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Polymerase Chain Reaction

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The present review reports Polymerase chain reaction (PCR) microbiological techniques currently in use, and explore its applications to different aspects of medical field. PCR has rapidly become one of the most widely used technique in molecular biology which is rapid, inexpensive and simple means of producing relatively large number of copies of DNA molecules from minute quantities of source DNA material even when source DNA is of relatively poor quality. PCR involves preparation of the sample, the reaction mixture and primers, followed by detection and analysis of the reaction products.

Key Words: Polymerase chain reaction, Primers, DNA, Taq Polymerase.

INTRODUCTION

Molecular microbiology has become an essential component in many fields of modern research[1,2]. Research into molecular mechanisms underlying many disease processes offer increased understanding of the pathogenesis of the disease and provide exciting therapeutic possibilities. Polymerase chain reaction (PCR) is a technique that is used to amplify a number of copies of a specific region of DNA, to produce enough DNA to be used to identify a very high probability disease causing viruses or bacteria, a deceased person or a criminal suspect. Polymerase chain reaction as currently practiced, requires several basic components. These components are:

- DNA template that contains the region of the DNA fragment to be amplified
- Two primers, which determine the beginning and end of the region to be amplified.
- Taq polymerase, which copies the region to be amplified.
- Nucleotides, from which the DNA polymerase builds the new DNA.
- Buffer, which provides a suitable chemical environment for the DNA polymerase

The polymerase chain reaction is carried out in a thermal cycler. This is a machine that heats and cools the reaction tubes with in the precise temperature required for each step of the reaction. To prevent the evaporation of the reaction mixture, a heated lid is placed on the top of the reaction tubes or a layer of oil is put on the surface of the reaction mixture.

Primers

The DNA fragment to be amplified is determined by selecting primers. Primers are short, artificial DNA strands not more than fifty (usually18-25 base pair) nucleotides that are complementary to the beginning and end of the DNA fragment to be amplified. They anneal (adhere) to the DNA template at these starting and ending points, where the DNA-Polymerase binds and begins the synthesis of the new DNA strand.

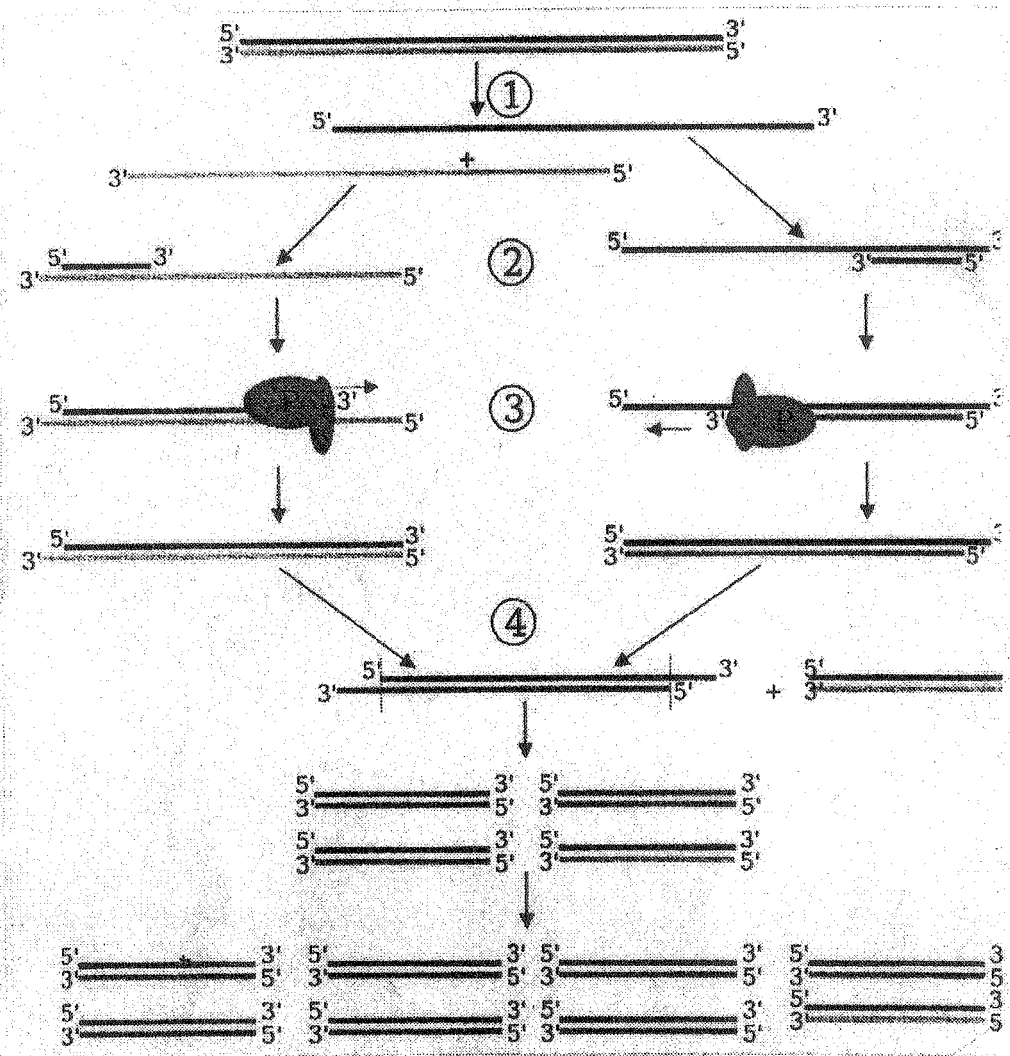

Figure 1: Schematic drawing of the PCR cycle. (1) Denaturing at 94-96°C. (2) Annealing at (eg) 68°C. (3) Extension at 72° (P=Polymerase). (4) The first cycle is complete. The two resulting DNA strands make up the template DNA for the next cycl thus doubling the amount of DNA duplicated for each new cycle.

Procedure

The Polymerase Chain Reaction process usually consists of a series of twenty to thirty five cycles. Each cycle consists of three steps (Fig. 1). The double-stranded DNA has to be heated to 94-96°C in order to separate the strands. This step is called denaturing; it breaks apart the hydrogen bonds that connect the two DNA strands. Prior to the first cycle, the DNA is often denatured for the extended time to ensure that

the template DNA and the primers have completely separated and are now single-strand only, timing 1-2 min up to 5 min; Taq-polymerase is also activated by this step.

After separating the DNA strands, the temperature are lowered so the primers can attach themselves to the single DNA strands. This step is called annealing. The temperature of this stage depends on the primers and is usually 5°C below their melting temperature (45-60°C). A wrong temperature during the annealing step can result in primer not binding to the template DNA at all, or binding at random; timing 1-2 min.

Finally, the DNA-Polymerase has to copy the DNA strands. It starts at the annealed primer and works its way along the DNA strand. This step is called extension. The extension temperature depends on the DNA-Polymerase. The time for this step depends both on the DNA-polmerase itself and on the length of the DNA fragment to be amplified. As a rule-of-thumb, 1 min per 1 kbp (kilo base pair). A final extension step is frequently used after the last cycle to ensure that any remaining single stranded DNA is completely copied, typically 10-15 min.

PCR optimization

Since PCR is very sensitive, adequate measures to avoid contamination from other DNA present in lab environment (bacteria, viruses, own DNA *etc.*) should be taken. Thus DNA sample process, in addition to the subsequent reaction product analysis, should be performed in separate areas. For the preparation of reaction mixture, a laminar flow cabinet with UV lamp is recommended. Fresh gloves should be used for each PCR step as well as displacements pipettes with aerosol filters. The reagents for the PCR should be prepared separately and used solely for this purpose. Aliquots should be stored separately from other DNA samples. A control reaction (inner control), omitting template DNA, should always be performed, to confirm the absence of contamination or primer multimer formation.

Difficulties with polymerase chain reaction

Polymerase chain reaction is not prefect, errors and mistakes can occur. These are some common errors and problems that may occur.

Polymerase errors

Taq polymerase lacks a 3' to 5' exonuclease activity. This makes it impossible for it to check the base it has inserted and remove if it is incorrect, process common in higher organisms.

Size limitations

PCR works readily with DNA of lengths two to three thousand base pairs, but above this length the polymerase tends to fall off and the typical heating cycle does not leave enough time for polymerisation to complete. It is often necessary to "restock" the reaction with polymerase part way through due to the limited half life of the polymerase.

Non specific priming

The non specific binding of primers is always a possibility due to sequence duplications, non-specific binding and partial primer binding, leaving the 5' end unatatched. Manipulation of annealing temperature and magnesium ion (which stabilize DNA and RNA interactions) concentrations can increase specificity. Non-specific priming can be prevented during the low temperatures of reaction preparation by use of hot start polymerase enzymes where the active site is blocked by an antibody or chemical that only dislodges once the reaction is heated to 95°C during the denaturation step of the first cycle.

Recent developments in pcr techniques and conclusions

- A more recent method which excludes a temperature cycle, but uses enzymes, is helicase-dependent amplification.
- TAIL-PCR, development is the thermal asymmetric interlaced PCR.
- Meta-PCR, developed by Adrew Wallace, allows to optimize amplification and direct sequence analysis of complex genes.

PCR can be used for a broad variety of experiments and analyses. Some examples are discussed below.

Genetic fingerprinting is a forensic technique used to identify a person by comparing his or her DNA with a given sample, such as blood from a crime scene can be genetically compared to blood from a suspect. The sample may contain only a tiny amount of DNA, obtained from a source such as blood, semen, saliva, hair, or other organic material. Theoretically, just a single strand is needed. First, one breaks the DNA sample into fragments, then amplifies them using PCR. The amplified fragments are then separated using gel electrophoresis. The overall layout of the fragments is called a DNA fingerprint. Since there is a very small possibility that two individuals may have the same sequences, the technique is more effective at acquitting a suspect than proving the suspect guilty. This small possibility was exploited by defence lawyers in the controversial O.J. Simpson case. A match however usually remains a very strong indicator also in the question of guilt.

Although these resulting 'fingerprints' are unique (except for identical twins), genetic relationships, for example, parent-child or siblings, can be determined from two or more genetic fingerprints, which can be used for paternity tests. A variation of this technique can also be used to determine evolutionary relationships between organisms.

The detection of hereditarty diseases in a given genome is a long and difficult process, which can be shortened significantly by using PCR. Each gene in question can easily be amplified through PCR by using the appropriate primers and then sequenced to detect mutations. Viral diseases, too, can be detected using PCR through amplification of the viral DNA. This analysis is possible right after infection, which can be from several days to several months before actual symptoms occur. Such early diagnosis give physicians a significant lead in treatment.

Cloning a gene, not to be confused with cloning a whole organism, describes the process of isolating a gene from one organism and then inserting it into another organism (now termed a genetically modified organism (GMO). PCR is often used to amplify the gene, which can then be inserted into a vector (a *vector* is a piece of DNA which carries the gene into the (GMO) such as a plasmid (a circular DNA molecule). The DNA can then be transferred into an organism (the GMO), where the gene and its product can be studied more closely.

Mutagenesis is a way of making changes to the sequence of nucleotides in the DNA. There are situations in which one is interested in mutated (changed) copies of a given DNA strand, for example, when trying to assess the function of a gene or in *in vitro* protein evolution.

Using PCR, it becomes possible to analyze DNA that is thousands of years old. PCR techniques have been successfully used on animals, such as a forty-thousand-year old mammoth, and also on human DNA, in applications ranging from the analysis of Egyptian mummies to the identification of Russian Tsar.

REFERENCES

1. K.B. Mullis, *Scientific American,* **56-65,** 262 (1990).
2. S. Paabo, R.G, Higuchi and A.C. Wilson, *J. Biol. Chem.,* **264,** 9709 (1989).