Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is a common laboratory technique used to amplify or make millions of copies of a particular region of DNA. This DNA region can be anything the experimenter is interested in. For example, it might be a gene whose function a researcher wants to understand, or a genetic marker used by forensic scientists to match crime scene DNA with suspects.

It was originally invented by Kary Mullis in 1985 and got the Nobel Prize in 1993.

Principle

The basic principle of PCR is that the double stranded DNA molecule, when heated to a high temperature, separate yielding two single-stranded DNA molecules. The single stranded DNA molecules can easily be copied with the help of a DNA polymerase and nucleosides resulting in the duplication of original DNA molecules. By repeating these events, multiple copies of the original DNA molecule can be generated.

Requirements

i) A thermal cycler (an instrument having a microprocessor-controlled temperature cycling)

ii) DNA segment to be amplified

iii) Two primers, which are oligonucleotides (about 10-18 nucleotides long), oriented with their ends facing each other so that DNA synthesis can occur between them

iv) The enzyme Taq polymerase (a DNA polymerase) which is stable at high temperature

v) $MgCl_2$

vi) dNTPs (deoxy nucleoside triphosphate: dATPs, dTTPs, dGTPs, dCTPs)

Procedure

The DNA, from which a segment is to be amplified, is mixed with an excess of the two primer molecules, all the four kinds of dNTPs, $MgCl_2$ and Taq polymerase in a reaction mixture. The DNA segment is amplified involving the following 3 steps:

i) Denaturation: The reaction mixture is heated to a high temperature (94-96ºC) so that the DNA molecule is denatured i.e. the two strands of DNA duplex get separated. Each strand of the target DNA then acts as a template for DNA synthesis.

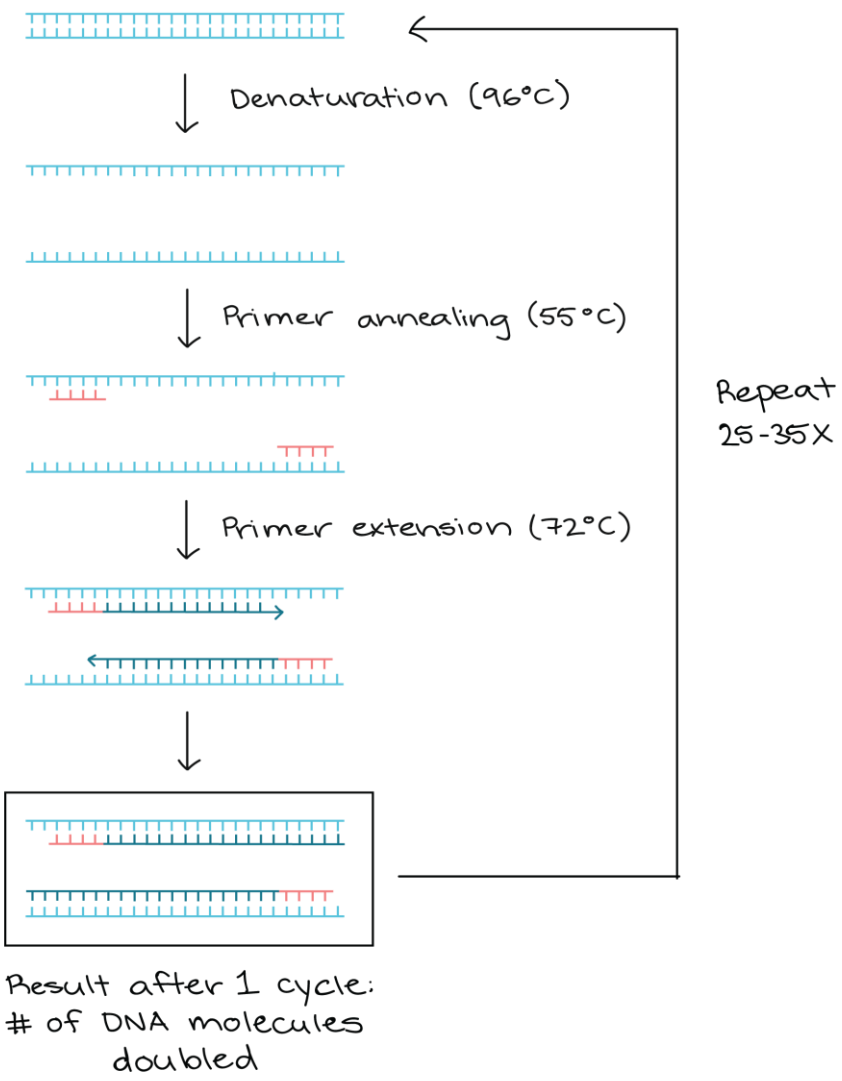
ii) Annealing: The mixture is then cooled by lowering the temperature upto 55-65ºC. At this temperature, the two primers anneal to each of the single-stranded template DNA. Annealing occurs due to presence of complementary sequences located at the 3’ ends of the template DNA.

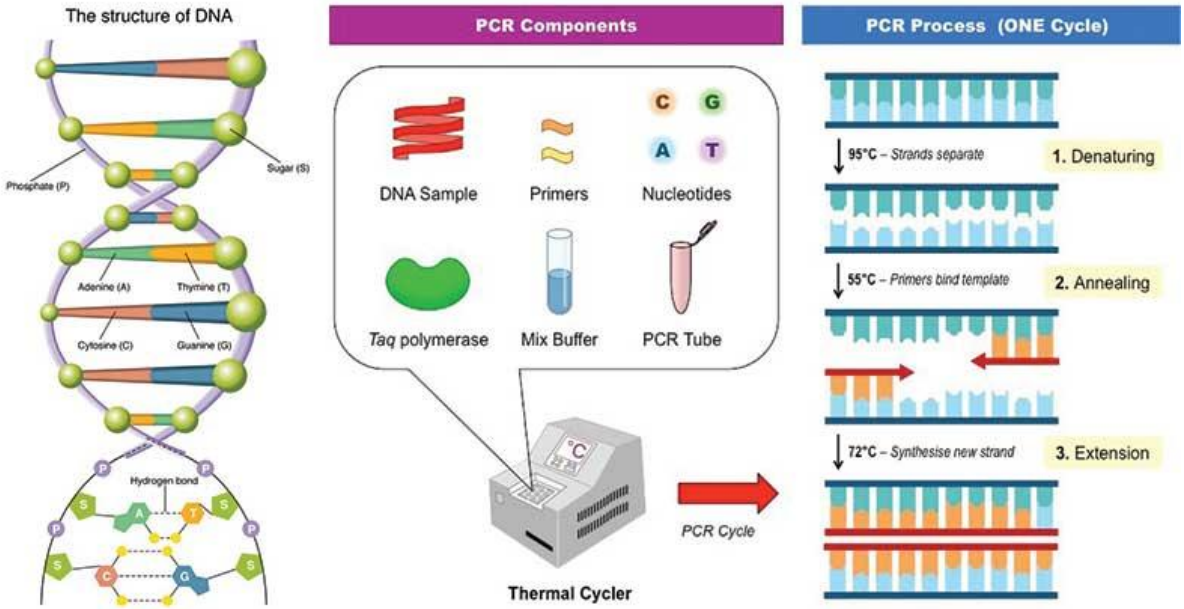
iii) Extension: In this step, the temperature is so adjusted that the Taq polymerase becomes active. Synthesis of new DNA strand begins in between the primers, dNTPs and Mg^{2+}. The optimum temperature for this polymerization is kept at 72ºC.

The next PCR amplification cycle begins as soon as all the stages of previous cycle end. During PCR operation, the extension product of one cycle serve as a template for subsequent cycles and each time the amount of DNA doubles. Thus, a single template molecule of DNA generates 2^n molecules at the end of n cycles.

Applications

PCR is useful in every aspect of modern biology including-molecular biology, genetic engineering, infectious and parasitic disease diagnosis, human genetic disease diagnosis, forensic validation, DNA fingerprinting, plant and animal breeding and environmental monitoring.




The structure of DNA
Phosphate (P)
Sugar (S)
Adenine (A)
Thymine (T)
Cytosine (C)
Guanine (G)
Hydrogen bond
PCR Components
DNA Sample
Primers
Nucleotides
Taq polymerase
Mix Buffer
PCR Tube
Thermal Cycler
PCR Cycle
PCR Process (ONE Cycle)
95°C – Strands separate
1. Denaturing
55°C – Primers bind template
2. Annealing
72°C – Synthesise new strand
3. Extension