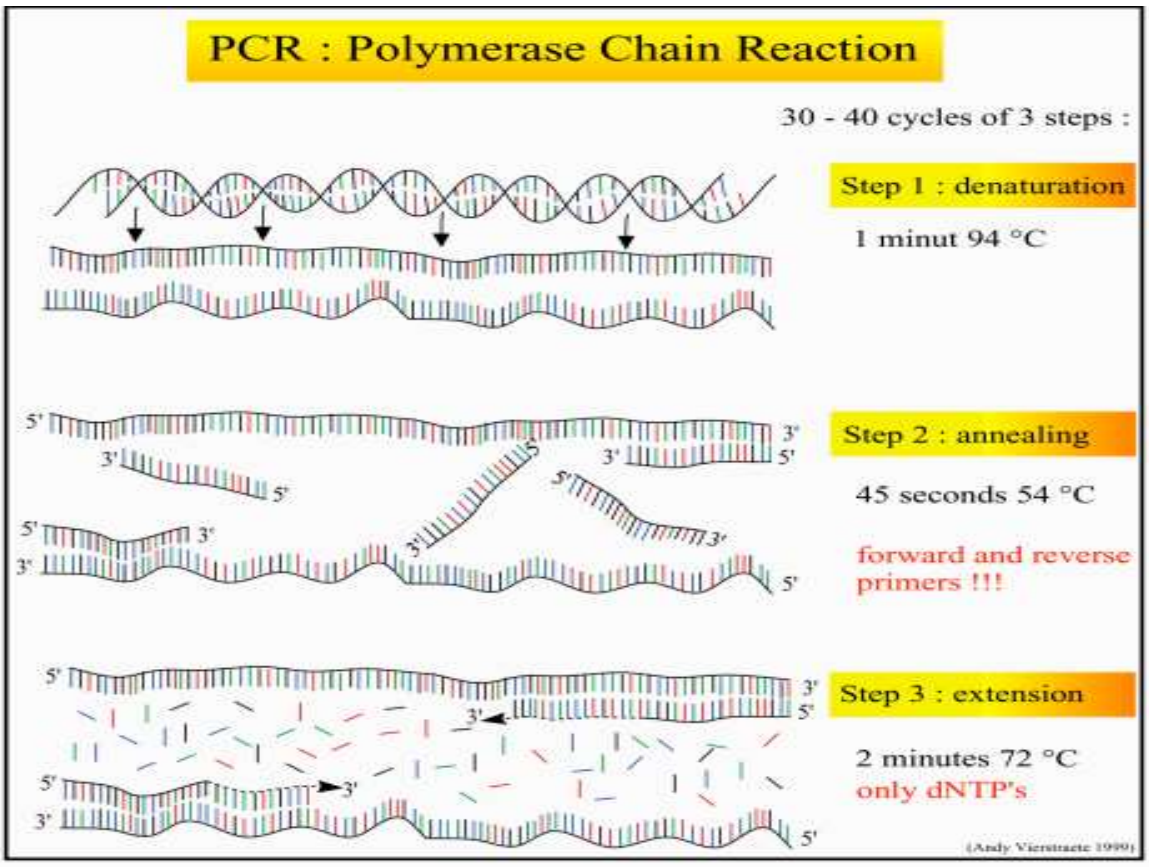
The **Polymerase Chain Reaction (PCR)** is a powerful laboratory technique used to amplify specific DNA sequences, allowing scientists to produce millions or even billions of copies of a target DNA fragment in just a few hours. The Polymerase Chain Reaction (PCR) was not a discovery, but rather an invention by Kary Mullis in 1983.

Important Outline

Primers are short, synthetic DNA sequences that play a critical role in amplifying specific regions of DNA during the Polymerase Chain Reaction (PCR). They are typically around 18-24 nucleotides long and are designed to match the DNA sequences flanking the target region that you want to amplify. **GC Content**: Ideally 40-60%. Each PCR reaction requires two primers:

1. **Forward Primer**: This primer binds to the 5' end of the target DNA strand, moving in the 3' direction.
2. **Reverse Primer**: This primer binds to the complementary strand at the 3' end of the target DNA, also moving in the 3' direction.

The enzyme DNA polymerase then adds nucleotide bases to the end **of each primer**, using the template DNA as a guide to extend the primer thereby producing **new double stranded DNA**.



PCR Materials

1. **Template DNA** (genomic, plasmid, bacterial colony, etc.).
2. **Primers** (small segment of DNA)
3. **MgCl2**.
4. **DNA polymerase** (as Taq polymerase).
5. **dNTPs** (nitrogen bases).

PCR steps (steps of the cycle)

1- **Denaturation:** The PCR reaction starts with a high-temperature denaturation step, usually **around 94-98°C**. This step separates the double-stranded DNA template into two single strands by breaking the hydrogen bonds between the complementary base pairs. Denaturation typically lasts for 20-30 seconds, allowing the DNA to become single-stranded.

2- **Primer annealing**: After denaturation, the reaction temperature is lowered to enable the primers to anneal (bind) to their complementary sequences on the single-stranded DNA template. The annealing temperature depends on the primer design and is typically 5-10°C below the melting temperature (Tm) of the primers. Annealing usually takes 20-30 seconds.

3- Extension (Elongation): Once the primers are bound to their target sequences, the temperature is increased to the optimal temperature for the DNA polymerase to extend the primers and synthesize new DNA strands. The extension temperature is generally around 68-72°C, depending on the DNA polymerase used. The extension time depends on the length of the target DNA segment and is typically around 1 minute per 1,000 base pairs. The DNA polymerase synthesizes a complementary DNA strand by adding nucleotides to the 3' end of each primer.

Note: These three steps (denaturation, annealing, and extension) constitute one cycle of PCR. After each cycle, the number of DNA copies doubles, leading to exponential amplification. The number of cycles required depends on the initial amount of the target DNA and the desired level of amplification. Typically, PCR runs between 25 and 40 cycles.

PCR applications

- PCR can be used for **forensic analysis**, when only a **trace amount** of DNA is **available as evidence**.
- Detecting of viruses and bacteria.
- Studying genetic causes (as **mutations** and variations).
- Identify the **genetic tree** of living organisms and **classify them**.

Questions and answers

1. What does PCR stand for?

PCR stands for Polymerase Chain Reaction.

2. What is the purpose of PCR?

The purpose of PCR is to amplify specific segments of DNA.

3. What are the essential materials for PCR?

The essential materials for PCR include DNA template, primers, DNA polymerase, nucleotides (dNTPs), buffer solution, magnesium ions (Mg2+), thermal cycler, PCR tubes/plates, and thermal cycler software.

4. What is the role of DNA polymerase in PCR?

DNA polymerase s**ynthesizes new DNA strands** by extending the primers and adding nucleotides.

5. Why are primers important in PCR?

Primers are important in PCR as they **define the target DNA region** for amplification.

6. What is the purpose of the DNA template in PCR?

The DNA template provides the original DNA sequence to be amplified.

7. What are dNTPs?

dNTPs are deoxynucleotide triphosphates, which are the building blocks for DNA synthesis in PCR.

8. What is the function of the buffer solution in PCR?

The buffer solution **maintains optimal pH and ionic conditions for the PCR reaction**.

9. Why are magnesium ions (Mg2+) necessary in PCR?

Magnesium ions are cofactors required for DNA polymerase activity.

10. What is the role of the thermal cycler in PCR?

The thermal cycler is an instrument that controls the **temperature cycles during PCR**, providing the necessary conditions for denaturation, annealing, and extension steps.