Polymerase chain reaction (PCR)

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Introduction

The polymerase chain reaction (PCR) is ascientific technique in molecular biologyto amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. Polymerase Chain Reaction was developed in 1984 by the American biochemist, Kary Mullis. Mullis received the Nobel Prize and the Japan Prize for developing PCR in 1993 (Bartlett, 2003).The polymerase chain reaction can be used to amplify both double and single stranded DNA and is a powerful technique that has rapidly become one of the most widely used techniques in molecular biology because it is quick, inexpensive and simple.

In order to perform PCR, one must know at least a portion of the sequence of the target DNA molecule that has to be copied. Generally, PCR amplifies small DNA targets 100-1000 base pairs (bp) long. It is technically difficult to amplify targets >5000 bp long (Sridhar Rao, 2006).

Basic concept of PCR

The basic PCR principle is simple. As the name implies, it is a chain reaction: One DNA molecule is used to produce two copies, then four, then eight and so forth. This continuous doubling is accomplished by specific proteins known as polymerases, enzymes that are able to string together individual DNA building blocks to form long molecular strands. To do their job polymerases require a supply of DNA building blocks, i.e. the nucleotides consisting of the four bases adenine (A), thymine (T), cytosine (C) and guanine (G). They also need a small fragment of DNA, known as the primer, to which they attach the building blocks as well as a longer DNA molecule to serve as a template for constructing the new strand. If these three ingredients are supplied, the enzymes will construct exact copies of the templates.The polymerase chain reaction relies on the ability of DNAcopying enzymes to remain stable at high temperatures (Joshi and Deshpande, 2011).

Steps in PCR

There are three major steps involved in the PCR technique: denaturation, annealing, and extension.

1. **Denaturation:** DNA is denatured at high temperatures (from 90 - 97 degrees Celsius).
2. **Annealing:** Two, primers anneal to the DNA template strands to prime extension.
3. **Extension:** Extension occurs at the end of the annealed primers to create a complementary copy strand of DNA. This effectively doubles the DNA quantity through the third steps in the PCR cycle.

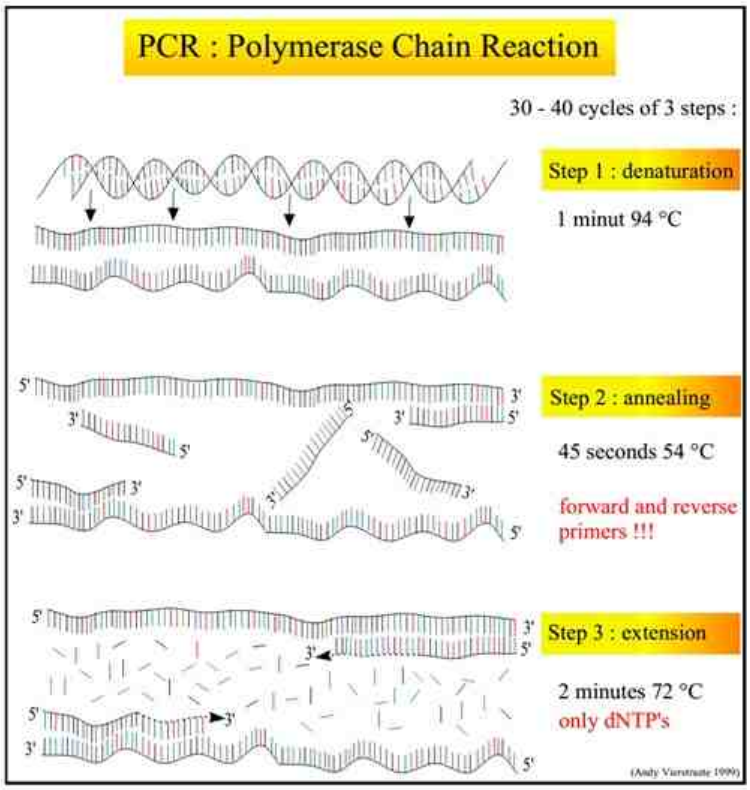
To amplify a segment of DNA using PCR, the sample is first heated so the DNA denatures, or separates into two pieces ofsingle-stranded DNA. Next, an enzyme called "Taq polymerase" synthesizes/builds two new strands of DNA, using the original strands as templates. This process results in the duplication of the original DNA, with each of the new molecules containing one old and one new strand of DNA. Then each of these strands can be used to create two new copies, and so on (Ochmanet al., 1988).The annealing phase happens at a lower temperature, 50-60°C. This allows the primers to hybridize to their respective complementary template strands, a very useful tool to forensic chemistry. The newly-formed DNA strand of primer attached to template is then used to create identical copies off the original template strands desired. Taq polymerase adds available nucleotides to the end of the annealed primers. The extension of the primers by Taq polymerase occurs at approx 72°C for 2-5 minutes. DNA polymerase I cannot be used to elongate the primers as one would expect because it is not stable at the high temperatures required for PCR. After 25-30 cycles, whoever is performing the PCR process on a sample of DNA will have plenty of copies of the original DNA sample toconduct experimentation. Assuming the maximum amount of time for each step, 30 cycles would only take 6 hours to complete.

Post amplification detection

Following PCR, the amplification product can be detected using gel electrophoresis followed by ethidium bromide staining and visualization with UV trans-illumination. Visualization of a band containing DNA fragments of a particular size can indicate the presence of the target sequence in the original DNA sample. Absence of a band may indicate that the target sequence was not present in the original DNA sample. Confirmation of the amplicons can be made by southern blotting using specific probes.

Modifications and different types of PCR

Nested PCR, Multiplex PCR, RT-PCR, Touchdown PCR, Arbitrarily Primed PCR, Inverse PCR, Allele Specific PCR, Asymmetric PCR, "Hot Start" PCR, Core Sample PCR, Degenerate PCR and PCR-Elisa.



Some important applications of PCR

1. In microbiology and molecular biology, for example, PCR is used in research laboratories in DNA cloning procedures, Southern blotting, DNA sequencing, recombinant DNA technology.
2. In clinical microbiology laboratories, PCR is invaluable for the diagnosis of microbial infections and epidemiological studies.
3. PCR is also used in forensics laboratories and is especially useful because only a tiny amount of original DNA is required, for example, sufficient DNA can be obtained from a droplet of blood or a single hair.
4. Also used in detection of adulteration of raw and cooked meat

Conclusion

PCR and its applications hold scientific and medical promise. PCR has very quickly become an essential tool for improving human health and human life. PCR has completely revolutionized the detection of RNA and DNA viruses and is valuable as a confirmatory test. PCR is a rapid technique with high sensitivity and specificity and has also been credited to have been able to detect mixed infections with ease in many studies. PCR, a more sophisticated technique, requires infrastructural support, is expensive but nevertheless, one cannot discount its utilitarian advantages which are many compared to the existing conventional diagnostic methods. ■