Amplification Techniques of Recombinase & Polymerase and their Application in Parasite Detection

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Abstract: To study the application principles of recombinase polymerase amplification (RPA) and the specific situations of detecting parasites, the principles of RPA are analyzed to find the optimal temperature conditions, advantages, and disadvantages. Then, the parasites are detected to observe the application characteristics of the RPA method. The results show that RPA is a kind of novel isothermal nucleic acid amplification technology, which is an open detection method. It has high sensitivity and specificity when being operated at 37-42°C, which makes it very suitable for early detection of pathogen infection. Besides, it also has high sensitivity and specificity in parasite detection. Therefore, the RPA technology has better performances and excellent applications in parasite detection, which has a certain significance for the future application of the technology in more fields.

1 Introduction

In vitro amplification of nucleic acids is one of the most commonly used techniques in molecular biology, genetics, medicine, and other fields of research. Of all the amplification techniques, the polymerase chain reaction (PCR) was invented by the PE-Cetus Corporation in the United States in 1985. PCR is similar to the in vivo replication of DNA, in which the in vitro synthesis and amplification of DNA is achieved through three steps, i.e., the denaturation, annealing, and extension. Later, the TwistDx Incorporation of the United Kingdom developed the amplification techniques of recombinase & polymerase, i.e., the recombinase polymerase amplification (RPA), which is an improvement of nucleic acid amplification technology. As the nucleic acid constant-temperature amplification technology does not require repeated thermal denaturation and no special instruments, it has a faster reaction speed, which is suitable for rapid on-site detection. Therefore, it has been widely used in various fields of life science [1].

Parasites can act as a pathogen and can be used as a medium to spread diseases. A parasite is any organism characterized by being in or attached to the hosts to obtain all the needed nutrients or shelter for maintaining its survival, development, or reproduction. A variety of small animals live in parasitic ways and attach to animals that are larger than them. Parasites can alter the behaviors of their hosts to survive and proliferate better [2]. As the ideal breeding habitat for parasites, the human body can provide the nutrients needed for their survival. Parasites will have certain effects on the human body and cause some diseases. Therefore, it is necessary to use detection methods to prevent the occurrence of some diseases.

Due to the various types of parasites, such as

hookworm, sarcoptic mite, ascarid, schistosome, tapeworm, pinworm, and toxoplasma gondii, there are different detection methods for different kinds of parasites. At present, parasite detection methods mainly include pathogen examination methods and immunological examination methods.

It can be seen that the analysis and application of amplification techniques of recombinase & polymerase in parasitic detection will have practical significances for future research of technology and disease.

2 Literature Review

The amplification techniques of recombinase & polymerase, as a technique for rapid amplification of trace nucleic acids in vitro, have excellent applications in many fields.

Qin Lide et al. (2017) analyzed the monitoring and cases, the primer and probe designs, and the influencing factors of amplification techniques of recombinase & polymerase, as well as the application of animal virus detection. The results showed that the amplification techniques of recombinase & polymerase had excellent application prospects for rapid on-site detections, as well as the detections for multiple and large samples [3]. Wang Qiuping et al. (2018) directly monitored the standard *Aspergillus fumigatus* strains by establishing the amplification techniques of recombinase & polymerase and explored the possibility of not requiring sample pretreatments. The results showed that the amplification techniques of recombinase & polymerase could amplify samples without pretreatments, which had the advantages of quickness and simplicity [4].

Chen Min et al. (2017) proposed through the comparative analysis that the AVE-562 automatic stool

analyzer and manual smear microscopy detection of parasites and artificial microscopy shared the high rate of coincidence. Besides, the degree of automation was also high, and the entire detection process did not require artificial exposure to specimens and with high biosafety [5]. Sanghyeob Lee et al. (2017) conducted CLM monitoring on sex pheromone traps in the main citrus farms in Jeju, South Korea, and investigated the parasites. The results showed that 6 species of parasites were found in 5 years, in which the parasitic rates of the two parasites were the highest, which also illustrated the effectiveness of the method [6].

In summary, previous studies have rarely applied the amplification techniques of recombinase & polymerase to parasite detection. Therefore, based on the analysis of the techniques, the application in parasite detection is researched to provide more ideas for the study of related technologies in parasite detection.

3 The Application in Parasite Detection

3.1 Amplification techniques of recombinase & polymerase

Amplification techniques of recombinase & polymerase, also known as recombinase polymerase amplification (RPA), are a kind of molecular diagnostic technique that

can be used as a molecular diagnostic method for the rapid detection of pathogens. RPA can be used for nucleic acid amplification at low-temperature and constant-temperature at 25-42°C for 10-20 min. The detection is fast, sensitive, specific, economical, and portable.

RPA achieves the index amplification of nucleic acid mainly through single-stranded binding protein (SSB) and strand-replacement DNA polymerase. The recombinase forms a protein nucleotide complex by binding with an oligonucleotide primer. Also, under the action of the recombinase, the catalytic primers are combined with the homologous target sequence. By scanning the template sequence in both directions, once the specific binding site is found, the recombinase can open the double-stranded DNA, and the primers undergo a strand exchange reaction with the homologous sequence. The DNA synthesis reaction is initiated and the specific target DNA fragment on the template, which is amplified under constant temperature conditions. The parental DNA strand replaced by the primer binds to the single-stranded binding protein to prevent further substitution from occurring. In such a reaction, a synthetic process is initiated by a pair of opposite primers, which can be rapidly completed in a short time, and the synthesized progeny DNA can still carry out the reaction rapidly, which is also the basis for the exponential amplification of RPA [7]. The amplification principles of RPA are shown in Figure 1:

Figure 1 The amplification principles of RPA

RPA has many advantages, such as constant temperature, simple operation, short time-consuming, lightweight, sensitive, and specific, which makes it widely used in disease diagnosis, food safety testing, genetically modified crop detection, and pathogen detection. RPA is most widely used in pathogen detection and has successfully detected a variety of bacteria, viruses, and parasites.

At present, research on RPA technology is focused on the design of primers and probes. The first is the primers. Primer screening for RPA is mainly divided into the selection of target regions, the design of candidate primers, the screening of candidate primers, and the screening of performance for re-screening.

The first is to select a template sequence with a relatively uniform nucleotide composition when selecting a target region. The contents of guanine (G) and thymine (C) are preferably between 40% and 60%, excluding long strings of polyfluorene or polypyrimidine. Besides, they do not contain forward/reverse repeats and palindromic sequences as possible, and the target region preferably avoids repetitive elements in the genome. The concentration of the primer required for the reaction also needs to be continuously optimized and screened according to different templates. Occasionally, the concentration of the primer can be adjusted slightly to improve its performance, which requires multiple tests to find the most suitable primer concentration.

It is followed by the design of the RPA probe. Most PCR probes are not well suited for RPA reactions since the probes used for PCR are mostly short probes and the recombination efficiency in the RPA reaction is low. Similarly, the RPA reaction probe concentration also needs to be continuously optimized and screened to ensure its efficient performance. In a reaction system, it is generally necessary to add a 120 nmol/L probe. Sometimes, the slight adjustment of the probe concentration will facilitate the reaction. The probe concentration can be optimized according to the specific application within a certain concentration range (from 50 to 150 nmol/L).

In the basic reaction system of RPA, Mg^{2+} and ddH_2O are also needed. The concentration of Mg^{2+} also affects the reaction. Generally, the concentration of Mg^{2+} is 14 nmol/L, which can be optimized between 12-20 nmol/L. It should be noted that during the reaction, once Mg^{2+} enters the reaction, the reaction begins, and then the calculation begins to expand, thereby the reaction time can be recorded [8]. To ensure rapid and efficient amplification of the template by RPA, the length of the template in the current reaction system should not exceed 500 bp. Due to the short-chain template, the replication can be completed more quickly during the constant-temperature reaction, and the ratio of the effective product/invalid product is high. Therefore, in the RPA reaction system, the length of the template is currently most suitable from 100 to 200 bp.

The sensitivity and specificity of RPA technology show better performance in the detection of transgenic crops. Many researchers have also suggested that RPA technology has high sensitivity and specificity in many tests, which also lays the foundation for the application in parasite detection.

3.2 The application in parasite detection

There are many methods for detecting parasites, which are mainly divided into two major categories, i.e., the pathogen examination methods and the immunological examination methods. The pathogen examination methods mainly include the smear method, the floating polymerization method, the precipitation method, the larval hatching method, the anal swab method, the blood smear staining method, the transparent adhesive tape method, and the biopsy (muscle compression method). The immunological examination methods mainly include the ring sputum sedimentation test, the ring egg sedimentation test, the dyeing test, and the intradermal test [9].

Human parasites refer to the parasites that use humans as the hosts. Human parasites can be divided into two major categories, i.e., the internal parasites and the external parasites. Most of the human parasites are protozoan, nematomorph, platyhelminth, annelid, and arthropod. Parasites are usually eaten in. Some larvae, such as cysticercosis, are also distributed in the lungs and skin, and even in the eyes and the brains. Parasites are harmful to the human body. They bring damages to the human body by plundering nutrition, causing inflammations, and blocking blood vessels. Most of the human parasites are protozoan, nematomorph, platyhelminth, annelid, and arthropod. In parasitology, protozoans are often referred to as protozoa, and nematomorph and platyhelminth are collectively referred to as helminths. Most of the important species of internal parasites are included in protozoa, nematodes, trematodes, and aphids.

Cryptosporidium sp. is the major parasite causing diarrhea in children, with a morbidity and mortality rate of 20%. At present, there are many kinds of detection methods. PCR has been considered as an important means for detecting *Cryptosporidium* sp., which determines the parasites by detecting the number of oocysts in the feces. However, during the application of PCR, due to the dependence of expensive experimental equipment and professional operators, PCR can only be carried out in the laboratory. Therefore, it is rarely used for the early diagnosis of diarrhea. Later, some experts used RPA technology to target the amplification of the 18S rRNA gene fragment of *Cryptosporidium* sp. and successfully detected the *Cryptosporidium* DNA in human fecal samples. If the number of fecal oocysts is $10^2/\text{mL}$, stable results can be obtained by using RPA, and the sensitivity is higher than that of PCR technology. By comparing with other pathogens that can cause diarrhea, RPA technology has also been proved to have excellent specificity.

Plasmodium sp. is a mosquito-borne sporozoite that is the causative agent of malaria. Parasitic human parasites mainly include *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium falciparum*, and *Plasmodium falciparum*. Malaria is also considered to be one of the parasitic diseases that seriously endanger human health. It is listed by the World Health Organization as the world's three major public health problems along with AIDS and tuberculosis. According to 2016 statistics, there are still 91 countries and regions with malaria popularity. Besides,

216 million new malaria cases emerge each year, and about 225,000 people die of malaria. The statistics of

China in the first half of 2011-2018 are shown in Figure 2:

Figure 2 Statistics on the number of malaria cases and deaths in China in 2018

The disease of malaria is cyclical. Early diagnosis and treatment are important means to control the spread of malaria. It usually occurs in areas with poor medical and economic conditions. The 18S rDNA gene fragment of malignant *Plasmodium* sp. is targeted by RPA technology, a unique probe is added at a constant temperature of 38°C, and the results are directly observed by using a simple chromatographic test paper. The detection of *Plasmodium* sp. can be completed without labeling, which shows high sensitivity and specificity. Also, 100 fg of malignant *Plasmodium* sp. genomic DNA is detected, in which 11 are negative *P. falciparum* samples, and 77 of the remaining *P. falciparum* samples are positive. Besides, at 25-42°C, by using DNA/RNA as a template and with a reaction time of 5-20 min, two primers can be detected by gel electrophoresis fluorescent probe laminar flow test paper, and high sensitivity and specificity can be obtained.

In summary, RPA technology can detect the parasites more accurately in the detection of various parasites, especially the detection of *Cryptosporidium* sp. and *Plasmodium* sp., which can improve the sensitivity and specificity of detection, thereby showing excellent performance.

4. Conclusion

In this study, the RPA technology is analyzed. Besides, its reaction principles, template, suitable temperature, reaction time, primers, enzymes, and detection methods are studied. Based on the analysis, the detection conditions of RPA technology in *Cryptosporidium* sp. and *Plasmodium* sp. are analyzed to obtain the optimum temperature and reaction time, thereby verifying the performance of RPA technology in parasite detection. As

a novel isothermal nucleic acid amplification technology, RPA provides a basis for the early detection of pathogens. The probe and primer concentrations are not further analyzed during the study, which will continue to be studied in subsequent researches. The application of amplification techniques of recombinase & polymerase in detecting parasites has a certain value for the improvement of primer and probe concentration, which also provides certain ideas for the future application in more fields.

Personal introduction

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