Chapter

PCR Techniques and Their Clinical Applications

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Abstract

Kary B. Mullis developed a revolutionary method name polymerase chain reaction (PCR) in 1983, which can synthesize new strand of DNA complementary to the template strand of DNA and produce billions of copies of a DNA fragment only in few hours. Denaturation, annealing, and extension are the three primary steps involved in the PCR process, which generally requires thermocyclers, DNA template, a pair of primers, Taq polymerase, nucleotides, buffers, etc. With the development of PCR, from traditional PCR, quantitative PCR, to next digital PCR, PCR has become a powerful tool in life sciences and medicine. Applications of PCR techniques for infectious diseases include specific or broad-spectrum pathogen detection, assessment and surveillance of emerging infections, early detection of biological threat agents, and antimicrobial resistance analysis. Applications of PCR techniques for genetic diseases include prenatal diagnosis and screening of neonatal genetic diseases. Applications of PCR techniques for cancer research include tumor-related gene detection. This chapter aimed to discuss about the different types of PCR techniques, including traditional PCR, quantitative PCR, digital PCR, etc., and their applications for rapid detection, mutation screen or diagnosis in infectious diseases, inherited diseases, cancer, and other diseases.

Keywords: PCR, quantitative PCR, digital PCR, rapid detection, mutation screen, infectious diseases, inherited diseases, cancer

1. Introduction

The polymerase chain reaction (PCR) is one of the most widely used techniques in molecular biology, which plays an important role in traditional molecular cloning experiments and also solves the problem of obtaining sufficient amount of nucleic acid [1]. PCR technology can solve many problems in molecular biology by synthesizing primers needed for genome sequence information to amplify specific segments of DNA and has been widely used in laboratories and clinics.

In 1971, Khorana et al. first proposed the idea of nucleic acid amplification *in vitro* [2]. "tRNA genes can be synthesized by the continuous repetition of the process that DNA denaturation, hybridization with appropriate primers, and extension of primers with DNA polymerase." However, due to the undiscovered heat-stable DNA

polymerase and the difficulty of primer synthesis, this idea was soon forgotten. In 1976, Jiayun Qian, a Chinese scientist, discovered stable Taq DNA polymerase, which made a fundamental contribution to the development of PCR technology [3].

In April 1983, Mullis came up with the idea for polymerase chain reaction when he was driving to his cottage in the country. In the same year, he obtained the first PCR fragment of 49 bp after 10 cycles by isotope labeling. In 1985, Mullis invented and patented PCR, with himself being the first inventor, while he worked at Cetus Corporation. In the same year, the first academic paper on PCR was published in *Science*, with Mullis as coauthor [4].

At present, PCR techniques have been widely used in life science and clinical practice to solve many problems by amplifying specific DNA fragments efficiently. Applications of PCR techniques for infectious diseases include specific or broad-spectrum pathogen detection, assessment and surveillance of emerging infections, early detection of biological threat agents, and antimicrobial resistance analysis [5, 6]. Applications of PCR techniques for genetic diseases include prenatal diagnosis and screening of neonatal genetic diseases such as Down syndrome and thalassemia [7, 8]. Applications of PCR techniques for cancer research include tumor-related genes detection, such as activating oncogenes, tumor suppressor genes, metastatic genes or metastatic suppressor genes, tumor-related viruses, and antitumor drug-resistant genes or mutation detection [9, 10].

This chapter mainly discussed about the principle, characteristics, common problems and their solutions of PCR, the different types of PCR techniques including traditional PCR, quantitative PCR (qPCR), digital PCR (dPCR), etc., and their applications for rapid detection, mutation screen or diagnosis in infectious diseases, inherited diseases, cancer, and other diseases.

2. The principle, characteristics, common problems, and their solutions of PCR

2.1 The principle of PCR

The basic principle of PCR is the synthesis of double-stranded DNA by the replication of a specific nucleic acid molecule as a template through primer extension. In addition to heat-resistant DNA polymerase, precise temperature cycle control is also a key reaction condition. PCR technology mainly includes the following three main steps: (1) Denaturation, template DNA double-strand dissociation to single strand at high temperature; (2) Annealed, specific primers and target DNA were complementary combined according to base pairing principle; and (3) Extension, using single-stranded DNA as a template, complementary DNA replicates with the extension of primers under the action of heat-resistant DNA polymerase.

The PCR reaction can be completed automatically in a special instrument, where the required PCR program can be edited. DNA denaturation temperature is usually around 94°C, and the required time is related to the structure of target DNA and PCR reaction system. Annealing temperature is affected by primer length and base composition, which is generally between 50 and 60°C. The quantity and specificity of PCR products can be improved by adjusting annealing temperature. Generally, the extension temperature is 72°C, and the reaction time depends on the length of PCR products. It is predicted that due to the longer length of PCR products, the longer reaction time will be needed (see **Figure 1A** and **B**).

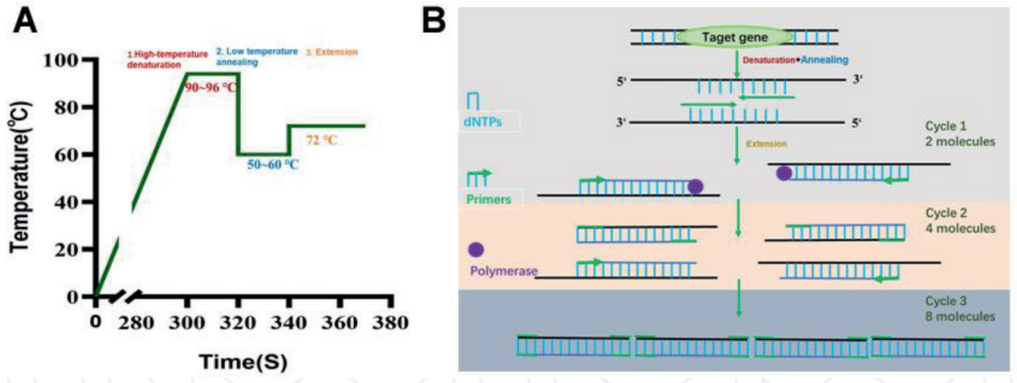

Figure 1.
Flowchart and schematic diagram of PCR technology. (A) General flowchart of PCR technology, predenaturation at 94°C for 5 min, denaturation at 90–96°C for 20 s, annealing at 50–60°C for 20 s (annealing temperature and time depends primer length and base composition), and extension at 72°C for 30 s (the reaction time depends on the length of PCR products), and (B) Schematic diagram of PCR technology, including amplification buffer, four dNTP mixtures, Taq DNA polymerase, etc., with two primers, the first 20–25 cycles of 2n index amplification, and then gradually stable after 30 cycles.

The advantage of PCR technology mainly includes its efficient amplification ability and specific replication of microtarget nucleic acid sequences. This simple technical design enables PCR technology to be widely used and play an important role in life science and related scientific fields [11].

2.2 The characteristics of PCR

The PCR reaction system includes template, primer, enzyme, dNTPs, and buffer. The template is the target DNA, which must meet the requirements of proper concentration and high purity. Too low or too high concentration of the template may lead to amplification failure, and low purity may affect the amplification efficiency. There is a pair of primers in PCR reaction, including upstream primer and downstream primer. The upstream primer is complementary to a short DNA sequence at the 5′ end of the single strand of DNA, and the downstream primer is complementary to a short DNA sequence at the 3′ end of this segment. Factors should be considered when designing primers such as its length, base composition, cross-complementary sequence within and between primers, specificity of primers, and continuous pairing at the template of 3′ ends. There are two kinds of heat-resistant DNA polymerase, that is, Taq and Pfu, from two different thermophiles [12]. In Taq enzyme, amplification efficiency is high but easy to mismatch, while in Pfu enzyme, amplification efficiency is lower but has error correction function. Deoxyribonucleoside triphosphate (dNTP) is a premixed solution containing deoxyadenosine triphosphate (dATP), dCTP, deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP) [13]. The composition of buffer solution is more complex. Besides water, the buffer system usually includes N-2-hydroxyethylpiperazine-N-ethane-sulphonicacid (HEPES) or 4-Morpholinepropanesulfonic acid (MOPS) buffer system [14] (univalent cation, generally using potassium ion; divalent cation, mainly magnesium ion; and auxiliary components, commonly using dimethylsulfoxide (DMSO) and glycerin), which is mainly used to maintain enzyme activity and help DNA unwinding structure.

PCR amplification follows the principle of base complementary pairing, which has high specificity in theory. PCR product synthesis increases exponentially and

amplifies the initial peak-grade template DNA to the microgram level with high sensitivity. PCR amplification is usually completed within 2–4 hours, which has the superiorities in saving time. Additionally, after the PCR reaction program is set, the amplification process can be automatically completed on the PCR instrument, which is easy to operate.

Four factors mainly determine the PCR reaction time: heating and cooling time of the template, temperature equilibrium time between the template and the PCR tube, extension time, and number of cycles [15]. Based on the principle of PCR, three temperature points of denaturation, annealing, and extension were set in three steps. The double-stranded DNA was denatured at 94°C and then rapidly cooled to 50–60°C; the primer was annealed and bound to the target sequence and then heated to 72°C. Under the action of Taq polymerase, the primer chain was extended along the template [16]. Among the four main factors, the extension time and the number of cycles play a more decisive role in the reaction time of PCR.

The number of PCR cycles is usually about 30–40. Although the number of products can be increased, the amplification of nonspecific products will also be increased correspondingly as the number of cycles increased. Additionally, the polymerase activities in the PCR reaction system are limited due to the temperature variation from high to low in the recirculation, and the amount of primers and raw materials in the PCR system is not infinite [17]. With the consumption of dNTP, primers and other raw materials in the PCR reaction system and the decrease of enzyme amplification ability, mismatch, and nonspecific amplification will also be increased as the number of cycles increased. Therefore, if the PCR products are sufficient for subsequent analysis, it is generally less likely to extend the number of PCR cycles excessively.

2.3 The common problems and their solutions of PCR

There are many possible reasons for false negative in PCR [18], and the corresponding treatment methods are different. First, the template was lost or degraded too much or contained too much protein and many impurities, which may be solved by the extraction quality of the template. Second, the PCR reaction system was prepared incorrectly, which may be solved by the reprepared experiment. Third, the DNA polymerase was inactivated or the concentration was too low, which may be solved by the replaced DNA polymerase and operated on the ice bath. Fourth, the PCR reaction procedure was not appropriately set, which may be solved by the adjusted PCR reaction conditions, such as reduction of denaturation time or annealing temperature. Fifth, the primer design was unreasonable or the synthesis quality was not high. In this case, primer design software should be used to further simulate and analyze its amplification quality or redesign and synthesize the primers if necessary.

There are relatively possible reasons for few false positive in PCR. First, primers have low specificity and certain homology with nontarget amplified sequences [19]. In this situation, PCR reaction conditions can be adjusted, such as increasing annealing temperature and redesigning the primers if necessary. Second, target DNA or amplified products were cross-contaminated [20]. In those circumstances, consumables such as PCR reaction tubes, centrifuge tubes, and pipette tips should be used once as far as possible. Additionally, before operation, the air in the laboratory, reaction reagent, or sampling gun could be exposed under ultraviolet light. Moreover, operation should be careful and gentle to prevent target DNA template from being sucked into the sampling gun or spattered out of the centrifuge tube.

3. The history of PCR development

3.1 The first generation: ordinary PCR

Usually what we call the first generation of ordinary PCR refers to the original PCR technology, which used the application of the ordinary PCR machine to amplify the target DNA and then analyzed PCR products by sequencing or agarose gel electrophoresis, and was generally used for qualitative analysis [21]. It was also used for semiquantitative analysis of gene transcription expression levels, before the second generation developed [22].

The first generation can effectively amplify trace amounts of DNA, especially in paleontological remains in fossils and samples coming from hair, skin debris, blood, or body fluids at crime scenes [23, 24]. Only a small number of DNA sequences were needed to be extracted, while enough copies can be obtained after amplification using PCR. Further sequencing analysis and homology comparison can smoothly go on.

In addition, the PCR products were analyzed by agarose gel electrophoresis for the amount, size, brightness of stripes, etc. According to the information from electrophoresis band, the target DNA fragment was prepared, the gene to be tested was qualitatively analyzed, and the gene expression was semiquantitatively analyzed [25]. However, a major drawback existed in the first generation of PCR technology, which was generally limited to qualitative analysis and unable to achieve accurate quantitative analysis. Besides, the nucleic acid dyes used at the beginning of agarose gel electrophoresis, such as ethidium bromide, are chemical carcinogens and have great harm to human body and environment [26]. And the detection process of sequencing or electrophoresis analysis takes quite a long time [27].

3.2 The second generation: quantitative PCR

The second generation of quantitative PCR (qPCR) refers to real-time PCR technology. qPCR adds fluorescent dyes or DNA probes into the reaction system to indicate the reaction progression and collects fluorescence signals to monitor PCR product accumulation. By analyzing the cycle threshold value (Ct value) of the amplification curve, the amount of PCR product is quantified in combination with the standard curve [28]. This technology can bring the results to repeatability and comparability, after being performed in accordance with standard operating procedures [29].

This technology is widely used in the medical field, due to the advantages of quantitative analysis, lower risk of cross-contamination, and easier operation. It has played an important role in the clinical application for infectious disease pathogen nucleic acid detection, anti-infection, antitumor and other drug resistance gene detection, genetic disease diagnosis, gene therapy, and so on [30–34].

qPCR overcomes the disadvantages of the first generation that cannot achieve accurate quantitative analysis, but it is still a relatively quantitative analysis method, which relies heavily on the level of operation technology and standard curve quality. For example, low-copy target DNA is difficult to detect, and background values skew test results and are greatly affected by PCR inhibitors [35]. Also, there are certain differences between the reagents and equipment produced by different manufacturers [36].

3.3 The third generation: Digital PCR

As next generation of PCR technology, digital PCR (dPCR) dispenses nucleic acid samples into a large number of independent, parallel microreaction units

(nanoliters), then dilutes the template in each reaction unit to the level of single molecules, and carries out amplification, detection, and distribution statistics on this basis, so as to achieve absolute counting of target molecules. According to its reaction unit carrier, dPCR is further divided into droplet digital PCR based on droplet microfluidics and chip microarray PCR based on chip microfluidics [37].

This technology reduces the concentration of nonspecific background sequences greatly, reduces amplification bias largely, and has low background values and strong specificity. Low-copy target DNA can be detected with high sensitivity due to small effects of template concentration. The quantitative analysis of target DNA does not require drawing a standard curve, which can achieve absolute quantification with good accuracy [38, 39]. Furthermore, this technology has obvious advantages in the reproducibility of reagents from different manufacturers and operator test results because of fewer interference factors.

dPCR is especially suitable for nucleic acid trace analysis with very high sensitivity requirements and accurate quantification of nucleic acids in complex samples such as tissues, body fluids, and excreta. For example, the accurate analysis of small changes in viral load is critical for determining the course of the disease and assessing efficacy [40]. Also, dPCR can accurately quantify mutation frequencies as low as 0.001–0.0001% due to its ultrahigh sensitivity, which can be used in the detection of rare mutation sites of genes related to anti-tumor-targeted drugs. dPCR can enrich target genes in the sample to be sequenced, validate, and accurately quantify sequencing results [41]. It has been reported of successfully applications for researches in single-cell or low-abundance gene expression analysis, gene expression fold change less than two times, and copy number variation with gene copy number greater than 5 [42–44].

However, dPCR has strict requirements for the amount of template, which in each reaction unit need to be diluted to the single-molecule level by limiting allocation, too much will lead to inability to quantify, and too little will lead to low signal. In addition, PCR amplification primers require a high specificity, and nonspecific amplification should be avoided as much as possible.

In addition to the PCR techniques described above, various methods have been developed to improve PCR. Examples include multiplex PCR, broad-range PCR, strip PCR, and direct strip PCR methods, which are used to detect 24 pathogens that cause ocular infections [45]. Oligoribonucleotide interference PCR (ORNi-PCR) can amplify the desired transcript variant in cDNA by inhibiting the amplification of unwanted transcripts and can simultaneously detect two single-nucleotide mutations in the same allele of epidermal growth factor receptor (EGFR) gene in lung cancer cells with high sensitivity, which is expected to be used for the early detection of cancers caused by single-nucleotide mutations [46]. Furthermore, there are other types of PCR, such as methylation-specific PCR, type-specific PCR, etc. [47, 48].

4. Clinical applications of PCR

4.1 Applications of PCR for infectious diseases

At present, PCR is widely used in clinical practice, especially in the detection, screening, or diagnosis of infectious diseases. Its applications include specific or broad-spectrum pathogen detection, assessment and monitoring of emerging infections, detection of biological threat agents early, and analysis of antimicrobial resistance [6]. The main methods for routine diagnosis of pathogens include bacterial

culture, virus culture, cell culture, serological diagnosis, and enzyme-linked immunosorbent assay. However, these detection methods have different disadvantages, such as tedious process of traditional cell culture and pathogen isolation and identification, time-consuming operation, high price, and low sensitivity [49, 50]. And serological diagnosis is prone to false positive results due to cross-linking reaction [51]. Compared with the above detection methods, the nucleic acid molecular detection method based on the nucleic acid amplification principle can significantly improve the sensitivity and specificity of detection. PCR technology breaks through the "time window" limit of immunological detection and can judge whether the disease is in recessive or subclinical state [52, 53]. Therefore, considering that one of the key points of prevention and treatment of infectious diseases is early and rapid diagnosis, PCR technology can overcome the lag of immunological detection and the complexity of gene sequencing. Since the epidemic of novel coronavirus infection (coronavirus disease 2019—COVID-19) for more than 3 years, PCR nucleic acid detection has always been the primary method for the diagnosis of infection [5]. With the development of PCR technology, multiplex PCR, multiplex quantitative PCR, microfluidic PCR, and other detection platforms have been developed to detect multiple pathogens at the same time [54–56].

In addition, PCR is used to identify pathogenic microorganisms by typing. For example, when testing for human papillomavirus (HPV), PCR can not only identify the infection, but also the subtype to which it belongs [57]. The pathogenicity of different HPV subtypes varies significantly. Assessing cancer risk based on subtype distribution and adopting individualized and precise prevention and control strategies can prevent cervical cancer more effectively [58].

PCR techniques also allow for the quantification of pathogenic microbial loads. For example, viral load is an important indicator for assessing efficacy of antiviral drug therapy. PCR is used to quantify changes in the load levels of hepatitis B virus (HBV) or human immunodeficiency virus (HIV) in blood to determine the effectiveness of treatment for HBV or HIV [59].

There are also more established applications of PCR techniques in antimicrobial resistance genetic testing. For example, *Klebsiella pneumoniae* is one of the important causative agents of hospital infections. With the widespread use of broad-spectrum antimicrobial drugs, such as β-lactams and aminoglycosides, bacteria are prone to produce extended-spectrum β-lactamases (ESBLs) and cephalosporinase (AmpC enzyme) as well as aminoglycoside-modifying enzymes (AMEs), resulting in drug resistance [60–62]. The drug resistance genes of clinical isolates of *K. pneumoniae* were quickly detected by PCR. Compared with the drug sensitivity test based on bacterial culture, it has unique advantages in guiding the rational use of antimicrobials [63, 64].

The third-generation digital PCR (dPCR) can detect trace drug resistance mutations of pathogenic microorganisms and achieve "accurate attack" in the selection of effective anti-infective drugs [65]. In view of the H275Y point mutation of neuraminidase H275Y in 2009 H1N1 influenza virus resistant to oseltamivir, Taylor and co-workers compared the ability of the second-generation fluorescent quantitative PCR (qPCR) and dPCR to detect H275Y under the background of high-abundance wild-type H1N1. The results showed that dPCR significantly improved the sensitivity of mutant virus detection (>30 times) [66]. Additionally, Mukaide and co-workers established a dPCR method to detect mutations in the core amino acid 70th site of hepatitis C virus (HCV) with 200-fold higher detection rate than qPCR (**Table 1**) [67].

4.2 Applications of PCR for inherited diseases

Traditional prenatal gene diagnosis mainly depends on the probe-based Southern blotting and restriction fragment length polymorphism (RFLP) to detect gene mutation including gene deletion and mismatch. However, due to its complex operation, high technical requirements, isotope labeling, and long time, its application is limited. After the rapid development of PCR in the late 1980s, it has become a common technique for the prenatal genetic diagnosis of genetic diseases. For example, PCR technology is applied to detect amniotic fluid, villi, and other samples for the rapid prenatal diagnosis of Down syndrome, thalassemia, and other genetic diseases.

Down syndrome, also known as trisomy 21 syndrome, can cause mental retardation and severe birth defects in newborns [69]. Traditional fetal aneuploidy screening is usually used in the first and second trimesters by serum biochemical testing (SBT). High-risk pregnancies identified by SBT are referred for invasive surgery, which is not only time consuming but also a risk of miscarriage. In addition, SBT can only indirectly estimate the risk or probability of pregnancy carrying aneuploid fetus based on alternative serological markers, which is not only a relatively low sensitivity, but also a high false positive rate [70]. dPCR-NIPT assay has better sensitivity and specificity than SBT, and the cost is much lower than NGS-NIPT. The simultaneous detection of fetal T21 in maternal plasma samples in a single reaction would be a better solution as the primary screening technique for fetal euploidy screening [7].

Thalassemia is an inherited blood disorder, with alpha-thalassemia and beta-thalassemia being the most common [71]. The prenatal diagnosis of monogenic diseases, such as beta-thalassemia, currently relies on invasive surgery. Since the discovery of cell-free fetal DNA (cffDNA) in maternal plasma, noninvasive prenatal diagnosis (NIPD) has made remarkable progress [72]. Guizhou Province took the lead in using next-generation sequencing (NGS) combined with the traditional screening technique for thalassemia—cross break point PCR (also named as Gap-PCR) is used

Year	PCR type	Clinical application	Reference
2010	TS-PCR	Detection of HPV subtypes (types 6, 11, 16, 18, 31, 33, and 35)	[58]
1996	PCR	Detection of the effect of lamivudine on HBV replication in HIV infected patients	[59]
2008	RAPD-PCR	Detection of *Klebsiella pneumoniae* (Molecular typing)	[63]
2016	PCR	Detection of *K. pneumoniae* (extended-spectrum β-lactamase (ESBL) producers)	[64]
2015	ddPCR	H1N1pandemic (detection of oseltamivir-resistant subpopulations)	[66]
2014	ddPCR	Detection of HCV (mutation at core amino acid 70)	[67]
2020	RT-qPCR	Detection of COVID-19 virus	[68]

Note: TS-PCR: type-specific polymerase chain reaction; PCR: polymerase chain reaction, RAPD-PCR: random amplification of polymorphic DNA-polymerase chain reaction; ddPCR: droplet digital polymerase chain reaction; and RT-qPCR: real-time-quantitative polymerase chain reaction.

Table 1.
Application of PCR for infectious diseases.

to screen newborn thalassemia genes, suggesting that NGS-Gap-PCR can effectively identify new mutations and reduce the misdiagnosis rate [8]. Microdrop digital PCR (ddPCR) is an efficient, simple, and cost-effective PCR-based technique that enables absolute quantification of target DNA molecules. D 'Aversa et al. demonstrated for the first time that a ddPCR-based NIPT method can be used to rapidly and sensitively identify materially inherited beta IVSI-110 and beta +039 thalassemia mutations [73]. Constantinou et al. optimized ddPCR assay to improve the sensitivity and specificity of monitoring and used it to determine fetal genotypes in couples noninvasively at risk of IVSI-110G > A β-thalassemia. It was suitable for all pregnancies of mothers of carriers of IVSI-110G > A, showing 97.06% accuracy [74]. In addition, PCR can also be used for NIPD of single gene diseases such as deafness and cystic fibrosis, and the results show that NIPD is accurate and effective and has the advantages of simple and rapid [75–77].

Screening for neonatal genetic diseases enables children to be diagnosed and treated within 10 days after birth, to prevent death, disability, or reduce the degree of disability due to genetic diseases. At present, qPCR and gene sequencing are the main technologies for screening neonatal genetic diseases. Genetic screening disorders range from a single disorder, such as hereditary deafness, to multiple disorders [78]. Hearing screening combined with genetic screening is helpful for the early diagnosis and intervention of clinical hearing impairment. Genetic screening for deafness can improve the detection rate of hereditary deafness. The current screening method is quantitative PCR for several genes and mutation sites, which has achieved remarkable results [79]. Garbuz et al. developed a genetic diagnostic method for Wilson's disease (WD) based on ARMS-PCR, DreamTaq green PCR premix, and modified primers to detect 14 mutations in Europeans. This diagnostic method is rapid and easy to use and has good accuracy and repeatability of mutation detection (**Table 2**) [80].

4.3 Applications of PCR for cancer and other diseases

Tumor-related genes include oncogenes, tumor suppressor genes, and genes that promote or inhibit metastasis. At present, in the field of clinical diagnosis and treatment of cancer, PCR technology is mainly applied to the detection of tumor-related genes, such as chromosome translocation [81], activated oncogene [82], tumor suppressor gene [83], metastasis gene or metastasis suppressor gene [84], tumor-related virus and antitumor drug resistance gene or mutation site [84–87], etc. By detecting gene mutation and activation expression, it can provide evidence-based medicine for analyzing tumor etiology, diagnosis and classification, metastasis, surgical treatment indication, selecting the best chemotherapy scheme, evaluating the curative effect, and judging the prognosis.

About 95% of chronic myelogenous leukemia (CML) have Philadelphia chromosome positive, which is the result of translocation of the long arm of chromosome 9 and 22. This translocation rearranges the proto-oncogene abl on the long arm of chromosome 9 (9q34) and the bcr gene on chromosome 22 (22q11) to form a fusion gene, known as Bcr-Abl fusion gene [88]. Overexpression and activation of this gene is considered to be one of the leading causes of CML, which can precede clinical symptoms, so it can be used as a basis for early diagnosis. In addition, it is known that patients with CML with negative chromosome in Philadelphia have a poor response to treatment and poor prognosis. At present, the detection of Bcr-Abl fusion gene by PCR has been deeply applied in the clinical diagnosis and treatment of CML [9].

Year	PCR type	Clinical application	Reference
2022	dPCR	prenatal diagnosis of Down syndrome	[7]
2021	PCR	prenatal diagnosis of thalassemia	[8]
2022	ddPCR	prenatal diagnosis of β-thalassemia	[73]
2022	ddPCR	prenatal diagnosis of β-thalassemia	[74]
2018	dPCR	prenatal diagnosis of deafness	[75]
2019	Fluorescent multiplex PCR	prenatal diagnosis of cystic fibrosis	[76]
2021	ddPCR	prenatal diagnosis of β-thalassemia	[77]
2019	qPCR	genetic screening for deafness	[79]
2022	PCR	genetic testing for Wilson's disease	[80]

Note: PCR: polymerase chain reaction; dPCR: digital polymerase chain reaction; ddPCR: droplet digital polymerase chain reaction; and qPCR: quantitative polymerase chain reaction.

Table 2.
Application of PCR for genetic diseases.

The study found that miR-122 is closely related to the classification of hepatocellular carcinoma [89]. The inhibition of miR-122 expression promotes the occurrence of hepatocellular carcinoma, which is characterized by enhanced invasion, easy recurrence, and reduced survival time [90]. Similarly, miR-1258 has also been reported to act as a tumor suppressor in liver cancer. miR-1258 inhibits the progress of breast cancer (BC) by increasing cell apoptosis and reducing cell viability and cell cycle transformation by inactivating key protein (KDM7A). Unlike miR-122, miR-1258 is also involved in gastric cancer (GC), colorectal cancer (CRC), oral squamous cell carcinoma (OSCC), and other diseases [91–93]. The detection of miR-122/1258 expression level by PCR can provide important reference for the classification and prognosis of hepatocellular carcinoma [10].

Ovarian cancer is a highly malignant tumor, which is insidious and progresses rapidly. Only about one-quarter of ovarian cancer is found in the early stage, and most patients have spread or metastasized at the time of treatment, so the early diagnosis of ovarian cancer is very important. In recent years, it has been found that human epididymis protein 4 (HE4) is an important biomarker for judging benign and malignant ovarian cancer [94]. Using PCR technology, the increased expression of HE4 before 4–5 months of the recurrence in advanced ovarian cancer after surgical treatment was found, suggesting that HE4 can be used to detect the recurrence of ovarian cancer.

Colorectal cancer (CRC) has also become increasingly common in recent years. Only in 2018, there were more than 1.8 million new cases of colorectal cancer and more than 880,000 deaths. Among all cancer cases in the world, CRC ranked third in the world in terms of incidence rate and the second in terms of mortality [95]. It is expected that by 2030, the global CRC burden will increase by 60%, with more than 2.2 million new cases and more than 1.1 million deaths [96]. The application of PCR in CRC is based on the fact that the microbiota spectrum associated with CRC is different from that of healthy subjects and is related to different mucosal gene expression profiles. The quantitative analysis of the host gene expression involved in CRC progression and immune response is performed by real-time quantitative PCR [97].

During tumor treatment, the dynamic detection of tumor-related gene expression in blood has obvious advantages for effect evaluation, individualized treatment, and

Year	PCR type	Clinical application	Reference
2023	PCR	Prostatic cancer, oophoroma (HE4)	[98]
2020	qRT-PCR	Lung cancer (TERT, HK-2, EGFR, KRAS)	[99]
2023	qRT-PCR	Identification of differentially expressed circRNA in GC of gastric cancer	[100]
2021	Digital droplet PCR (ddPCR)	Colorectal cancer (EVL, NTRK3)	[101]
2008	Methylation specific PCR	Prostate cancer (ASC, CDH13)	[102]
2008	Methylation specific PCR	Breast cancer (PITX2)	[103]
2013	Methylation specific PCR	Ovarian cancer (APC, RASSF1A, CDH1, RUNX3, TFPI2, SFRP5, and OPCML)	[104]
2013	Methylation specific PCR	Lung cancer (p16INK4A, CDH13, APC, DAPK)	[105]

Note: PCR: polymerase chain reaction; qRT-PCR: quantitative real-time polymerase chain reaction; and ddPCR: droplet digital polymerase chain reaction.

Table 3.
Application of PCR technology for cancer research.

metastasis. Especially for patients with nonsolid tumors and postoperative chemotherapy, there is no need to measure solid lesions. This method is particularly important for evaluating the sensitivity of chemotherapy (**Table 3**).

Additionally, PCR is also widely used in the diagnosis and treatment of other diseases, such as in parasitic diseases [106], metabolic diseases [107–109], autoimmune diseases (rheumatoid arthritis and diabetes) [110], pediatric pulmonary diseases [111], spinal muscular dystrophy diseases [112], and so on. In a word, PCR has been used in almost the whole process of disease prevention, diagnosis, treatment, and prognosis.

5. Conclusion

In conclusion, PCR is a powerful technique for obtaining nucleic acid quickly and accurately, which can synthesize new DNA strands that complement the DNA template strands and produce billions of DNA fragments. This chapter reviews the principle, characteristics, common problems, and solutions of PCR, different types of PCR techniques, including traditional PCR, quantitative PCR, digital PCR, and their rapid detection, mutation screening or diagnostic applications in infectious diseases, genetic diseases, cancer, and other diseases. Currently, PCR has become a powerful tool in the field of life science and medicine. Based on PCR techniques, detection systems with higher sensitivity and specificity are constantly developed. In the future, with the continuous upgrading of PCR, it will play an important role in molecular diagnostic technology and have a wider application prospect in clinical and more fields. Further studies on PCR techniques and their clinical application not only provide theoretical basis for the analysis of disease etiology and the prevention of disease development, but also provide new ideas for the indication of surgical treatment and the selection of the best disease treatment plan through the detection of genes mutation or differential gene expression.

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Conflict of interest

The authors declare no conflict of interest.

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