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Role of PCR in Mutation Detection and Precision Medicine: Methods, Case Studies, and Optimization Strategies

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Abstract

Polymerase Chain Reaction (PCR) is one of the foundations of molecular genetics because it can be used to detect genetic mutations rapidly, sensitively, and specifically. This study examines the principles, types, and uses of PCR methods in the analysis of mutation such as conventional PCR, real-time PCR, digital PCR, multiplex PCR, and allele-specific PCR. The research paper analyzes the optimization strategies of PCR, influences on accuracy, sensitivity and specificity, and the clinical diagnosis limitations. The essential role played by PCR in the identification of single nucleotide polymorphism (SNP), insertion and deletion mutant and genetic changes in cancer is discussed as well as its use in inherited genetic disorders and pharmacogenomics. Comparative studies to next-generation sequencing (NGS) highlight the benefits of PCR in a fast, sensitive, and specific method of detecting mutations in the human genome, with future outlooks in precision medicine demonstrating its changing impact in the areas of personalized diagnostics, liquid biopsy, and point-of-care tests. The examples of PCR application in oncology, prenatal screening, and the management of genetic disorders are characterized by case studies. The study finds PCR-based methods to be invaluable in both research and clinical practice and deliver accurate, reproducible, and cost-effective instruments in the detection of mutations. The future of technology holds more possibilities of being integrated in the field of precision medicine that provides better early diagnosis, monitoring of therapy, and personalized treatment plans.

Keywords

PCR, mutation detection, real-time PCR, and digital PCR, allele-specific PCR, SNPs, insertion/deletion mutations, oncology, inherited genetic disorders, precision medicine

Introduction

Different diseases, such as cancer, hereditary and infectious diseases are associated with genetic mutations. These mutations need to be detected and analyzed accurately in order to be diagnostic, treatable, and personalized medicine. The Polymerase Chain Reaction (PCR) has transformed the science of DNA into a revolution as it offers a fast, sensitive and highly specific way of amplification and detection of target DNA sequences. PCR has progressed since the days of conventional amplification, introduced in 1983 by Kary Mullis, into more sophisticated methods like the real-time PCR, digital PCR, multiplex PCR and allele-specific PCR each having its own benefits in regards to detection of mutations. Some of the fields where PCR is utilized include oncology, pharmacogenomics, prenatal screening and the diagnostics of infectious diseases.[1] They are flexible to identify single nucleotide polymorphisms (SNPs), insertions, deletions and low abundance mutations frequently in heterogeneous or limited samples. Even with its wide application, PCR has its own difficulties such as sensitivity to contamination, specificity of the primers, and inability to detect unknown mutations. However, the use of PCR in clinical diagnostics and studies has broadened with optimized PCR protocols, in conjunction with the new technologies. This paper offers an in-depth review of PCR-based technologies in mutation detection, their principles, applications, optimization approaches, shortcomings, and prospects and identifies them as the key to the development of precision medicine.[2]

PCR procedures in genetic analysis Overview.

Polymerase Chain Reaction (PCR) is one of the most basic molecular biology methods that are applied in amplifying specific DNA sequence and make genetic analysis sensitive and specific to a high degree. PCR is an exceptionally effective technique in the current research and clinical practice to identify mutations, detect pathogens, and research gene expression. The method has developed into a number of versions that

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suit a particular requirement. Ampification and detection of DNA fragments can be done by conventional PCR which offers qualitative results. qPCR (Real-Time PCR) enables both amplification and quantification in real time, which makes it convenient in the quantification of a gene expression level and in the quantification of viral loads. Digital PCR (dPCR) offers absolute quantification by subdividing the samples of DNA into thousands of reactions, which enhances the accuracy of detecting mutation, even at low frequency. Multiplex PCR allows simultaneous amplification of multiple targets on a single run, thus saving of resources and time and offers a comprehensive analysis of complex genetic profile.[3] The methods of PCR can be used in various areas, such as oncology, where it can be used to identify cancer-related mutations; infectious disease diagnostics, where it can be used to detect bacterial and viral genomes; pharmacogenomics, where it can be used to establish genetic variability unique to patients and thus their response to drugs. The versatility of the PCR procedures lets it be combined with downstream techniques including sequencing, restriction fragment length polymorphism (RFLP) assay and genotyping arrays. Although very versatile, PCR based methods rely on such factors as the specificity of the primers, the fidelity of the enzymes and the quality of the sample. Ongoing PCR technology improvements such as the creation of high-fidelity polymerases, automated systems, and the like have increased the accuracy and reproducibility of genetic studies. All in all, PCR techniques will continue to be essential in contemporary molecular genomics as they are ready and efficient solutions to genetic mutation detection and the development of personalized medicine, and are relatively inexpensive.[4]

In Molecular Diagnostics, PCR has a long historical path of development.

The technology known as Polymerase Chain Reaction (PCR) was initially established in 1983 by a scientist named Kary Mullis, and it brought revolution to the world of molecular biology and diagnostics. Until the invention of PCR, genetic analysis was performed by use of labor intensive methods like, cloning and Southern blotting that were time consuming and consumed significant quantities of DNA. Mullis had a concept of selective amplification of the DNA sequences in vitro by specific primers and enzyme of DNA polymerase. The initial PCR procedures were not practical especially enzyme inactivation by high temperatures needed during denaturation processes. These limitations were overcome by the discovery and use of thermostable DNA polymerases, including Taq polymerase, a derivative of Thermus aquaticus, and made it possible to perform automated thermal cycling, which greatly enhanced reproducibility and efficiency.[5] In the late 1980s and 1990s, PCR quickly evolved out of its laboratory use as a diagnostic method and made it possible to identify infectious agents, genetic diseases and hereditary mutations. In the 1990s, the term real-time PCR was coined, combining fluorescent dyes or probes to be quantified and around the beginning of the 2000s, digital PCR was introduced, which enabled detection of low-frequency mutations. The advancements increased the scope of PCR use to oncology, prenatal diagnostics, and pharmacogenetics. In the last decades, the diagnostics based on PCR have been improved by the advances in primer design, optimization of the reaction and combination with high-throughput platforms. By the 2010s, PCR had become a standard of molecular diagnostics, as using it during outbreaks of contagious diseases, timely detection of pathogens became possible thanks to rapid PCR testing. In the current world, PCR is one of the most popular methods in molecular diagnostics with the speed, sensitivity and flexibility, which underlies personalized medicine, genomic studies, and epidemiological surveillance. Polymerase Chain Reaction Principals.[6]Polymerase Chain Reaction (PCR) is established on the premise of in vitro amplification of DNA by repeated thermal cycling, which allows the amplification of a target DNA sequence exponentially. The PCR mechanism is based on the three key elements; DNA template, short sequence-specific primers, and thermostable DNA polymerase. There are three fundamental stages of each PCR cycle: denaturation, annealing and extension. In the denaturation process, the DNA is heated to a high temperature (usually, 94-98degC) to cause the nucleotide bases hydrogen bonds to break and leave behind single-stranded DNA templates. During the annealing step, the temperature is reduced (usually to 50-65degC) to enable the primers to attach themselves to complementary sequences on the single stranded DNA. This procedure enhances the specificity of the amplification since the primers define the fragment of DNA which is replicated. In the extension step, the DNA polymerase produces a new strand of DNA complementary in sequence to the template between 5 3' and typically at 72degC temperature in Taq polymerase. The number of replications (25-40 cycles) will lead to millions of copies of the target sequence after several hours. The efficacy of PCR relies on the design of the primer, quality of the template, and faithfulness of the enzyme and ideal reaction conditions.[7] PCR variations like quantitative PCR, digital PCR and multiplex PCR build on these core principles by offering these added sensitivity, quantification and concomitant detection limits. PCR has been highly applied in genetic studies, diagnostics, forensics, and individual medicine due to the fact that it is simple, specific, and amplifies quickly. Through taking advantage of the natural DNA replication process occurring in a controlled laboratory setting, PCR enables a researcher and clinician to identify even

those tiny genetic mutations using high level of precision and reproducibility.[8]

Study of the PCR Methods to detect mutation.

Techniques based on PCR have been broadly scaled up to identify genetic mutations, and there are benefits associated with each of the techniques where they are used. The simplest type is conventional PCR, as the target DNA sequences are multiplied and are observed using gel electrophoresis. Although it is useful in identifying larger inserts, deletions, or existing mutation sites, conventional PCR is mostly qualitative and has little sensitivity. A Real-Time PCR (qPCR) is a polymerase chain reaction that is used to quantify the DNA amplification in real-time by the use of fluorescent dyes or probes to detect small mutations and single nucleotide polymorphisms (SNPs) in a highly sensitive way. Digital PCR (dPCR) enhances quantification by dividing DNA into thousands of single reactions, so that it can be feasible to find rare mutations, low-frequency alleles, or minimal residual disease in cancer patients.[9] Multiplex PCR is a technique that enables an amplification of multiple targets using a single reaction thereby conserving resources and time, but offers detailed mutation profiles. The second method is an Allele-Specific PCR (AS-PCR) that is tailored to amplify specific alleles and would be suitable in the identification of point mutations or SNPs. There are other variations called as Nested PCR and touchdown PCR which increase specificity and decrease non-specific amplification, particularly when using complex or degraded DNA samples. The selection of the PCR technique is influenced by the characteristics of the mutation, kind of sample, sensitivity necessary and throughput.[10] As an example, digital PCR finds usage in the field of oncology research because of its ability to identify low-frequency mutations, whereas multiplex PCR is popular in the field of clinical genetics because of its ability to screen many mutations associated with a disease. Altogether, these varied methods of PCR offer flexible technology of correct, fast, and delicate identification of genetic mutations in research, diagnostic science, and individualized medicine.[11]

Traditional PCR vs. Real-Time PCR.

Both conventional PCR and Real-Time PCR (qPCR) are necessary methods of molecular analysis of DNA amplification, yet they differ greatly in their methodology, output, and uses. The traditional PCR method is based on amplification of target DNA based on thermal cycling followed by visualization on an agarose gel electrophoresis. It gives qualitative data, that is, whether a certain DNA fragment is present or absent. This is a relatively easy, low-cost technique that is commonly applied in mutation detection, cloning of genes, and preliminary screening. Nevertheless, the traditional PCR lacks the real-time quantification, and it might be under the risk of contamination during the post-amplification manipulations.[12] Real-Time PCR in contrast can be used to both amplify and detect DNA simultaneously with the use of fluorescent dyes (including SYBR Green) or sequence-specific probes (including Taqman). The quantitative data generated by qPCR allows measurement of initial concentrations of templates and detection of small changes in copy number of genes unlike the conventional PCR; hence its use in viral load monitoring, minimal residual disease monitoring, and SNP detection. In addition, qPCR minimizes the chances of contamination as the post-amplified work is not necessary. Although it is more expensive and requires specialized equipment, qPCR is a method of choice in clinical diagnostics and research because of its quantitative ability and fast outcomes. As a whole, though traditional PCR is still useful in simple genetic analysis, Real-Time PCR will offer better accuracy, velocity, and quantitative data, especially with regard to the detection of mutation and molecular diagnostics.[13]

Genetic Mutation Identification in the Allele-Specific PCR.

Allele-Specific PCR (AS-PCR) is a very specific method that is aimed at detecting known genetic mutations especially single nucleotide polymorphisms (SNPs) and point mutations. As a contrast to standard PCR, which amplifies any DNA fragment between the pairs of primers, AS-PCR works with primers, which are complementary to particular alleles, so its amplification depends on whether the selected mutation is present. The main rule is to make the primers such that there is a nucleotide at the 3' end that corresponds to the mutation of interest. When the DNA template contains that specific mutation, the primer anneals ideally and polymerase is then extended. On the other hand, a 3' end mismatch inhibits amplification allowing the separation of wild-type and mutant alleles.[14] The AS-PCR is common in the clinical diagnostics, pharmacogenetics, and cancer research. Indicatively, it is able to detect mutations in tumor suppressor genes or oncogenes, which can be used to make decisions on targeted therapy. The method is also used in the recognition of hereditary genetic diseases and patient-drug interaction. Its strengths are that it is simple, fast and less expensive than sequencing but it is restricted to known mutations and it also has to be designed very carefully. Other differences like competitive AS-PCR and real-time AS-PCR increase sensitivity and can be used in a quantitative manner. In general, the Allele-Specific PCR is a powerful, reliable and fast method of determining particular genetic mutations and therefore is an essential resource in molecular genetics and personalized medicine.[15]

Quantitative PCR Applications in Mutation Analysis

Real-Time PCR or quantitative PCR (qPCR) has now become a necessity in the study of mutations because

it is capable of detecting DNA amplification in real-time with high sensitivity and specificity. In comparison to traditional PCR which can only give qualitative results, qPCR enables researchers and clinicians to determine the number of target DNA molecules, convenient with detecting subtle genetic variations and low-frequency mutations. qPCR is based on fluorescent dyes, e.g., SYBR Green or sequence-specific probes, e.g., TaqMan, which fluoresces in proportion to the quantity of amplified DNA.[16,17] This real-time surveillance allows accurate calculation of the numbers of alleles copies, mutation burden and modification of gene expression. Applications of qPCR in mutation analysis qPCR has extensive use in detecting single nucleotide polymorphisms (SNPs), deletions and inversions, and cancer somatic mutations. As an example, in oncology, qPCR may be used to determine the abundance of mutant alleles in circulating tumor DNA, which can be detected early on, used to monitor treatment response, and to determine minimal residual disease. qPCR is also used to determine the abundance of specific genetic mutations in pathogens which confer drug resistance or increased virulence. In addition, the quick turnaround time and reproducibility of qPCR are appropriate in high-throughput screening of clinical laboratories and research laboratories. Limitations of qPCR are that it requires designed primers and probes carefully, optimization of reaction conditions and that it requires the use of reference standards to be accurately quantified. Nevertheless, the difficulties have not stopped the application of qPCR, as its quantitative and highly sensitive character has enhanced its application as a vital tool in the detection and analysis of mutations, promoting precision medicine and genetic studies.[18]

The Digital PCR and Its Benefits.

Digital PCR (dPCR) is an important development in the technology of PCR that can provide absolute quantification of nucleic acids and increased sensitivity over traditional and real-time PCR techniques. As opposed to the PCR, which is a conventional method that offers a relative quantification of a DNA sample, dPCR subdivides a DNA sample into thousands to millions of micro-reactions, each partition containing one or a theoretically zero target molecule. Once amplified, presence or absence of target sequence in each partition is then identified and Poisson statistics are used to analyze the finding to give absolute quantification.[19] This splitting enables dPCR to identify very rare mutations and alleles with low frequency with high accuracy that is highly desirable in oncology where circulating tumor DNA can be in incredibly low quantities. Also, dPCR is less susceptible to PCR inhibitors and differences in amplification efficiency, and it is more reproducible and accurate. DPCR is used in the detection of minimal residual disease, tracking of treatment response, somatic mutation identification, and copy number variation.[20] It is also used in the diagnostics of infectious diseases to measure the viral loads and identify low-abundance drug-resistant variants. The other characteristics are high sensitivity, robustness, and the capacity to work with degraded or low DNA samples that in many cases is a constraint with traditional PCR. Although digital PCR is more expensive and demands specialized equipment, it offers the highest level of accuracy, as well as sensitivity, and is a powerful method in research, clinical diagnostics, and custom-made medicine. The fact that it enables absolute quantification and detects rare mutations makes dPCR an NGS standard of molecular diagnostics and mutation analysis.[21]

Simultaneous Multiplex PCR Multiplex PCR Multiplex PCR Multiplex PCR Multiplex

Multiplex PCR is a special method of PCR which allows simultaneous amplification of multiple target sequences in a single reaction. Multi-plex PCR is also an effective, cost effective, and time saving technique of identifying different genetic mutations simultaneously using several sets of primers that target individual DNA sequences. This is of special use in clinical diagnostics, genetic screening and research where multiple mutations, genes or polymorphisms are required to be analyzed at the same time. As an example, multiplex PCR has been extensively used in cancer genetics to simultaneously screen a set of oncogenes or tumor suppressor genes in a single experiment to enable the results to be used to comprehensively profile mutations. Likewise, the method is applicable in the study of infectious diseases to identify multiple pathogen or resistance genes within a single reaction.[22] In multiplex PCR, the arrangement of primers is very important in order to avoid the generation of primer-dimer and specific amplification, thus the optimization of annealing temperatures, primer concentrations, and reaction conditions should be highly observed. The technique has been improved by the use of fluorescent-labeled probes and real-time multiplex PCR, which can now be used to quantitatively detect multiple targets simultaneously.[23] Multiplex PCR has the ability to reduce reagent usage, manual effort, and turnaround time relative to standard singleplex PCR, besides offering a bigger range of genetic data. Advantages consist of the complexity of assay design and the possibility of the primers competing with each other in resources and, hence, influencing amplification efficiency. Although it has these limitations, multiplex PCR has become a useful and potent instrument in detecting mutations by providing high-throughput examinations and facilitating genetic screening in large amounts, personalized medicine, and therapeutic decision-making of a genetic nature.[24]

PCR-Based Methods Sensitivity and Specificity.

Sensitivity and specificity are very important parameters used in the assessment of the PCR-based

methods performance in terms of detecting genetic mutations. Sensitivity is the capacity of a method to identify properly samples which contain the desired mutation whereas specificity is the capacity of a method to rule out properly samples without the mutation. The high sensitivity is what makes the detection of low-abundance or rare mutations possible, which is especially needed in oncology, in prenatal diagnostics and in the study of an infectious disease. Indicatively, minimal levels of mutations in cancer patients can be identified with digital PCR with as few as 0.01% of the total DNA content hence suitable in treatment of residual disease. Real-time PCR also has high sensitivity with potential of detecting the single copy of DNA sequence when optimized appropriately.[25] Specificity, in its turn, depends also largely on the design of the primers and the probes, because the false-positive results can be caused by non-specific binding or amplification. Specific methods such as Allele-Specific PCR were specifically aimed at making such methods more specific by allowing amplification to happen only when a desired allele is present. PCR method and reaction conditions are the factors that affect sensitivity and specificity. Primer length, melting temperature, enzyme fidelity, and reaction buffer composition could be among the factors influencing the capacity of the balance between false positives and true positives. Although efficient, multiplex PCR must be optimally employed in order to retain specificity when multiple targets are going to be amplified. [26]The sensitivity and specificity should be evaluated to validate the PCR assays in clinical diagnostics to assure proper detection of mutations, patient management, and decision making on treatment. In general, when designed and optimized, PCR-based techniques are highly sensitive and specific, and thus, are useful in genetic studies and molecular diagnostics.[27]

Variables that Influence PCR Accuracy in mutation.

Determining the validity of PCR-based methods in the detection of mutations is a critical issue that is affected by a number of factors, each of which contributes to the validity of the findings. The most critical is primer design because the primers should specifically anneal to the target sequences to prevent unintended amplification. Inaccuracy can be due to mismatches, hairpin structures or a primer-dimer. Quality and quantity of templates also influence the outcome of PCR; poor and inadequate DNA can result in partial amplification or it may fail to identify mutations. The other factor is enzyme selection because polymerase fidelity dictates the rate of error in amplifications.[28] Polymerases with a high-fidelity reduce errors in the process of synthesizing DNA, which is especially essential when a single nucleotide mutation is under consideration. The reaction conditions such as the annealing temperature, magnesium ion concentration and number of cycles should also be well tuned to obtain a specific and efficient amplification. PCR is very susceptible to contamination: even minute traces of foreign DNA may give a false-positive due to their presence in a PCR run, particularly with a very sensitive technique, such as digital PCR.[29] This is also dependent on the detection method; in real-time PCR fluorescence-based or in digital PCR partitioning can enhance precision relative to the traditional gel-based visualization. Lastly, detection can be affected by biological factors including heterogeneity of the sample, presence of inhibitors or low concentration of mutant alleles. These factors should be taken into careful consideration in order to detect mutation accurately and by the use of positive and negative controls. With the solution of these variables, PCR will be able to give a reliable and reproducible result that can be used in clinical and research levels thus confidence in genetic analysis.[30]

PCR Disadvantages and Problems in Clinical Diagnostics.

In spite of clinical diagnostics, PCR has a number of limitations and challenges regardless of its widespread use. Sensitivity to contamination is one of the main restrictions. The ability of PCR to amplify DNA is so high that the method is highly prone to pollution by foreign DNA that results into false-positive outcomes. This risk should be curbed by proper laboratory practice, and by the use of negative controls. Another issue is primer design and specificity; ineffective primer design can lead to non-specific amplification or primer-dimerization which can decrease the reliability of the assays.[31,32] Moreover, PCR can have a problem with mutations with a low abundance in nonhomogeneous samples, which necessitates sophisticated techniques such as digital PCR to identify rare alleles. The presence of inhibitors in clinical samples, e.g. hemoglobin, urea, or some chemicals, may result in disruption of the polymerase reaction, which may induce variations in amplification efficacy and precision. PCR does not also give much information about the context of mutation; although it identifies individual sequences, it cannot identify the larger genomic mutations, structural variation, and new mutations, frequently requiring the support of other techniques such as sequencing.[33] Additionally, cost and equipment will be a constraint especially when their high-throughput and resource limited laboratories are in need because real-time and digital PCR demand specialized equipment. Lastly, interpretation of results in clinical setting may be complicated, particularly when it is applied to heterozygous mutations or cells that are mixed. In spite of these, PCR has been a pillar of molecular diagnostics owing to its speed, sensitivity and flexibility. Its limitations can be overcome by appropriate assay design, sample

preparation, and applying sophisticated PCR variants so that it can be used to the fullest extent by clinicians and researchers to detect mutations and treat patients.[34,35]

PCR Optimization Planning to find mutations better.

The sensitivity, specificity and overall reliability of the mutation detection method is to be maximized by optimizing the PCR conditions. There are a number of strategies that can enhance the performance of PCR, and the first one is the primer design. Primers should also be of the right length, melting point and GC-content so they will only anneal to the target sequence. Primer-dimer formation can compete with target amplification hence avoiding self-complementary regions would improve this.[36] The quality of templates and the way they are prepared is also of the highest importance; purified and high-quality DNA will enhance the amplification efficiency, and degraded samples might demand special PCR protocols (e.g. nested PCR). Parameters in thermal cycling need to undergo a fine tune. Specificity and yield can be improved by changing the denaturation, annealing and extension temperatures and times, in particular with complex or GC-rich templates.[37] Enzyme activity and primer binding depend on the concentration of magnesium ions in the reaction buffer; optimization of magnesium 2+ levels can usually enhance fidelity in the amplification. Selection of enzymes is also an important factor. The high-fidelity DNA polymerases decrease the number of errors made during replication and this is important in the analysis of mutations. To amplify difficult targets, additives may be used that enhance amplification of GC-rich or structurally complex areas, e.g. DMSO, betaine or BSA. In more complex techniques, touchdown PCR sequentially lowers the annealing temperature to enhance specificity of the primer, whereas nested PCR is used with two amplification cycles on low-abundance targets.[38,39] Real-time PCR can also be used to improve the detection of mutation through the provision of quantitative information and real-time monitoring of the amplification process thus minimizing the chances of a false positive. Further, the results are reliable as due controls are put in place such as positive, negative and no template controls. The combination of these optimization strategies makes PCR very sensitive and specific and can be used to detect rare mutations and more complicated genetic variations accurately. In clinical diagnostics, research, and personalized medicine, the successful optimization is of paramount importance because the quality of mutation detection directly influences the final diagnosis and treatment of a patient, as well as their survival.[40]

Application of PCR in the detection of Single Nucleotide Polymorphisms (SNPs).

The most prevalent genetic variation is known as single nucleotide polymorphisms (SNPs) which are usually linked to disease susceptibility, drug response and phenotypic characteristics. The significance of PCR in the identification of SNPs is as a result of its specificity, sensitivity, and flexibility. Methods like Allele-Specific PCR (AS-PCR) are aimed at specifically amplifying DNA sequence that includes a given nucleotide to thus be able to distinguish between wild-type and mutant alleles. SNP detection can be done in real-time by using Real-Time PCR with fluorescent probes, which can detect the degree of fluorescence of each amplification cycle with Taqman assays.[41] Multiplex PCR has the advantage of screenings of multiple SNPs at once to offer an efficient and thorough analysis of genetic investigations. Pharmacogenomics uses SNP detection via PCR to identify SNP variations specific to different patients and tailors the type of drug administered to them to reduce side effects and enhance the therapeutic benefit of the drugs they take. In clinical genetics, PCR is used to identify SNPs in hereditary diseases, in research it can be used to conduct an association study involving SNPs and traits or risk of disease.[42] High-resolution methods e.g. PCR-restriction fragment length polymorphism (PCR-RFLP) are a combination of a PCR and an enzyme digestion to identify the differences in sequences and define alleles. Digital PCR has high sensitivity in the detection of low frequency SNPs in heterogeneous samples, including circulating tumor DNA. The high-throughput SNP genotyping of population studies and the high-level genetic screening is also provided by the fact that PCR can be amplified and precisely targeted very quickly. In general, PCR offers an inexpensive, precise, and versatile method of SNP detection, which has become the foundation of numerous diagnostics and research and individual medicine studies.[43]

PCR in the Determination of the Mutations.

Indels are genetic changes such as insertion and deletion mutations, which have the potential to impair the functionality of genes, with the result of diseases or phenotypic variation. PCR is also an effective way of identifying indels because it has the capacity to amplify small segments of DNA and also helps in identifying the difference in the sizes of the amplified products. The standard PCR technique with gel electrophoresis enables the length changes with insertions or deletions to be visualized, hence it is appropriate when the size of an indel is moderate- to large-sized.[44] In small or weak indels, PCR methods that have high resolution like real-time PCR or capillary electrophoresis are more sensitive and precise. Indels can be detected by Allele-Specific PCR, whereby primers are specifically designed to anneal to the site of insertion or deletion, whereupon their amplification is only possible in the presence of the target mutation. Digital PCR is another method which improves the detection, particularly of low frequency indels in heterogeneous samples, because

it divides the sample and individually amplifies each amplicon.[45] PCR techniques have popular applications in clinical diagnostics, genetic screening and research. As an example, they are able to detect deletions in the dystrophin gene in Duchenne muscular dystrophy or small inserts into the oncogenes that influence the development of cancer. Multiplex PCR Multiplex PCR can be used to identify multiple indels in different genes simultaneously, and offers more efficiency in diagnostic panels.[46] In spite of these benefits, primers should be designed carefully and the optimal reaction should be established in order to prevent non-specific amplification and correct indel detection. Altogether, PCR is a cost-effective, sensitive, and quick method of detecting insertion and deletion mutations, which can be used to support genetic diagnosis, research, and personalised medicine.[47]

Comparison of Next-Generation sequencing Techniques and PCR.

Both Polymerase Chain Reaction (PCR) and Next-Generation Sequencing (NGS) are important molecular instruments, but they are fundamentally different in the way they work, their scope and their intended use. PCR is a highly sensitive method used mostly to detect known mutations and this technique is highly sensitive and results can be obtained in a short period of time since it is mainly a targeted technique of amplifying specific sequences of DNA.[48] It is economical, uses little input of samples and can be implemented in conventional, real-time, digital or multiplex formats. PCR is most effectively applied in cases where the target region is clearly defined (e.g., the detection of single nucleotide polymorphisms (SNPs), insertions, deletions or detection of specific pathogen genes). PCR is, however, only limited in scope; it is not able to reveal unknown mutations not included in the designed primers and also does not provide a comprehensive information about the genome.[49] However, NGS can be used to do high-throughput, parallel sequencing of millions of fragments of DNA, which makes it possible to study the whole-genome, exome, or targeted panel. NGS is a more global view where it can detect known and novel mutations, structural variants, copy number variations, and complicated genetic rearrangements. Even though NGS is both a complete and unbiased source of data, it is also more costly, necessitates advanced bioinformatics, and has a longer turnaround time than PCR. PCR can occasionally be more sensitive than NGS in identifying low-frequency mutations in heterogeneous samples particularly in digital PCR. PCR and NGS can also be used complementary in the clinical practice: PCR is used as a high-speed screening technique or to validate an NGS-identified variant, whereas NGS is used in the detailed examination of the genome and revealed new mutations.[50,51] In general, the decision between PCR and NGS is determined by the purpose of the study, the type of mutation, the sensitivity that is needed, the cost factors, and the necessity to perform a targeted or genome-wide study. The two technologies used in conjunction with each other provide benefits to molecular diagnostics, genetic research, and personalised medicine.[52]

PCR in Oncology: The use of PCR in the detection of mutations that are related to cancer.

PCR has proven to be a very important instrument in the field of oncology used in the detection and monitoring of genetic alterations associated with cancer. Genetic changes in oncogenes or tumor suppressor genes or DNA repair pathways (point mutations, insertions, deletions, and copy number changes) cause cancer. The sensitive and quick identification of these mutations in small or heterogeneous specimens is possible with PCR, including tumor biopsies or tumor ctDNA in blood.[53] Such methods as Allele-Specific PCR and digital PCR are especially useful in cancerology because they enable the identification of rare mutant alleles in the background of wild-type DNA. A real-time PCR gives quantitative information, which can be used to measure the burden of mutations and track changes in the course of treatment. Its uses include the detection of mutations in genes, which include KRAS, EGFR, BRAF, and TP53, which are used to guide the decision to use targeted therapy and predict response to treatment. PCR can also be used in the detection of minimal residual disease following surgery or chemotherapy and this gives early warning of the relapse.[54] Oncogenes, or mutations Multiplex PCR panels enable the screening of many oncogenes or mutations at a time, which increases the efficiency of clinical diagnosis. Moreover, PCR-based studies are used in the liquid biopsy methods, providing non-invasive control of tumor progression and treatment effectiveness.[55] PCR is a sensitive technique that fails to identify unidentified mutations beyond the target regions, and thus it is generally combined with next-generation sequencing in order to achieve genomic profiling. All in all, PCR is a fast, sensitive and inexpensive method of cancer-related mutation detection, which is essential in personalized oncology, treatment planning, and monitoring of patients.[56]

PCR is applicable in Inherited Genetic Disorders.

PCR has found extensive use in diagnosis and research of genetic disorders which can be inherited because it is capable of selectively amplifying sequences of DNA related to the disease. It facilitates early and correct detection of mutations, deletions, insertions and single nucleotide polymorphisms (SNPs) that cause hereditary diseases. As an example, CFTR gene mutations in cystic fibrosis, HBB gene mutations in sickle cell disease, and deletions in the DMD gene causing the Duchenne

muscular dystrophy are identified using PCR. Allele-Specific PCR (AS-PCR) is able to identify point mutations whereas multiplex PCR can simultaneously examine a number of loci that is handy in screening panels of hereditary disorders.[57] The RTC PCR offers a quantitative measurement, which is applicable in copy number variation evaluation to measure the dosage of gene. PCR is also important in prenatal and carrier screening that enables detection of disease causing mutation in embryos, fetuses or parents prior to conception. Besides diagnostics, PCR is also used in research to learn the molecular etiology of inherited diseases, learn more about genotype phenotype relationships, and determine new pathogenic variants. PCR is sensitive and specific, so it is suitable to test small or degraded samples of DNA (like those used in sampling dried blood spots or amniotic fluid). Even with these benefits, the use of PCR-based techniques is restricted to the known mutations and special precautions are needed to prevent false positives. However, PCR also continues to play a fundamental role in the treatment of inherited genetic diseases, where it is used to diagnose the illness early, conduct genetic counseling, identify carriers, and develop specific treatment programs. It is fast, precise and flexible, which is why it cannot be dispensed with in clinical and research practice.[58,59]

The Future Prospects of PCR in Precision Medicine.

Polymerase Chain Reaction (PCR) has already changed the way the molecular diagnostics are done, and the role of molecular diagnostics in precision medicine is expected to continue growing. Treatment customization according to a personal genetic profile is the foundation of precision medicine, and PCR is highly sensitive, specific, and flexible, which is why it is an essential part of the strategy. PCR is predicted to be further incorporated with high-throughput technologies in the future, including the microfluidics and lab-on-a-chip systems, enabling the rapid, automated and economical analysis of mutations using small sample volumes.[60,61] Digital PCR (dPCR) is likely to have a critical role in identifying the mutations that are rare and track the disease progression with a higher level of accuracy, especially in tumor, infection, and pharmacogenomics. In addition, real-time multiplex PCR will facilitate wholesome genetic profiling in clinical labs as it will be possible to screen several mutations simultaneously. Introduction of next-generation sequencing (NGS) and bioinformatics tools will enable PCR to be used as a screening tool and as a validation tool of complex genomic data. Also, PCR-based assays might be further used in liquid biopsy, which allows to monitor tumor-derived DNA, response to treatment, and minimal residual disease non-invasively, thereby eliminating the repetitive invasive biopsy.[62,63] Other trends are point-of-care PCR machines that can provide high-quality and quick results in a clinical or a remote environment and assist in making individualized therapeutic decisions real-time. Nevertheless, by overcoming such difficulties, including the fact that assay design and optimisation have to be carefully considered, the versatility of PCR and ongoing technological advancements will enhance its use in precision medicine.[64,65] The future will witness convergence between PCR and digital health, real-time monitoring, and personalized therapeutics, making it one of the main ways to further the advancement of patient-centered healthcare, enhance the level of diagnostic accuracy, and allow using PCR as a source of providing timely and targeted interventions. Generally, the development of PCR will keep on improving personalized medical approaches to improving the outcome and also creating improved and effective treatment mechanisms.[66]

Case Studies that have emphasized use of PCR in detection of mutations.

The use of PCR-based methods in the identification of genetic mutations is proven, as it has been demonstrated in countless case studies, making these techniques and techniques clinically relevant and versatile. As an illustration, in oncology, EGFR mutations have been monitored in non-small cell lung cancer patients with the help of digital PCR. One of them showed that analysis of ctDNA in patient plasma with the help of digital PCR could identify low-frequency EGFR mutations that can be used to predict the early signs of treatment resistance and make the necessary changes in targeted therapy.[67] Likewise, the real-time PCR has also been utilized in colorectal cancer to identify KRAS mutations that can be used in the selection of anti-EGFR therapy. PCR has enabled CFTR mutations to be identified in cystic fibrosis in inherited genetic disorders. Multiplex PCR panels enabled screening of common CFTR variants in newborns simultaneously and as such, the early diagnosis and early intervention tremendously enhanced the patient outcome. In another example, deletion mutations in the DMD gene were detected by the PCR technology using deletion primers and detected by allele-specific methods and enabled carrier identification and genetic counseling of the affected families. PCR has also been found useful in the management of infectious diseases like the detection of mutation in HBB gene that causes sickle cell disease during prenatal diagnosis. PCR was fast, sensitive and specific in all these instances and was in most cases better than the conventional methods of diagnosis.[68] The examples highlight the flexibility of PCR to a wide range of different samples, including blood and tissue biopsies and circulating DNA, and its ability to detect the presence of rare mutations in heterogeneous samples.[69] The total use of case studies indicates the influence of PCR on personalized medicine, early diagnosis, treatment

monitoring, and risk assessment, which further indicates the importance of the technique in the current clinical and research practice. PCR is still relevant in terms of actionable genetic information, the gap between laboratory research and patient care.[70,71]

Conclusion

The methods based on PCR have proven to be indispensable in the detection of genetic mutations with the unquestionable ability to provide sensitivity, specificity and versatility. Researchers and clinicians can precisely use single nucleotide polymorphisms, insertions, deletions, and cancer-related mutations with the aid of conventional PCR, real-time PCR, digital PCR, multiplex PCR and allele-specific PCR. PCR has greatly contributed to the diagnosis of genetically inherited diseases, prenatal screening, and oncology applications, which allows early diagnosis, individualized therapy, and the evaluation of the disease. Although PCR has weaknesses, such as contaminability, difficulties in primer design and inability to detect unfamiliar mutations, optimization schemes and combination with other technologies such as digital PCR and real-time multiplex PCR have alleviated these problems. PCR has advantages compared to next-generation sequencing in that it is fast, enables targeted detection of mutations, and in the future, point-of-care diagnostics and high-throughput platforms hold the potential to increase its use in precision medicine. The effectiveness of PCR in clinical and research applications is proven by case studies that have shown its usefulness in patient care, monitoring treatment, and in genetic counseling. Comprehensively, PCR has been the workhorse of molecular diagnostics offering a solution to mutation detection that is cost effective, accurate, and reliable. The progressive development of the PCR technologies is projected to enhance their use in the realm of individual medicine and enable people to intervene on time and achieve better health care results.

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