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*Review Article*

# Progress in Recombinant Polymerase Nucleic Acid Amplification Technology

**Hong Shen[1,2] and Mohamad Hesam Shahrajabian[3*]**

[1]College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou, Zhejiang, CHINA
[2]Biological Inspection Department, Zhejiang Institute for Food and Drug Control, Hangzhou, Zhejiang, CHINA
[3]Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, CHINA

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## ABSTRACT

**In vitro nucleic acid rapid technology is a technology that enables rapid amplification of trace nucleic acids in vitro. Since its inception, it has been widely used in molecular biology, medicine and legal identification, and it has been continuously improved to its function and adaptability. In vitro rapid amplification nucleic acid technology developed on the basis of the existing in vitro nucleic acid amplification principle. Polymerase spiral amplification is a new visual in vitro amplification technology that is continuously upgraded from existing LAMP technology. This article mainly introduces the recombinase polymerase amplification (RPA) technology, as well as recombinase-aid amplification (RAA) technology, the polymerase spiral reaction (PSR), and application prospects.**

**Keywords**: **Nucleic acid amplification, RPA, RAA, PSR, Rapid detection**

## 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. In 1985, the United States PE-Cetus company invented the epoch-making polymerase chain reaction (PCR). The reaction is carried out in three steps of denaturation, annealing and extension, and the in vitro synthesis and amplification of DNA is achieved by the participation of specific oligonucleotide primers complementary at both ends of the target sequence. Since the PCR reaction has the defects of temperature change, long time and expensive equipment, more and better nucleic acid in vitro amplification technology had emerged. Among them, by the British TwistDxInc. The company developed recombinase polymerase amplification (RPA) to improve nucleic acid amplification technology. Compared with the classical PCR technology, the main advantage of RPA technology is isothermal, and its optimum temperature is 37~42 ºC.

In addition, the second advantages is that the detection time is short, and the whole process can be completed within 10~20 minutes. It does not need to be denatured, which is not only greatly shortens the detection reaction time, but also eliminates the need for special temperature control equipment that is similar to the PC instrument, thereby realizing portable rapid nucleic acid detection; in addition, it has the advantages of sensitivity and diversification of reading results. Recombinase-aid amplification (RAA) is also a method for rapid amplification of nucleic acids at constant temperature. Unlike RPA, RAA amplification is obtained from bacteria or fungi. The recombinase can bind tightly to the primer DNA at a constant temperature of 37 ºC from a complex with recognition function. The entire reaction is simple and fast, because it does not require high temperature cycling, so it is especially suitable for use in non-laboratory testing sites with large number of samples.

Unlike RPA and RAA amplification, polymerase helic counter A spiral amplification method should be adopted, which is superior to the existing LAMP amplification technology, and can realize various visualization results presentation methods. This article intends to summarize the principles and applications of PPA, RAA and PSR technologies.

* Corresponding author: hesamshahrajabian@gmail.com

## RPA PRINCIPLE

## RPA AMPLIFICATION PRINCIPLE

The RPA technology consists of three key components, a recombinase, a single-stranded binding protein, and a strand-replacement DNA polymerase. In the RPA reaction, the recombinase first binds to the upstream and downstream primers to find homologous double-stranded DNA. Once localized, strand exchange occurs, and the single-stranded binding protein (SSB) binds to the parental strand to prevent interaction with the detached template stand (James and Macdonald 2015). Figure 1 shows recombinase polymerase amplification reaction mechanism.

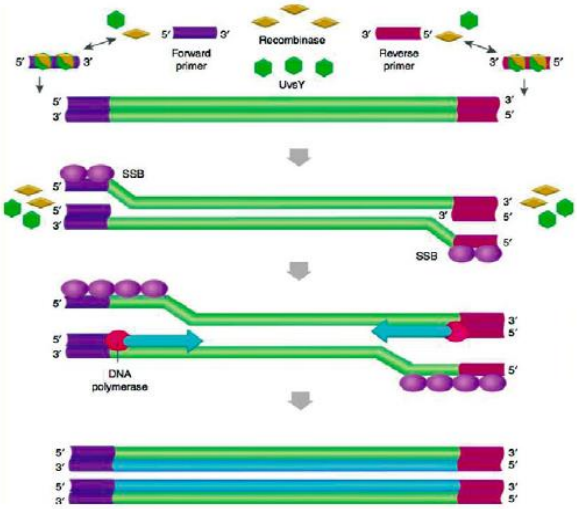


**Figure 1.** Recombinase polymerase amplification reaction mechanism [1] Next, the DNA polymerase initiates template synthesis from the 3' end of the upstream and downstream primers to form two double-stranded DNAs, and thus repeats the amplification to effect amplification. If RT-RPA is established by introducing reverse transcriptase, it is also suitable for rapid detection of RNA.

## TECHNICAL CHARACTERISTICS

Compared with other DNA detection technologies, RPA technology has the following characteristics:

(1) Amplification of a nucleic acid under constant temperature conditions. Compared with PCR technology, the biggest feature of RPA technology is that it does not need to denature and amplify template DNA under high temperature cycle, but only needs to open template chain with special enzyme at 37~42 ºC. Amplification of the nucleic acid is also completed, and other isothermal replacement amplification (SDA), rolling circle amplification (RCA), helicase-dependent isothermal amplification (HAD), nucleic acid sequence-dependent amplification (Compared with NAS-BA) and loop-mediated isothermal amplification (LAMP), SDA, RCA, HAD and LAMP need to be annealed at 65 ºC, and the length of the amplified target sequence of LAMP technology is preferably controlled at 300 bp. In the following, the requirements for primers are high and there are thousands of primers designed by the online primer design software. It takes a lot of work to screen our suitable primers; NASBA can only be used for detecting RNA. In addition, they all require long reaction times and special equipment.

(2) It takes a short time. One of the biggest advantages of RPA technology is that the detection time is short, and the whole reaction can be completed in 5~20 minutes, much faster than other isothermal amplification or PCR techniques.

(3) Simpler operation. The basic system of RPA amplification (Twist Amp® Basic) contains all the

reagents required for DNA amplification and can be reacted by simply adding primers and templates. By adding different enzymes and probes to this basic system, various RPA tests can be realized such as real-time fluorescence quantitative RPA and lateral flow test strips.

(4) Low cost. Compared with the traditional PCR, since the RPA technology does not require a special PCR instrument, it can be detected by a general thermostat device, which greatly reduces the detection cost.

(5) Not subjected to the restrictions of the testing site. RPA technology has low requirements for testing equipment low detection temperature and can even detect the sample using the temperature of the human armpit. It has high sensitivity and does not require complex pre-treatment of the sample, especially suitable for on-site detection of complex nucleic acid extraction. Other methods require nucleic acid extraction.

(6) High sensitivity and specificity. RPA has high sensitivity and is capable of amplifying limited amount of nucleic acid template (1-10 copies) to a detectable level; its specificity is not different from the real-time PCR detection limit. Table 1 shows a comparison of RPA with other nucleic acid amplification methods.

**Table 1.** Comparison of RPA with other nucleic acid isothermal amplification methods.

| Amplification method | Template | Number of primers | Reaction temperature/℃ | Reaction time/min | Denaturation step | Product detection[2] | Multiple channels |
|---|---|---|---|---|---|---|---|
| NASBA | DNA & RNA | 2 | 42 | 90-120 | √ | GE, RT | √ |
| LAMP | DNA[1] | 4-6 | 60-65 | 60-90 | × | GE, RT, TE | × |
| SDA | DNA[1] | 4 | 37 | 120 | √ | GE, RT | √ |
| RCA | DNA[1] | 1 | 37 | 60 | × | GE | × |
| HDA | DNA[1] | 2 | 65 | 75-90 | × | GE, RT | √ |
| TMA | DNA & RNA | 2 | 42 | 15-60 | × | GE, ECL | × |
| RPA | DNA[1] | 2 | 30-42 | 20 | × | GE, RT | √ |

Note 1: RNA can be amplified by introducing reverse transcriptase into the system.
Note 2: GE for gel electrophoresis, RT for real-time monitoring, TE for turbidity, and ECL for chemiluminescence.

Primer, probe design. In primer design, RPA is similar to PCR, but differs in primer length, sequence composition, and selection criteria. RPA primers are usually at least 30-35 bp in length, since single-stranded binding proteins require oligonucleotides that 30 to 35 bp in length to integrate into double-stranded DNA (Wang et al., 2016).

The length of the amplified product is usually within 500 bp, preferably 100-300 bp, which can ensure the sensitivity and reaction speed of the detection. In addition, the sequence composition of RPA primers has specific requirements: 3 to 5 nucleotides at the 5′ end should avoid multiple G; GC should be present at the 3′ end; avoid primers to form dimers and secondary structures; GC content and Tm value. The 3′ terminal mismatch number exceeds one base and hinders the progress of the RPA reaction. A mismatch between three bases at the ends and at the center of the primer will affect the detection efficiency of the RPA. The length of the probe in the RPA experiment is about 46-52 nucleotides, and the blocker is designed at the 3′ end, such as C3-space, phosphate, amine, biotin tetraethylene glycol (BTG), etc. A cleavage site is added inside the probe for exo, nfo, and fpg enzyme cleavage. Cleavage occurs only when the probe is bound to the template DNA. The cleavage site of the probe is at least 30 bases from the 5′ end and at least 15 bases from the 3′ end.

## DETECTION RPA PRODUCTS

There are various methods for detecting RPA products, including gel electrophoresis, real-time fluorescence quantitative determination and lateral flow test strip detection. The latter two methods are fast, sensitive, and highly specific, and are currently the most widely used methods.

### Real-time RPA (Piepenburg *et al.* 2006; Xia *et al.* 2014).

At present, real-time fluorescence quantitative determination methods have been widely used. For real-time detection, the TwistAmpexo probe can be applied. This probe carries a fluorophore and a fluorescence quencher, each bound to a thymine, separated by a tetrahydrofuran (THF) base. Low strength. The 3′ end of the probe is

blocked, blocking the amplification of this oligonucleotide sequence as a primer. When the probe binds to the target sequence, the THF base site connecting the fluorophore and the quenching group is recognized and digested by the endonuclease, the downstream quenching group is released, and the fluorescence intensity is enhanced. The free 3′-OH produced after digestion is used as a target of DNA polymerase and the probe is amplified, and the fluorescence intensity is also enhanced as it is amplified. The fluorescence signal was analyzed by fluorescence detector (Twista, Twist DX, Cambridge, UK), and the sensitivity was 1~10 copies. The specificity was no difference compared with real-time PCR, and the detection time was greatly shortened.

**Lateral flow test strip detection**

RPA-LFD technology is based on the principle of RPA amplification, using a biotin-labeled primer and a carboxyl-containing (FAM)-labeled probe to carry out an amplification reaction with a target nucleic acid, so that the final amplified product carries both FAM and biotin markers The LFD front end is coated with nano gold particles with FAM antibody, and the detection line is coated with biotin antibody. When the reaction solution enters the test strip, the amplification product with FAM and biotin will bind by antigen and antibody. A biotin antibody-nucleic acid-nanogold complex is formed on the detection line and developed. There is also a quality control line on the LFD, which is coated with immobilized antibodies and can be directly combined with FAM antibodies.

Particle bonding, color development on the quality control line to ensure the validity of the test strip (Rohrman and Richardskortum, 2012; Jaroenram and Owens, 2014).

As a fast, simple and visualized nucleic acid detection method, RPA-LFD has successfully detected pathogens such as bacteria, viruses and parasites. LFD technology greatly simplifies RPA detection and truly eliminates the dependence on detection instruments, which is very suitable for disease prevention or bedside diagnosis.

**Gel electrophoresis detection**

The RPA basic reaction system is the same as PCR except that it requires the addition of recombinase and a single-stranded binding protein. RPA amplification can be performed in any constant temperature equipment, and the appropriate temperature can be selected according to different amplification targets, and the target fragment can be obtained generally at 37 ºC for 20 min. In order to avoid the tailing phenomenon of the amplified product during gel electrophoresis detection, it is necessary to extract the amplified product by phenolchloroform-sioamyl alcohol (25:24:1) and perform agarose gel electrophoresis through EB or GelRed. Dyeing, the target band is detected under ultraviolet light. The detection method is simple in operation, and the LAMP method requires 6-8 primers to complete the amplification. The RPA technology only needs a pair of primers to complete the amplification, and the sensitivity is not lower than LAMP isothermal amplification technology, so it is more suitable for nucleic acid. Detection, at present, this method has been widely used in the detection of genetically modified rice and other fields.

## RPA DEVELOPMENT AND APPLICATION PROSPECTS

Searching for RPA keywords in Chin Knowledge Network, we found that after 1990, RPA technology began to receive attention, and the number of papers began to increase. After 21st century, there has been a surge (as shown in Figure 2), indicating that there has been more and more attention to RPA technology in recent years. Figure 2 shows annual growth trend of RPA keyword papers published.

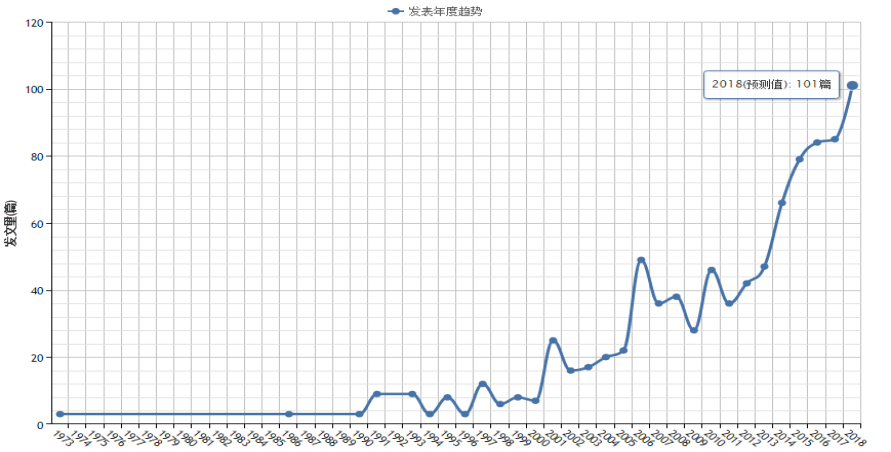


**Figure 2.** Annual growth trend of RPA keyword papers published.

## VIRUS DETECTION

In terms of virus detection, RPA is applied to detection of HIV infection in infants, and it is possible to detect low-copy HIV-1 subtype pre-DNA within 20 minutes without complication, and increase reverse transcription (RT) RPA. The reaction, which detects HIV-1 RNA, is sensitive, rapid and easy to operate. In the temperature range of 31~43 ºC, the detection effect of RPA-HIV technology can quaranteed and it is suitable for outdoor use in sub-Sahara Africa.

Yehia et al. (2015) designed a three-stream upstream primer, three downstream primers and an exo probe for the Egyptian avian influenza virus strain, 970 bp in the H5 gene HA2 fragment (13, 14). The H5RT-RPA method can detect a single RNA molecule in 7 minutes while the real-time RT-PCR method takes at least 90 minutes and the real-time RT-LAMP technique takes 30 to 60 minutes. While ensuring sensitivity and specificity, RPA reduces reaction time compared to other assays. Conserved region fragment of VP2 gene of canine parvovirus type 2 (CPV-2) (about 210 bp).

The RPA detection method was developed and the reaction can be carried out in an isothermal water bath, which can be used as a potential alternative to canine parvovirus detection in resource-limited environments. RT-RPA method was established for the serotypes of serotypes of DENV 3′UTR, primers and probes were designed. Compared with RT-LAMP and RT-q PCR, RT-RPA showed the fastest. The molecular diagnostic method has also become a supplement to the laboratory routine serological testing methods.

Xtract (SE) during the outbreak of Ebola in Guinea, and the detection of N gene using RT-RPA method. Sexuality and sensitivity are good. 15 copies of RNA in plasma samples can be detected in 8 minutes. In the test clinical samples, the recombination rate with the RT-qPCR method is 100%. The real-time quantitative RT-RPA detection method of foot-and-mouth disease virus is simpler than the RT-LAMP method. Compared with the RT-PCR method, the reaction time is saved, and it is suitable for border animal monitoring and screening of infected animals in the foot-and-mouth disease outbreak area. At present, specific primers and probes have been designed for the Middle East Respiratory Syndrome Coronavirus, Bovine Coronavirus, Vaccinia Virus, Ebola Virus, Sudan Virus, Marburg Virus, Sigma Virus, Sheep Aphthous Virus, etc., and the RPA method has been established. It has been confirmed to have good specificity and sensitivity.

## BACTERIAL TESTING

In terms of bacterial detection, the earliest report on RPA was the detection of drug-resistant Staphylococcus aureus. Lutz (2010) and others developed a cassette based on platinum microfluidics technology with a detection

limit of 20 copies of DNA, and the results were obtained in 15 minutes. Ren (2016) designed four upstream primers and four downstream primers for Brucella omp31 gene fragment, combined and detected the reaction time and fluorescence intensity, and selected the optimal primer combination. A rapid, multiplexed detection technique for Neisseria gonorrhoeae, Salmonella, and methicillin-resistant Staphylococcus aureus (MRSA) was developed by combining RPA technology with solid-phase chip technology. The RPA detection method developed for neonatal group B streptococci, Mycobacterium tuberculosis complex (MTC), Yersinia pestis, Bacillus anthracis, and T. facilis has significantly shortened the detection time and has a promising clinical molecular diagnostic application prospect. In the field of food safety testing, especially in the detection of contaminated bacteria milk, RPA enzyme and buffer are mixed, at 37 °C, without extracting DNA, can detect Salmonella, E. coli O157:H7, parahemolytic in milk samples Vibrio, the total time taken for the entire test to read is only 30 minutes.

## PARASITE DETECTION

In terms of parasite detection, Crannell et al. (2016) used RPA to detect Giardia in fecal samples and read the results using lateral flow chromatography strips. The lowest detectable number of 103 to 103.5 oocysts per ml of stool samples. Sensitivity is 73% of the PCR method, and the specificity is 95% of the microscopy method. The method is expected to be used for "point-of-care" (POC, bedside detection, on-site inspection). Crannell et al. (2014) further developed a multi-lateral flow chromatography strip based on RPA technology. By constructing different labeled probes, it is possible to simultaneously detect and identify protozoa causing diarrhea: Giardia, Cryptosporidium and Amoeba DNA. The live parasite DNA was extracted from the stool sample, and the detection limit was 444, 6, and 9 parasites per reaction, respectively. At present, urinary tract schistosomiasis, amoeba amoeba, Plasmodium falciparum lateral flow chromatography test strips have been developed, which have been confirmed to have good specificity and sensitivity, and are convenient to use.

## OTHER PATHOGENS

Fluorescence quantitative RPA method and RPA lateral flow chromatography test strip were developed for Mycoplasma gondii, tsutsugamushi rickettsia, Chlamydia trachomatis (O.tsutsugamushi or R. typhi), and the detection sensitivity was comparable to that of real-time PCR. Ahmed et al. (2014) developed a real-time quantitative RPA method for the detection of Leptospira in blood clinical samples and is expected to detect pathogens present in environments such as reservoirs. Figure 3 shows schematic diagram of recombinase-mediated amplification.

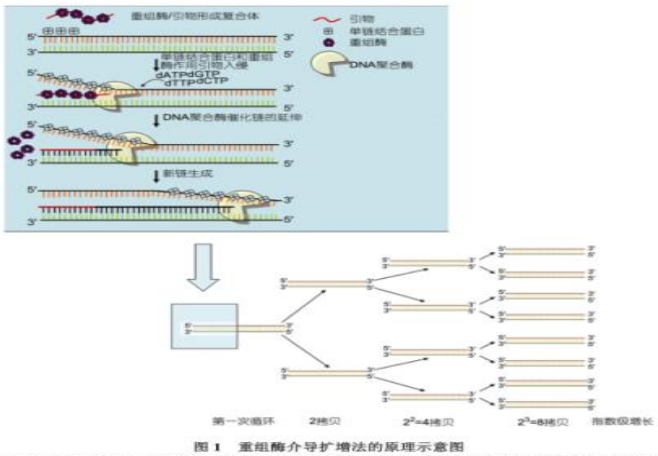


图 1　重组酶介导扩增法的原理示意图

RAA 法中主要使用了 3 种酶：重组酶、单链 DNA 结合蛋白和 DNA 聚合酶。首先，重组酶与引物结合形成复合体，在单链 DNA 结合蛋白的辅助下，模板 DNA 解链并使引物与模板配对，然后在 DNA 聚合酶的作用下，生成新的 DNA 链。经过几十个循环后，DNA 新链的数量以指数级增长。RAA 法用酶的作用使双链 DNA 解链，因此反应条件较温和，不需要高温使模板 DNA 变性，RAA 反应的时间也较短，在 15~30 min 就可以得到能检测到的扩增片段

**Figure 3.** Schematic diagram of recombinase-mediated amplification.

Three enzymes were used in RAA: recombinase, single-stranded DNA binding protein and DNA polymerase. First, recombinant enzymes combine with primers to form complexes. With the assistance of single-stranded DNA-binding proteins, template DNA is unlinked and paired with primers. Then, new DNA chains are generated by DNA polymerase. After dozens of cycles, the number of new DNA chains increases exponentially. RAA method uses enzymes to unchain double-stranded DNA, so the reaction conditions are mild, the template DNA does not need to be denatured by high temperature, and the reaction time of RAA is shorter. The amplified fragments can be detected in 15-30 minutes.

## INTRODUCING to RAA TECHNOLOGY

## RAA TECHNOLOGY PRINCIPLE

Recombinase proteins play an important role in bacterial or fungal DNA replication. Recombinase proteins bind to single-stranded DNA and prevent the degradation of single-stranded DNA by nucleases in the nucleus. Recombinases are also responsible for basic base pairing during DNA recombination. Of course, in the body, there will be other helper proteins, such as recB, recC and single-stranded DNA binding proteins, which are involved in the recombination of DNA. Therefore, when performing nucleic acid in vitro amplification, the recombinant enzyme protein is undoubtedly a reaction catalyst that plays a decisive role. In order to verify the role of recombinase, a targeted RAA amplification system was designed. In addition to SC-recA, BS-recA and Rad51 recombinase, DNA polymerase and accessory protein Bsu were added, and the amplified target fragment was long. 310 bp, which is part of the coding region of the rnd gene previously cloned into the vector. The experimental results show that in the current reaction system, the reaction system containing SC-recA and BS-recA recombinase can correctly amplify the target fragment (Figure 3), indicating that both SC-recA and BS-recA recombinase have catalysis. Function, and the specificity of the reaction is very high (Kuzminov 2001).

Recombinase-mediated amplification reaction establishment and optimization. In the RAA reaction, the enzyme is undoubtedly the most important component. The purity of the enzyme directly determines whether the reaction proceeds smoothly, because the reaction is only carried out at 37 °C, and any unnecessary impurities will affect the reaction results, especially the nuclease. The contamination, while nucleases can be almost ignored in traditional high temperature PCR. Therefore, in the purification of the enzyme, the removal of the nuclease is particularly emphasized, and the activity of the enzyme is maintained. Most impurities can be removed by Ni-NTA affinity chromatography (Ni-NTAagarose) to obtain a high purity enzyme. The quantification of the enzyme in each reaction is also important. In addition to the recombinase, there are 3 to 4 enzymes involved in the amplification reaction, which are required between them. A reasonable ratio can be used to obtain the expected amplification results. In practical applications, it has been found that for different templates and primers, the reaction system needs to be optimized in a targeted manner, and not a system is suitable for all reactions. Once the reaction system is adjusted, it can basically achieve 100% repeatability. This is also a feature of normal temperature nucleic acid amplification, and the optimized system has better sensitivity and stability, and does not produce different results due to environmental or operator changes. In addition, the research team has recently created an optimized RAA system that can perform amplification of large-sized DNA similar to roll-over replication (results not published). RAA normal temperature nucleic acid amplification method is not critical to the template, it can be plasmid DNA, or it can be a bacterial solution containing plasmids. Animal and plant DNA can be used as a template, and even RNA can be used directly as a template without additional reverse transcription process. However, in order to improve the accuracy of amplified fragments, RAA has stricter requirements on primers. Usually, a pair of primers needs to be composed of 30 to 34 nucleotides, and the sequence of the primer is strictly complementary to the amplified fragment, so that the desired amplified band can be obtained. The pair of primers used in this experiment were 34 nt and 32 nt, respectively. It can be seen that the product amplified by the pair of primers is very specific. The RAA method is characterized by the strict complementary of the primer and the template, so that it can be used for the detection of SNP and methylation. As long as there is a difference on the template DNA paired with the primer, it can be detected by the RAA method.

Buffers are also a critical part of the optimization of the RAA reaction system. Since the reaction catalyzed by the recombinase which is an energy-consuming reaction that requires the participation of ATP, ATP and creatinekinase which promotes ATP regeneration and essential components in the buffer. At the same time, polyethylene glycol (PEG 20000), besides dithiothreitol (DTT) which stabilize and enhance the activity of the enzyme are added. The entire reaction was carried out in a weak base environment with a pH of 8.0 in Tris buffer. The optimized reaction system has been able to stably amplify a target fragment of 310 bp in a stable manner under simple reaction conditions (1h at 37 °C), and has high specificity.

## PROSPECTS FOR THE DEVELOPMENT of RAA TECHNOLOGY

Because it is not limited by the instrument, the RAA method can be used for rapid detection of a large number of samples. If the sample needs to be timed, it is also possible to add a probe to the RAA reaction. The amplified fragment can be detected at 5 to 30 minutes with the addition of another protein. If the ambient temperature is lower than room temperature (25 °C), only one additional one is needed.

The characteristics of the RAA method make it competitive in virus detection. Currently, traditional PCR methods are commonly used to detect hepatitis B virus (HBV), human papillomavirus (HPV) and HIV (HIV) (Steketee et al. 1997). The primers obtained by the RAA method have high specificity, and the operation is simple and time-saving. In the future, the traditional PCR can be used to detect the virus, and even multiple pairs of primers can be used in one reaction, and different viruses can be detected and identified at the same time.

The advantages of the RAA amplification method are obvious relative to conventional PCR and other thermostatic nucleic acid amplification methods such as the LAMP method. Due to the simple reaction of the RAA method, it is also possible to produce a portable detection device similar to a blood glucose meter, and to regularly and quantitatively detect a specific disease, which will largely change the existing medical detection procedures, which can not only enable the majority of medical personnel to Freed from heavy labor, you can get inspection results anytime, anywhere. Since the development of nucleic acid amplification technology, it has been inseparable from expensive instruments and professional training. Therefore, the emergence of the RAA method has revolutionary significance. At present, the RAA method is still in its beginning, but for a new technology that has just emerged, it is a good result to be able to rapidly amplify the target fragment at a lower temperature in vitro (optimal temperature 37 °C). It is expected to develop a home molecular diagnostic kit. In addition, RAA technology is currently the only backup technology that may be developed for in vivo nucleic acid amplification technology. It is believed that with the continuous optimization of the reaction system and reaction conditions, the RAA method can take advantage of its advantages and play a wide range of roles in medicine, agriculture and other fields, especially in testing (medical testing, animal and plant quarantine and field microbial testing).

## INTRODUCTION of PSR TECHNOLOGY

## PRINCIPLES of PSR TECHNOLOGY

Techniques such as HDA require DNA single-stranded binding proteins to maintain the stability of the DNA single strand (Vincent et al. 2004). There is a large amount of betaine in the polymerase helix reaction system. When the reaction temperature is reached, betaine will make the DNA in a single double strand. The dynamic balance reduces the type of enzyme and relatively improves the stability of the reaction.

Take a single strand on the target sequence in Figure 4 as an example (the other single-strand amplification mechanism is the same), as shown in Figure 5, Ft F.

The segment specifically recognizes and binds to the Fc segment of the target sequence (James and Macdonald, 2015), and extends to the 3' end gap under the action of Bst DNA polymerase to form a DNA double-stranded structure in 2, the double strand in betaine under the action, it is once again melted into a free DNA single strand, and the B segment of the other main primer Bt is combined with one of the single-stranded

Bc segments (the other single strand is no longer considered for the subsequent reaction) and extends to the 3' end gap (Hatch et al., 1999). The generated single chain will be disconnected again, and at this time, the Nc segment and the N segment of the single chain are inversely complementary, and the Nc segment is rotated and combined with the N segment according to the principle of base complementary pairing. The first hook-shaped structure is formed, which is the initial step of the polymerase helix reaction, and the 3'-end gap formed by the Bst DNA polymerase is filled with bases and continues to extend toward the 3' end.

The structure of Figure 6 extends to the 3' end to form the U-shaped structure of Figure 7, and the B segment of the primer Bt is then bonded to the Bc segment of the structure, extending around the U-shaped structure toward the 3' end. After expansion, a DNA double strand of 7 is generated. Similarly, the DNA double strand is melted into a single strand in the presence of betaine, and the Nc stretch of the single strand is complementary to the N segment, forming a hook structure again, and continuing to the 3' end. After the gap is extended, the cycle of primer binding, extension, melting, single-strand rotation and extension is repeated repeatedly, and finally a series of complex structures with different molecular weights are formed to achieve the purpose of nucleic acid amplification under isothermal conditions. We named this isothermal strip nucleic acid amplification method as "polymerase spiral reaction", the English name is "Polymerase Spiral Reaction", and the English abbreviation takes the initial letter "PSR".

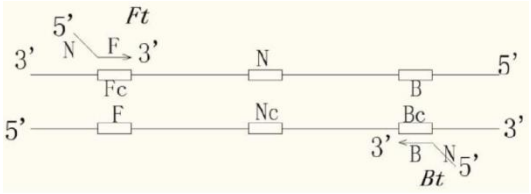


**Figure 4.** Primer composition of the polymerase helix reaction and its binding site on the target sequence.

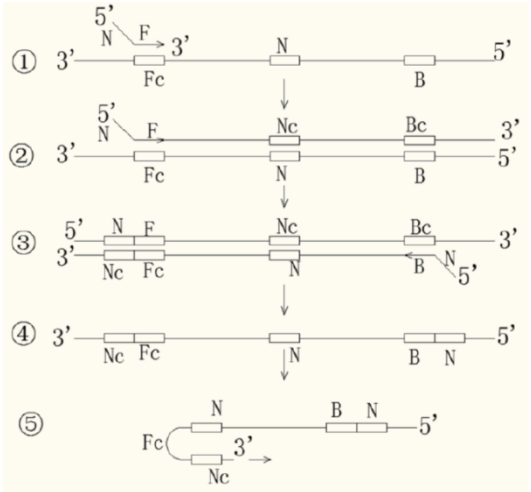


**Figure 5.** Schematic diagram of the initial process of polymerase helix reaction (top).

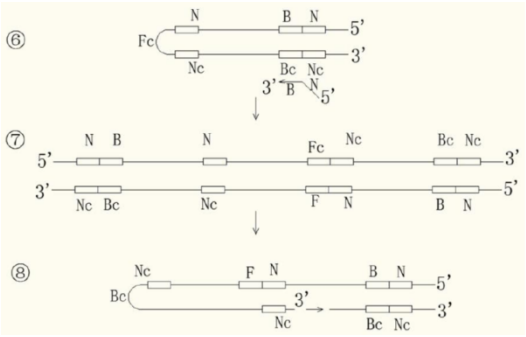


**Figure 6.** Schematic diagram of the initial process of polymerase helix reaction (below).

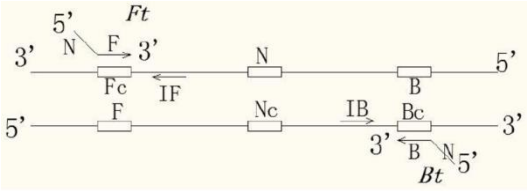


**Figure 7.** Addition of accelerated primer schematic.

## OPTIMIZATION OF PSR RECTION

In order to meet the requirements of fast and sensitive on-site detection, we introduced the concept of accelerating primers, including IF and IB, between F and N, B and N, respectively, from the 5' end to the 3' end with Ft. In contrast to Bt, the positions of IF and IB on the target sequence are as follows.

The reaction system of PSR includes potassium chloride, magnesium sulfate, betaine, etc., and their substance concentrations are mostly referred to the system in Bst DNA polymerase reaction buffer provided by NEB, but the buffer system does not include Betaine. Betaine is an important substance for decomposing DNA double-strands at a constant temperature, and its concentration must be optimized.

## DEVELOPMENT OF PSR TECHNOLOGY

The results of PSR reaction are generally calculated by real-time monitoring of turbidity using a real-time turbidimeter. The peak time (Ct value) is definitely obtained, but the price of the real-time turbidimeter is higher, which invisibly increases the research and popularization threshold of PSR. Agarose gel electrophoresis is undoubtedly a method of interpretation, but it still needs glue. Complex operations such as electrophoresis, contrary to the fast and convenient concept of isothermal amplification, and the lack of these complex instruments in the field detection environment are unrealistic. Therefore, after several years of efforts, based on the PSR reaction system. Add color indicator (Calcein, hydroxynaphthol blue and SYBR Green I, etc.), and quickly judge the experimental results by the color difference of the positive reaction. The polymerase spiral reaction detection platform has been applied to the clinical detection of bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*) and fungi (*Candida albicans*), and to establish a virus (hand, foot and mouth disease) and a strong infectious disease (*Vibrio cholerae*). The polymerase spiral reaction detection method. However, its technology is still far from mature, and it is hoped that with the continuous optimization in the later period, there will be a wider range of applications.

## CONCLUSIONS

The innovation of nucleic acid amplification technology is more and more convenient and accurate, especially

the development of RPA, RAA and PSR technologies, and has significant advantages in clinical detection and rapid on-site diagnosis. Although the detection cost of RPA, RAA and PSR technologies is higher than other nucleic acid amplification technologies such as PCR, RPA technology, RAA technology or PSR technology is expected to become a routine rapid diagnosis with the further development of technology, improvement and progress of production technology.

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