxicogenomic approach.The toxicity of other chemicals with limited toxicological inception may therefore be predicted using these signals. According to recent bioinformatic methods, the best chance to distinguish between different groups of samples' gene expression profiles is found in the examination of just 50 gene products. To supplement and extend initial microarray analyses for the identification of toxicological signatures, the development of RTAQ-RT-PCR discrimination assay sets may thus be a suitable second-tier analysis.

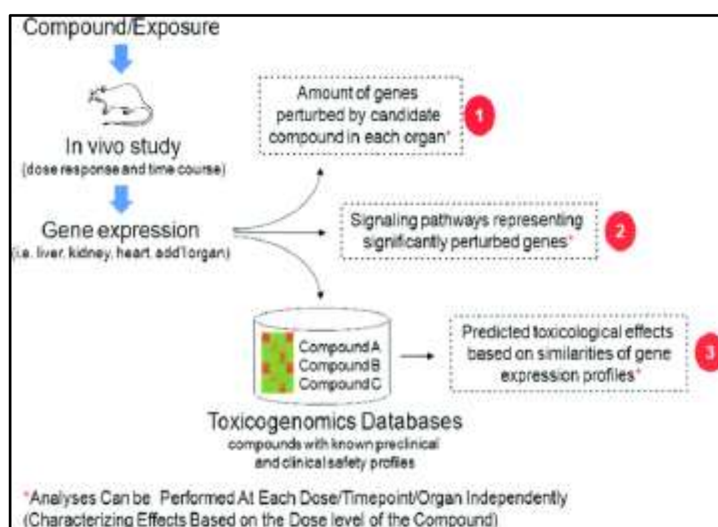

Figure 6: Toxicogenomic

APPLICATIONS OF MULTIPLEX PCR :

➢ **Gene deletion and mutation detection:**By using PCR or a subsequent study of PCR products, multiplex assays can directly detect mutations and minor deletions in genes. For RB1 gene exons amplified to reveal minor deletions causing retinoblastoma and for multiplex ARMS responses of frequent population-specific beta-thalassemia and cystic fibrosis mutations, results can be obtained right away by gel electrophoresis. As in a multiplex reaction that recognizes a point mutation, a 4-base deletion, and a full deletion of the alpha-globin genes, numerous mutation types may be investigated simultaneously. For diagnosis, other mutation-amplifying multiplexes use a post-PCR modification of the reaction product. Breast cancer-related mutations in the human p53 tumor suppressor gene are found using SSCP. Cycle sequencing of mice as oncogenes reveals activating mutations.Multiplex systems have been developed to identify the numerous, frequently population-specific mutations in the cystic fibrosis transmembrane conductance regulator gene. These multiplex systems include allele-specific primers, restriction enzyme digestion of naturally occurring restriction sites at amplified mutations, and hybridization of exons to mutation-specific oligonucleotides.

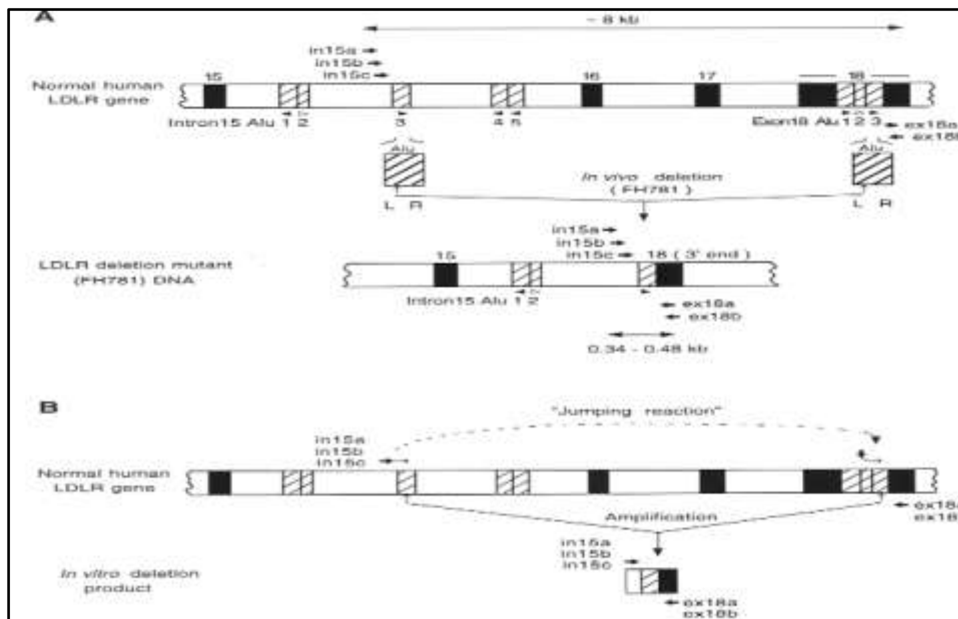

Figure 7: Gene deletion and mutation.

➢ **Polymorphic Repetitive DNA:**

For mapping, illness linkage, gender determination, and DNA typing/identification, repetitive DNA polymorphisms are multiplexed. Because they are plentiful, highly polymorphic, and may be coamplified without overlapping size ranges, short tandem repeats (STRs) of 1-6 bp are useful for multiplexing. While chromosomally unlinked repetitions are used to identify individuals, multiplexes of relatively near repeats are used to link diseases. Multiplex tests for detecting gender incorporate repetitive DNA regions. By co-amplifying a Y-specific repetitive DNA locus with a gene sequence on both the X and Y chromosomes, embryos from families with an X-linked disease can be sexed. Subsequent analyses have verified the multiplex results. In a multiplex for forensic samples, the Y-specific STR is amplified with an X-specific STR.

➢ **Applications of PCR in malaria:**

A series of procedures for employing quantitative real-time PCR to track the in vivo kinetics of entomopathogenic fungi in Anopheles in the presence or absence of malaria parasites. Two well-known fungal entomopathogens, Beauveria bassiana isolate IMI391510 and Metarhizium anisopliae var. Acridumisolate IMI330189 and a "generic" fungal test for assessing any fungal load were effectively separated from each other. Both have previously been found to be virulent to Anopheles mosquitoes using three different qPCR assays. Quantification of co-infecting malarial parasites was made possible by a fourth assay to Plasmodium chabaudi. Every qPCR assay offers accurate, reliable quantification with a linear range higher than five orders of magnitude (seven orders of magnitude for the fungal assays). The B. bassiana-specific test was used to track B. bassiana growth in mosquitoes exposed to three distinct conidial challenge dosages. This is the first description of entomopathogenic fungus replication in an insect host. This showed that, regardless of the challenge dose, a sudden on-set of considerable nuclear division occurs after many days of relatively modest replication, followed by physical fungal growth (hyphae) within the mosquito haemocoel just before death. At each time point studied, increasing conidia exposure led to both a noticeably greater mosquito pickup and higher fungal loads. With increasing challenge dose, high fungal loads, comparable to those found in cadavers, were obtained more quickly and mortalities occurred earlier post-exposure. The qPCR assays discussed here will enable new study areas that will help to improve fungal biopesticides against malaria and other vector-borne diseases.

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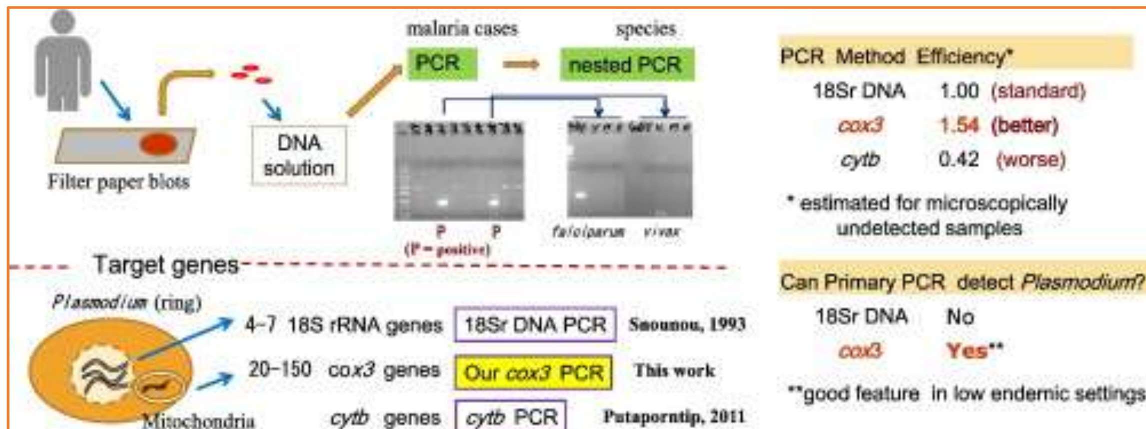

Figure 8: PCR in Malaria

➢ **The Applications of PCR to the Diagnosis of Primary Cardiac lymphoma :**

An aggravated stomach ulcer in a 75-year-old man who had first complained of pollakiuria and low abdominal pain caused significant bleeding before he passed away. Primary cardiac lymphoma was identified postmortem. Only the epicardium and myocardium were affected by the tumour, which was classified as a primary cardiac lymphoma by the Armed Forces Institute of Pathology. Large cells made up the lymphoma, which also expressed CDZO, a B cell marker. Polymerase chain reaction (PCR) was used to detect monoclonality at the DNA level in lymphoma cells, which were demonstrated to comprise a monoclonal population. This was done despite the fact that chronic inflammation caused by chronic renal failure was seen in the pericardium around the lymphoma.

IV. CONCLUSION:

PCR has emerged as a fundamental tool in modern molecular biology and has transformed various scientific disciplines and industries. Its versatility, sensitivity, and specificity have made it indispensable for a wide range of applications, including genetics, medicine, forensics, agriculture, and biotechnology. As technology continues to evolve, PCR is expected to remain a cornerstone of molecular research, contributing to further breakthroughs and discoveries in the years to come.The ability to recreate the history of life has greatly improved with the use of genotyping techniques for all living things. At the population level, natural selection's impacts, the distribution of genetic polymorphisms within a species, and demographic change may all be inferred from the frequency and distribution of known genetic polymorphisms within a species. The molecular phylogenies that presently dominate in categorization were first derived from comparisons of the sequences of the same genes in various species and from analyses of whole genomes.In the field of paleogenetics, where DNA sequences of more or less ancient organisms are recovered and analyzed, PCR has thus taken the lead. This is true for remains preserved in museum collections as well as for historical sites where extinct organisms' skeletal or mummified remains have been present for hundreds of thousands or even hundreds of thousands of years. Thus, the applications of PCR swiftly expanded to include other sectors or specialties and were no longer restricted to the study of biology.

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