Polymerase Chain Reaction (PCR)

Paul C Winter, *Belfast City Hospital, Belfast, UK*

The polymerase chain reaction is a technique that allows DNA molecules of interest (usually gene sequences) to be copied in a simple enzyme reaction producing a sufficient quantity of the copied DNA for detailed analysis or manipulation. The method is a basic tool in molecular biology with widespread applications in biological and medical research.

Introductory article

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Principle of the PCR

The polymerase chain reaction (PCR) is a powerful and widely used technique that has greatly advanced our ability to analyze genes. Genomic deoxyribonucleic acid (DNA) present in cells contains many thousands of genes. This makes it difficult to isolate and analyze any individual gene. PCR allows specific DNA sequences, usually corresponding to genes or parts of genes, to be copied from genomic DNA in a simple enzyme reaction. The only requirement is that some of the DNA sequence at either end of the region to be copied is known. DNA corresponding to the sequence of interest is copied or amplified by PCR more than one million fold and becomes the predominant DNA molecule in the reaction. Sufficient DNA is obtained for detailed analysis or manipulation of the amplified gene.

Components of the PCR

DNA is amplified by PCR in an enzyme reaction that undergoes multiple incubations at three different temperatures. Each PCR has four key components.

Template DNA

This contains the DNA sequence to be amplified. The template DNA is usually a complex mixture of many different sequences, as is found in genomic DNA, but any DNA molecule that contains the target sequence can be used. Ribonucleic acid (RNA) can also be used for PCR by first making a DNA copy using the enzyme reverse transcriptase.

Oligonucleotide primers

Each PCR requires a pair of oligonucleotide primers. These are short single-stranded DNA molecules (typically 20 bases) obtained by chemical synthesis. Primer sequences are chosen so that they bind by complementary base pairing to opposite DNA strands on either side of the sequence to be amplified.

DNA polymerase

A number of DNA polymerases are used for PCR. All are thermostable and can withstand the high temperatures (up to 100°C) required. The most commonly used enzyme is *Taq* DNA polymerase from *Thermus aquaticus*, a bacterium present in hot springs. The role of the DNA polymerase in PCR is to copy DNA molecules. The enzyme binds to single-stranded DNA and synthesizes a new strand complementary to the original strand. DNA polymerases require a short region of double-stranded DNA to get started. In PCR, this is provided by the oligonucleotide primers, which create short double-stranded regions by binding on either side of the DNA sequence to be amplified. In this way the primers direct the DNA polymerase to copy only the target DNA sequence.

Deoxynucleotide triphosphates

These molecules correspond to the four bases present in DNA (adenine, guanine, thymine and cytosine) and are substrates for the DNA polymerase. Each PCR requires four deoxynucleotide triphosphates (dNTPs) (dATP, dGTP, dTTP, dCTP), which are used by the DNA polymerase as building blocks to synthesize new DNA.

How the PCR Works

PCR allows the amplification of target DNA sequences through repeated cycles of DNA synthesis (**Figure 1**). Each molecule of target DNA synthesized acts as a template for the synthesis of new target molecules in the next cycle. As a result, the amount of target DNA increases with each cycle until it becomes the dominant DNA molecule in the reaction. During the early cycles, DNA synthesis increases exponentially but in later cycles, as the amount of

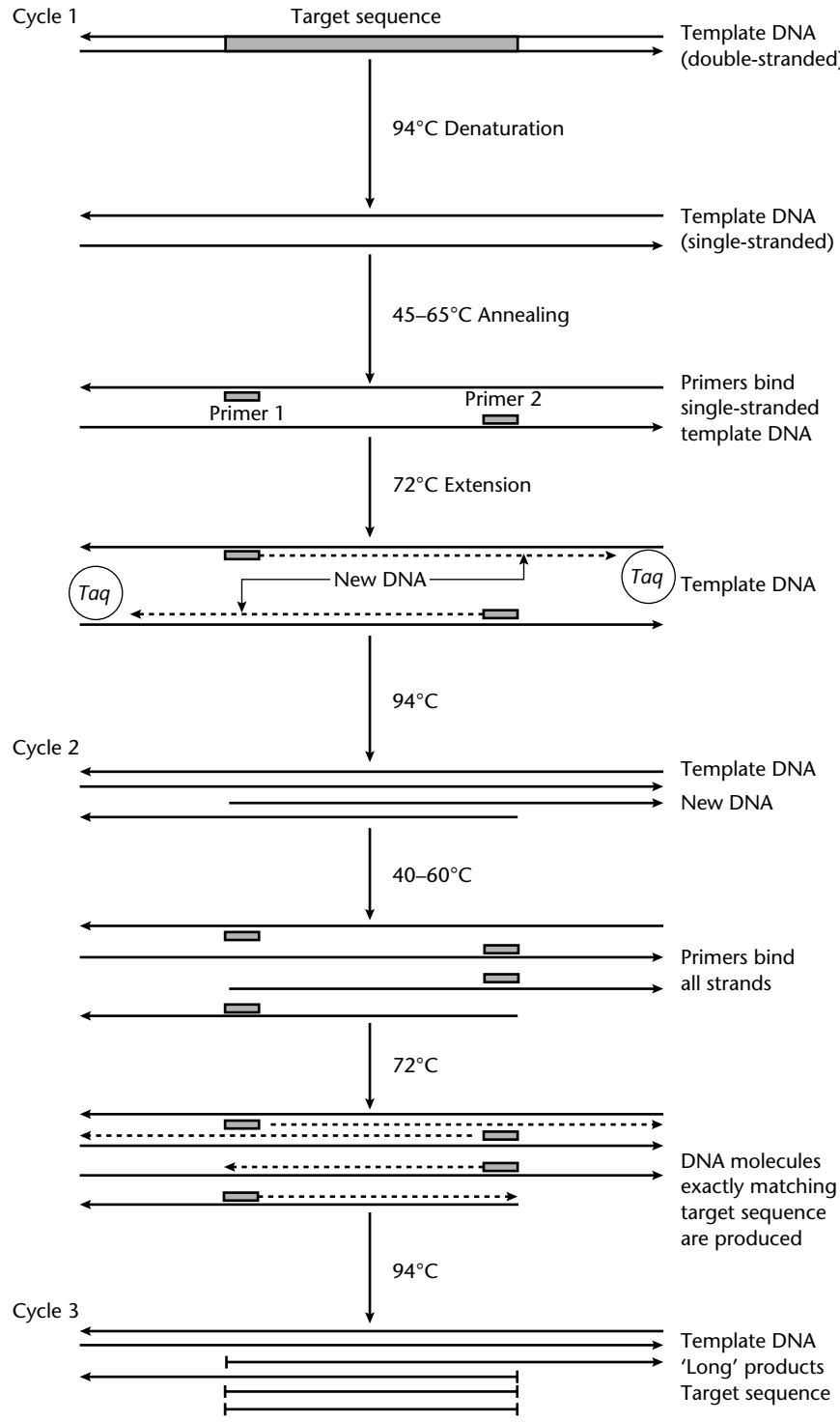

Figure 1 Polymerase chain reaction involves repeated cycles of incubation at three temperatures. The reaction is initially heated to above 90°C. At this temperature, the double-stranded DNA template is denatured and becomes single-stranded. The temperature is next reduced to 45–65°C. At this temperature, the oligonucleotide primers bind to their target sequences on the single-stranded template. The temperature is then raised to 72°C and the *Taq* DNA polymerase begins to synthesize a new DNA strand complementary to the template strand beginning at the primers. Further cycles of incubation result in the synthesis of increasing amounts of the target sequence.

target DNA to be copied increases and the reaction components are used up, the increase becomes linear and then reaches a plateau.

Each cycle of DNA synthesis involves three stages (denaturation, primer annealing, elongation), which take place at different temperatures and together result in the synthesis of target DNA.

Denaturation

The reaction is heated to greater than 90°C. At this temperature the double helix is destabilized and the DNA molecules separate into single strands capable of being copied by the DNA polymerase.

Primer annealing

The reaction is cooled to a temperature that allows binding of the primers to the single-stranded DNA without permitting the double helix to reform between the template strands. This process is called annealing. The temperature used varies (typically 45–65°C) and is determined by the sequence and the number of bases in the primers.

Extension

This stage is carried out at the temperature where the DNA polymerase is most active. For *Taq*, this is 72°C. The DNA polymerase, directed by the position of the primers, copies the intervening target sequence using the single-stranded DNA as a template. A total of 20–40 PCR cycles is carried out depending on the abundance of the target sequence in the template DNA. Sequences of up to several thousand base pairs can be amplified. To deal with the large number of separate incubations needed, the PCR is carried out using a microprocessor-controlled heating block known as a thermal cycler. In the first cycle, DNA molecules that extend beyond the target sequence are synthesized. This is because there is nothing to prevent the DNA polymerase from continuing to copy the template beyond the end of the target sequence. In subsequent cycles, however, newly synthesized DNA molecules that end with the primer sequence act as templates and limit synthesis to the target sequence so that the amplified DNA contains only the target sequence.

Variations of the PCR Method

A number of spin-off techniques based on the original PCR method have been developed that have a range of specific applications.

Reverse transcriptase PCR

This technique involves using RNA rather than DNA as the template for amplification. The procedure is very similar to conventional PCR but includes an initial step in which a DNA copy of the RNA template is produced using the enzyme reverse transcriptase. This enzyme, which is of viral origin, is a polymerase and has the unique ability to synthesize DNA from a RNA template. One of the main uses of the reverse transcriptase PCR (RT-PCR) technique is in the analysis of gene expression. Using gene-specific primers, the presence of an individual messenger RNA (mRNA) species in the total RNA derived from cells or tissues can be detected. A refinement of the technique allows the amount of a particular mRNA to be measured. This is known as quantitative RT-PCR and can be used to obtain information on levels of gene expression.

RT-PCR can also be used to copy the complete coding sequence of a gene. The amplified DNA can subsequently be used to produce gene clones, which direct the synthesis of large amounts of the encoded protein. By altering the sequence of the cloned DNA, using another PCR-based method known as site-directed mutagenesis, variant proteins with altered properties can be produced. This is sometimes referred to as protein engineering.

Degenerate oligonucleotide primer PCR

This technique can be used to amplify related DNA sequences, such as the members of a gene family, or to amplify a DNA sequence from one species based on sequence information from another. The method involves using a mixture of oligonucleotide primers in which alternative nucleotides occur at certain positions. By incorporating all of the possible bases at a known point of variation, related sequences can be amplified. This technique has been successfully used to identify new members of gene families such as the homeobox and cyclin genes.

An extension of the degenerate oligonucleotide primer PCR (DOP-PCR) technique involves carrying out PCR with primers that are a mixture of completely random sequences. This results in indiscriminate amplification of sequences from throughout the genome. This technique is referred to as whole-genome PCR and is a useful means of generating many sequences for analysis when only a small amount of template DNA is available, such as in the analysis of ancient DNA or single-cell samples.

Inverse PCR

One of the limitations of conventional PCR is that in order to design primers it is necessary to know part of

the DNA sequence to be amplified. However, in some cases only a portion of the sequence of a gene is known. Inverse PCR is a variation of conventional PCR that allows unknown sequences flanking a known sequence to be amplified for analysis. The technique involves digesting the template DNA with a restriction enzyme such that the target sequence for a given PCR will be contained within a larger fragment containing unknown sequence. This fragment can be circularized using the enzyme DNA ligase and the flanking regions amplified using the original primer pair facing outwards so that they amplify the remainder of the circle, generating a PCR product containing the unknown flanking sequence.

Ligase chain reaction

A ligase chain reaction (LCR) technique can be used to detect variations in the DNA sequence of a gene. Two pairs of complementary primers that bind to adjacent positions on the DNA template are used. The first two primers bind to the template and are joined to each other by the action of a DNA ligase enzyme. After one denaturation and annealing cycle, more copies of the first pair anneal to the target DNA and the product of the first round ligation acts as a template for the other primer pair. Repeated cycles of denaturation, annealing and ligation result in the exponential generation of ligated product. The reaction is very sensitive to the sequence of the target DNA and if any variation is present no ligation product will be produced.

Repetitive element PCR

A significant portion of the human genome is composed of repeated DNA sequences. A well-known example is Alu elements, which have an average length of 250 bp and occur as almost one million copies dispersed throughout the genome. Repetitive element PCR is a method used to amplify intervening sequences between repetitive elements. The method involves the use of a primer that binds a core sequence in the repetitive element. The success of the method relies on the repetitive elements occurring in close proximity and often having opposing orientations.

Applications of the PCR

PCR and its variants are used extensively as a research tool. Most studies in molecular biology involve the use of PCR at some stage, normally as part of an overall strategy and in association with other techniques. For example, DNA amplified by PCR can be used for DNA sequencing, as a probe in Northern and Southern blotting, and to generate clones.

PCR has applications in many areas of research in biology and medicine as well as in unexpected subjects such as anthropology and archeology. It is also an important technique used in the biotechnology industry. PCR has made important contributions to research in many areas, some of which are described below.

Inherited diseases

These disorders are caused by gene mutations passed on from parents to their children. Examples include hemophilia and cystic fibrosis. PCR is used to amplify gene sequences, which can then be screened for disease-causing mutations. The information obtained has dramatically improved our understanding of these disorders and has produced the important additional benefit of allowing carriers of the disorders to be identified.

Cancer research

PCR has been widely used in studies of the role of genes in cancer. For example, mutations in oncogenes and tumor-supressor genes have been identified in DNA from tumors using PCR-based strategies. This has improved our understanding of how cancer develops.

Forensic science

By amplifying repetitive sequences, PCR can be used to identify individuals from samples of their DNA. This can be used, for example, to link individuals with forensic DNA samples from the scene of a crime. Analysis of variable sequences is also used in tissue typing to match organ donors with recipients and in anthropology to study the origins of races of people.

Biotechnology

PCR has played an important role in the production of recombinant proteins such as insulin and growth hormone, which are widely used as drugs, and in the development of recombinant vaccines such as that for the hepatitis B virus.

See also

Polymerase Chain Reaction (PCR): Design and Optimization of Reactions

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