

The principle and application of new PCR Technologies

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Abstract: Polymerase chain reaction (PCR) is essentially a selective DNA amplification technique commonly applied for genetic testing and molecular diagnosis because of its high specificity and sensitivity. PCR technologies as the key of molecular biology, has realized that the qualitative detection of absolute quantitative has been changed. It has produced a variety of new PCR technologies, such as extreme PCR, photonic PCR, o- amplification at lower denaturation temperature PCR, nanoparticle PCR and so on. In this paper, the principle and application of PCR technologies are reviewed, and its development is prospected too.

1. Introduction

PCR (polymerase chain reaction, PCR) that is polymerase chain reaction, is a method of in vitro enzymatic synthesis and amplification of specific DNA fragments. In 1985, the American Karray and other scholars pioneered the PCR technology, and developed by the United States Cetus company^[1]. With the development and breakthrough of science and technology, PCR technology has been widely used in many fields, such as microbial detection, veterinary medicine, aquaculture and so on. This technique has strong sensitivity, accuracy and specificity, And it can be detected quickly, so its application field is extending continuously^[2-3]. With the development of PCR technology, many new technologies, such as extreme PCR, photonic PCR, o- amplification at lower denaturation temperature PCR, nanoparticle PCR and so on. They have been developed on the basis of conventional PCR technologies, and many new PCR technologies have been developed.

PCR technology is based on the known DNA sequence, to be amplified with the synthetic DNA two chain end complementary two oligonucleotide primers, in vitro to be detected DNA sequences (template) were amplified in enzymatic action. The whole technical process of PCR by several cycles, one cycle consists of 3 steps: the first step is the continuous DNA template degeneration under high temperature conditions, namely the template DNA at 93~94 °C under the condition of denaturation chain; the second step is that annealing 2 synthetic oligonucleotide primer and template DNA chain 3. By the end of the cooling to 55 °C annealing; the third step is to extend, which exist at the same time in 4 kinds of d NTP substrate, with the help of Taq DNA polymerase, primers chain along the 5'-3' direction and new chain of complementary template. After this cycle, a new chain is synthesized that can be continued as a DNA template and thus recycled. During the cycle, the amount of amplified products increased exponentially, and the single copy gene was 25~30 times^[4], and DNA could amplify 100 million times. The steps of the PCR reaction are simple, but the specific operations are complex, such as the determination of the annealing temperature, the length of the extension and the number of cycles. Therefore, different reaction systems should determine appropriate reaction conditions to avoid false negatives or false positives.



2. Classification of new PCR technologies

2.1 Extreme PCR

Conventional PCR process usually takes time to 1.5-2h because of the metal plate heating. In order to improve the test speed of PCR, the Swiss company Roche (Fast/Rapid) proposed the concept of PCR, and developed by Lightcycler PCR quartz capillary instrument air heating and rapid heat conduction, the instrument temperature rise speed (about 5°C/s), and solve the temperature uniformity of the sample, the whole experiment the process is shortened to 40 min. Subsequently, Farrar^[5] found that the concentration of primers and polymerase 10-20 times increased, the amplification rate of instrument reaches about 0.4-2.0 s/; when the concentration of more than 10 mol/L primers, polymerase concentration is 1 mol/L, 28s only need to complete 35 cycles, will shorten the reaction time of PCR to the limit, was born the limit of PCR (Ex-treme PCR). Extreme PCR is suitable for rapid detection of virulent infectious and bioterrorism pathogens.

2.2 Photonic PCR

Photonic PCR is proposed by the Luke Lee team, whose basic principle is to achieve fast heating based on energy conversion, thus shortening the PCR time^[6]. The specific process is caused by the electronic resonance light emitting diode irradiation on the surface of the gold film quickly converted to heat to DNA degeneration; then, close the light emitting diode, gold film cooling fast, complete annealing and extension. Because the energy conversion process is more rapid than the conventional cooling process, the amplification of target DNA can be achieved within 5 min. Optical PCR is a breakthrough in traditional PCR heating mode, which makes PCR detection more convenient and fast.

2.3 Cold-PCR

Low denatured temperature PCR (co-amplification at lower denaturation temperature PCR, Cold-PCR) is a new PCR technique for enriching mutant genes by reducing the reactive temperature of PCR. The principle is that the base mismatch in any strand of DNA affects the denaturation temperature, so the denaturation temperature of mutant DNA is often lower than that of wild type DNA. The denaturation temperature of PCR reaction can be reduced, and the mutant DNA can be denatured, broken and amplified further, while wild type DNA can not amplify^[7], and the mutant gene can be enriched at the denatured temperature. The assay is often used for viral gene mutations^[8] detection, cancer associated gene mutations (p53^[9-10], EGFR, KRAS, etc.), and beta globulin (HBB) mutations that cause beta thalassemia^[11] etc.

2.4 Nanoparticle PCR

PCR nanoparticles (nanoparticle PCR) proposed by Li, the gold nanoparticles as a new additive high (G+C) template amplification, amplification of PCR can significantly improve the specificity, sensitivity and reaction efficiency. Gold nanoparticles have superior electrical, optical, thermal and catalytic activity, and have the same properties as SSB proteins (single stranded binding proteins), which bind to single stranded DNA and do not interact with double stranded DNA^[12]. In addition, it can interact with many components such as primers in PCR reaction system, d NTP and DNA polymerase. In addition, gold nanoparticles also have excellent heat dissipation capability. In the absence of gold nanoparticles, non-specific amplification is very serious, and the yield of target bands is very low. But by adding appropriate amount of gold nanoparticles, it can inhibit non-specific amplification, but too much gold nanoparticles can also inhibit the amplification reaction. Therefore, the amplification effect of high GC template can be significantly improved by adding gold nanoparticles as additives to Slowdown, PCR or Touchdown PCR reaction systems.

2.5 HPE-PCR

Pulsed PCR (heat pulse extension-PCR, HPE-PCR)^[13] is an amplification technique for templates with long DNA chains and large numbers of CTG repeats. Because it contains a lot of CTG repeats

(G+C) content is high, easy to form the complex structure of the two level (or two hairpin dimer etc.), often due to degeneration of template is not sufficient as a result of PCR amplification failure, is a big problem in PCR technology. HPE-PCR by increasing the denaturation temperature of PCR to solve this problem, two level degeneration in the pre degeneration, when the temperature rises to 98°C, two level structure can denature solution chain, to solve the problem of high content of DNA (G+C) template degeneration is not sufficient; then, the temperature dropped to 68 °C for primer in the process of annealing; extension extension temperature gradually increased from 76 °C to 83 °C pulse by heat, then quickly cooled to 76 °C, cycle, a total of 21 times Heat pulses complete the extension of the new chain. After the amplification, the PCR products were separated on 1% agarose gel, using ethidium bromide staining in micro gel imaging system for imaging. Therefore, HPE-PCR has a significant advantage over the amplification of high (G+C) content DNA templates ^[14].

3. Summary

In the context of modern accurate diagnosis, how to improve the technology, ensure high sensitivity and specificity while improving And the ease of use of the repeatability of PCR detection, will become the new direction of the future development of PCR technology; PCR technology and sequencing technology integrated automation will likely become the new requirements of clinical molecular diagnosis and public health emergencies nucleic acid detection.

Reference

- [1] Chang Shimin. Application of PCR in detection of food microorganism [J]. Journal of Handan agricultural college, 2004, 21 (4) : 23-25.
- [2] Tang Yongkai, Yu Juhua, Xu run, et al. Real-time quantitative PCR technique and its application in aquaculture [J]. China Agricultural Science Bulletin, 2010 (21): 422-426.
- [3] Wu Xuegui. LPS. Cloning and differential expression analysis of immune related genes in grouper *Epinephelus* [D]. Haikou: Hainan University, 2011.
- [4] Xie Haiyan. Partial Sequence Cloning of LHR and expression of tissue and organ in Striped Hamster [D]. Qufu: Qufu Normal University, 2011.
- [5] Farrar JS, Wittwer CT. Extreme PCR: efficient and specific DNA amplification in 15—60 seconds[J]. Clin Chem, 2015, 61(1): 145—153
- [6] Son JH, Cho B, Hong S, et al. Ultrafast photonic PCR[J]. Light: Science & Applications, 2015, 4(7): e280
- [7] Castellanos-Rizaldos E, Richardson K, Lin R, et al. Singletube, highly parallel mutation enrichment in cancer gene panels by use of temperature- tolerant COLD- PCR[J]. Clin Chem, 2015, 61(1): 267—277
- [8] Wong DK-H, Tsoi O, Huang F-Y, et al. Application of coamplification at lower denaturation temperature-PCR sequencing for early detection of antiviral drug resistance mutations of hepatitis B virus[J]. Journal of Clinical Microbiology, 2014, 52(9): 3209—3215
- [9] Liu C, Lin J, Chen H, et al. Detection of hepatitis B virus genotypic resistance mutations by coamplification at lower denaturation temperature-PCR coupled with sanger sequencing[J]. J Clin Microbiol, 2014, 52(8): 2933—2939
- [10] Lewandowska MA, Jozwicki W, Jochymski C, et al. Application of PCR methods to evaluate EGFR, KRAS and BRAF mutations in a small number of tumor cells in cytological material from lung cancer patients[J]. Oncol Rep, 2013, 30(3): 1045—1052
- [11] Galbiati S, Brisci A, Lalatta F, et al. Full COLD- PCR protocol for noninvasive prenatal diagnosis of genetic diseases[J]. Clin Chem, 2011, 57(1): 136—138

- [12] Li H, Huang J, Lü J, et al. Nanoparticle PCR: nanogoldassisted PCR with enhanced specificity[J]. *Angew Chem Int Ed Engl*, 2005, 44(32): 5100—5103
- [13] Orpana AK, Ho TH, Alagund K, et al. Novel heat pulse extension- PCR- based method for detection of large CTG-repeat expansions in myotonic dystrophy type 1[J]. *J Mol Diagn*, 2013, 15(1): 110—115
- [14] Yan Wen, Yu Chong ge. New PCR technology [J]. *Journal of Lanzhou University (Medical Edition)*, 2017,43 (01): 60-65.