Polymerase Chain Reaction (PCR)

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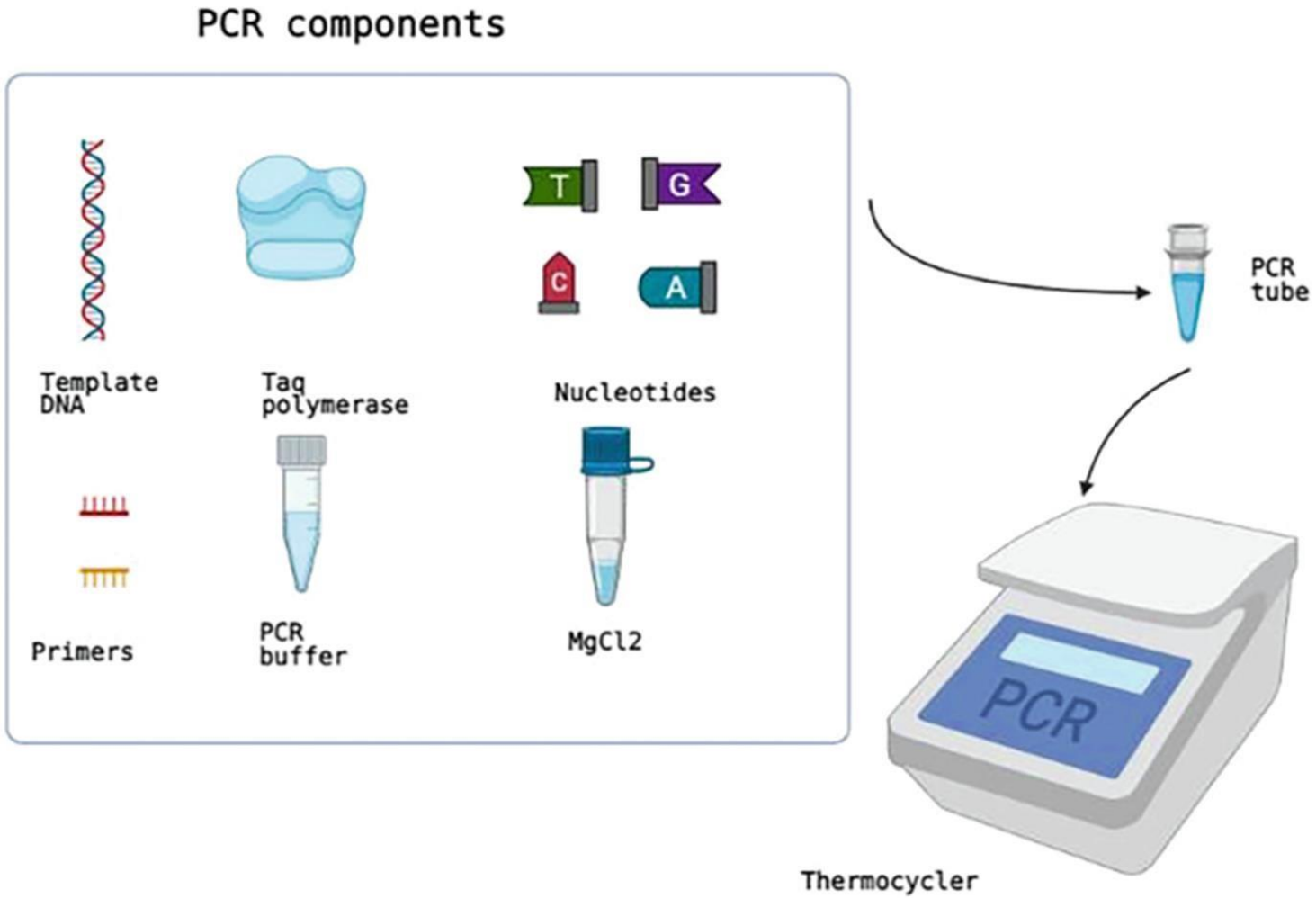
- **Polymerase chain reaction (PCR):** is a molecular technique used to amplify small quantities of DNA, producing millions of copies of a specific DNA sequence within a short period.

- An efficient and cost-effective technique that combines the principles of complementary nucleic acid hybridization with those of nucleic acid replication that are applied repeatedly through numerous cycles.

- It results in the exponential production of the specific target DNA/RNA sequences by a factor of 107 within a relatively short period.

PCR Component

1. Target DNA
2. Two primers
3. Nucleotides
4. Buffers
5. Taq polymerase enzyme



PCR Component

1. **Template DNA**: The DNA sample containing the target sequence to be amplified.

2. Primers: Short DNA sequences that bind to specific regions of DNA, helping DNA polymerase to attach to DNA to work.

2 primers, Forward and Reverse.

3. DNA Polymerase: Typically Taq polymerase, a heat-resistant enzyme that synthesizes new DNA strands by **adding nucleotides**.

PCR Component

4. dNTPs : (deoxy nucleotide triphosphate) The building blocks for new DNA synthesis (adenine, thymine, cytosine, and guanine).

5. Buffer Solution: Maintains the optimal pH environment for the reaction.

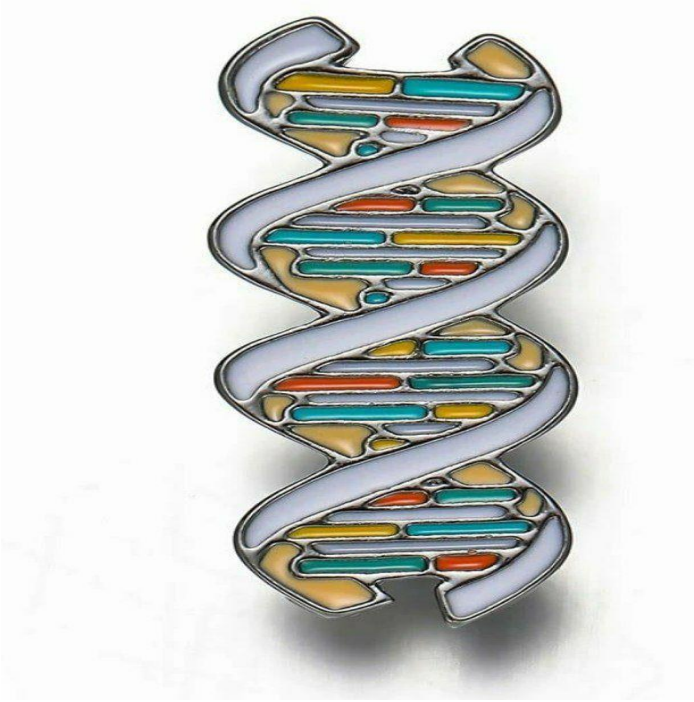
6. Nuclease-free water

Purpose of PCR

DNA Amplification, PCR increases the quantity of a specific DNA sequence, allowing for detailed analysis, detection, or manipulation.

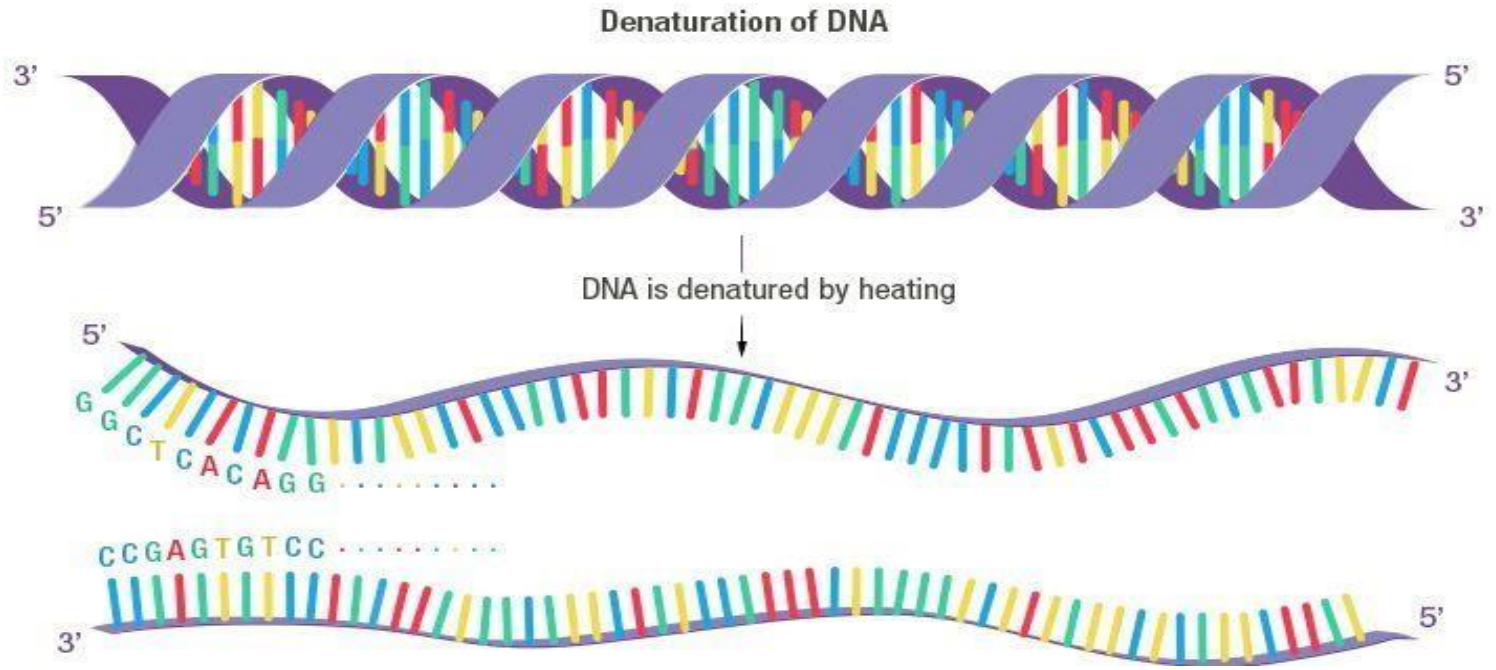
Step of PCR:

- Denaturation
- Annealing
- Extension



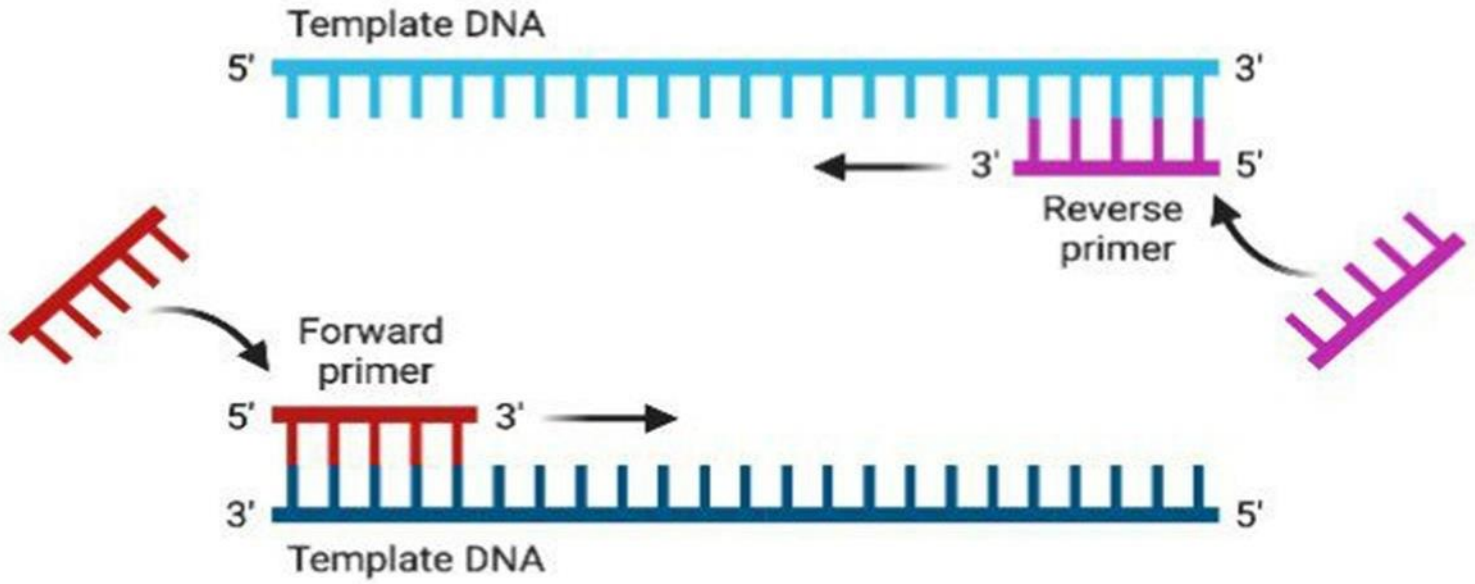
1- Denaturation

- Temperature:92-94 °C
- Double stranded DNA melts to single stranded



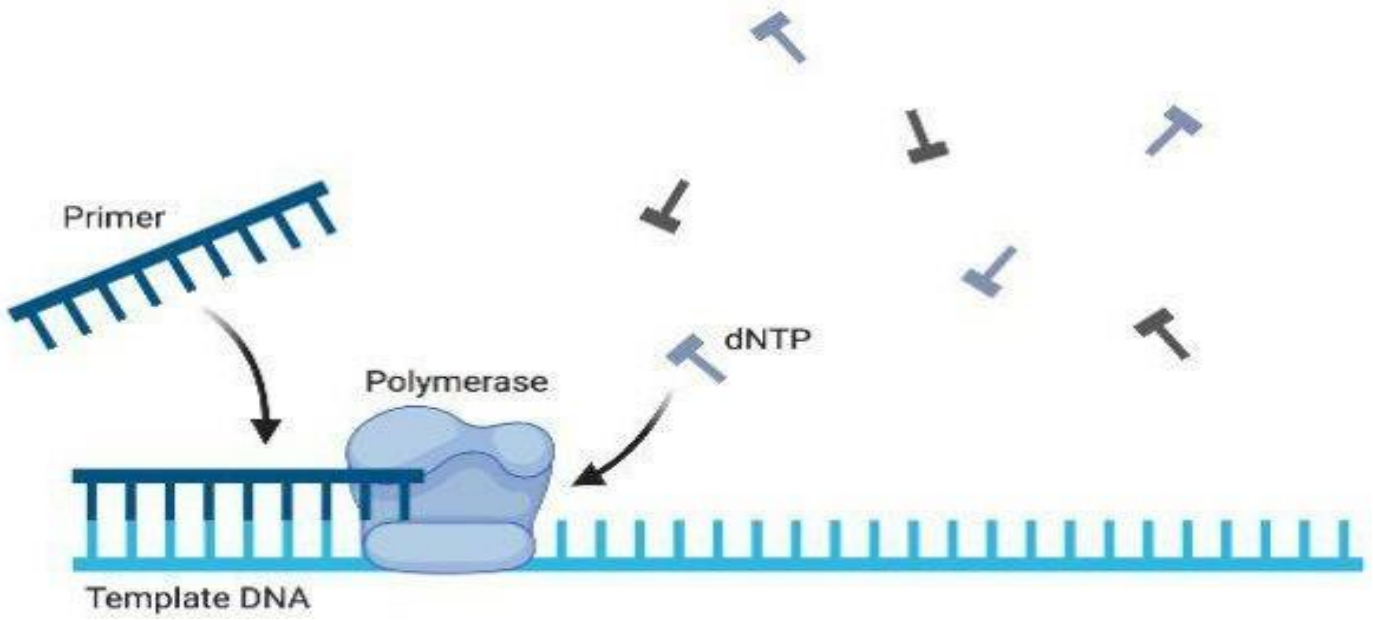
2- Annealing

- Temperature: ~ **50–65°C** (dependent on the melting temperature of the expected duplex)
- Primers bind to their complementary sequences.



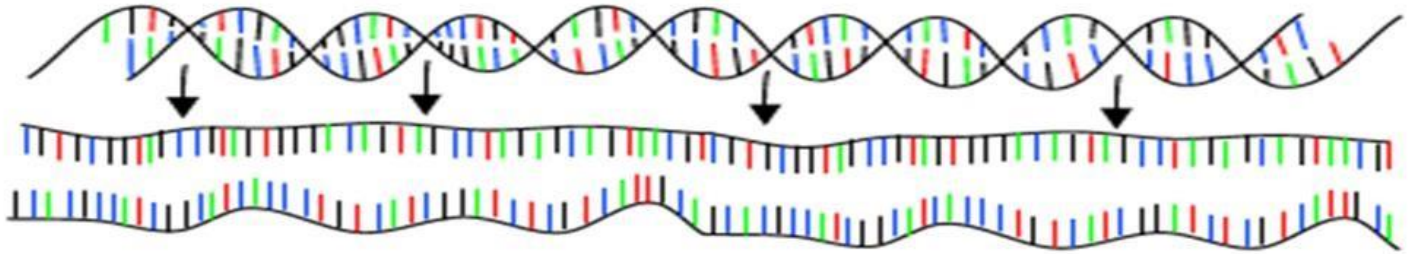
3- Extension

- Temperature: ~ **(72°C)**
- DNA polymerase binds to the annealed primers and extends DNA at the 3' end of the chain



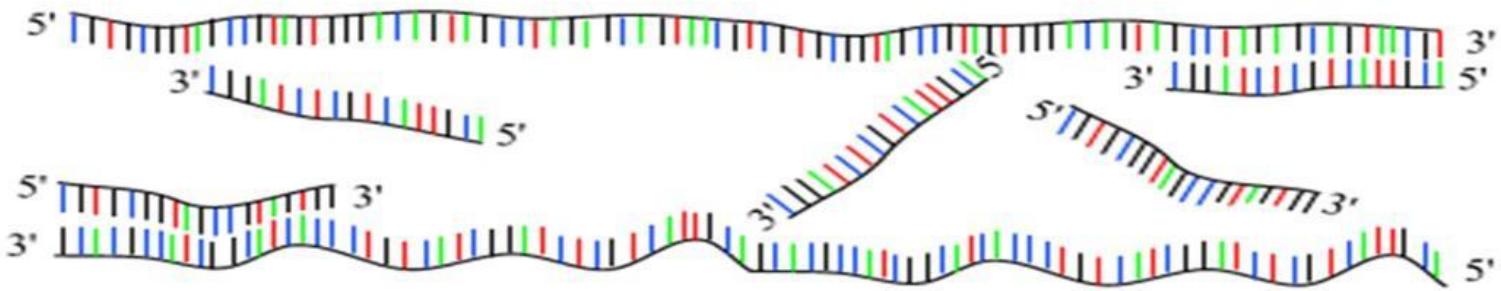
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

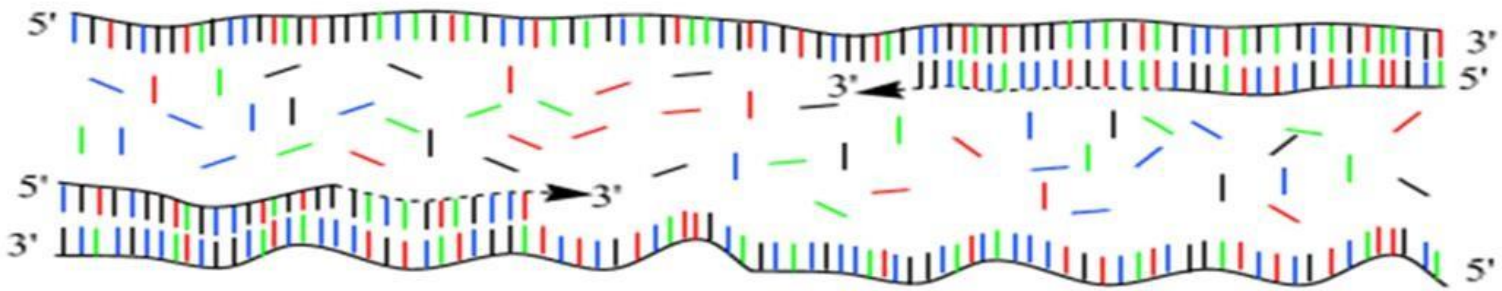
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

2 minutes 72 °C
only dNTP's

(Andy Vierstraete 1999)

Basic requirements for PCR reaction

1) DNA sequence of target region must be known.

2) Primers - typically 20-30 bases in size. These can be readily produced by commercial companies. Can also be prepared using a DNA synthesizer

3) Thermo-stable DNA polymerase - e.g. Taq polymerase which is not inactivated by heating to 95C

4) DNA thermal cycler - machine which can be programmed to carry out heating and cooling of samples over a number of cycles.

Applications of PCR

1. Drug discovery
2. Forensic science
3. Human genome project
4. Gene sequencing
5. Vaccine production by recombinant DNA technology
6. Identification of microorganisms grown in culture
7. Direct detection of microorganisms in patient specimens


Thank You!