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(Short Notes on Polymerase Chain Reaction (PCR))

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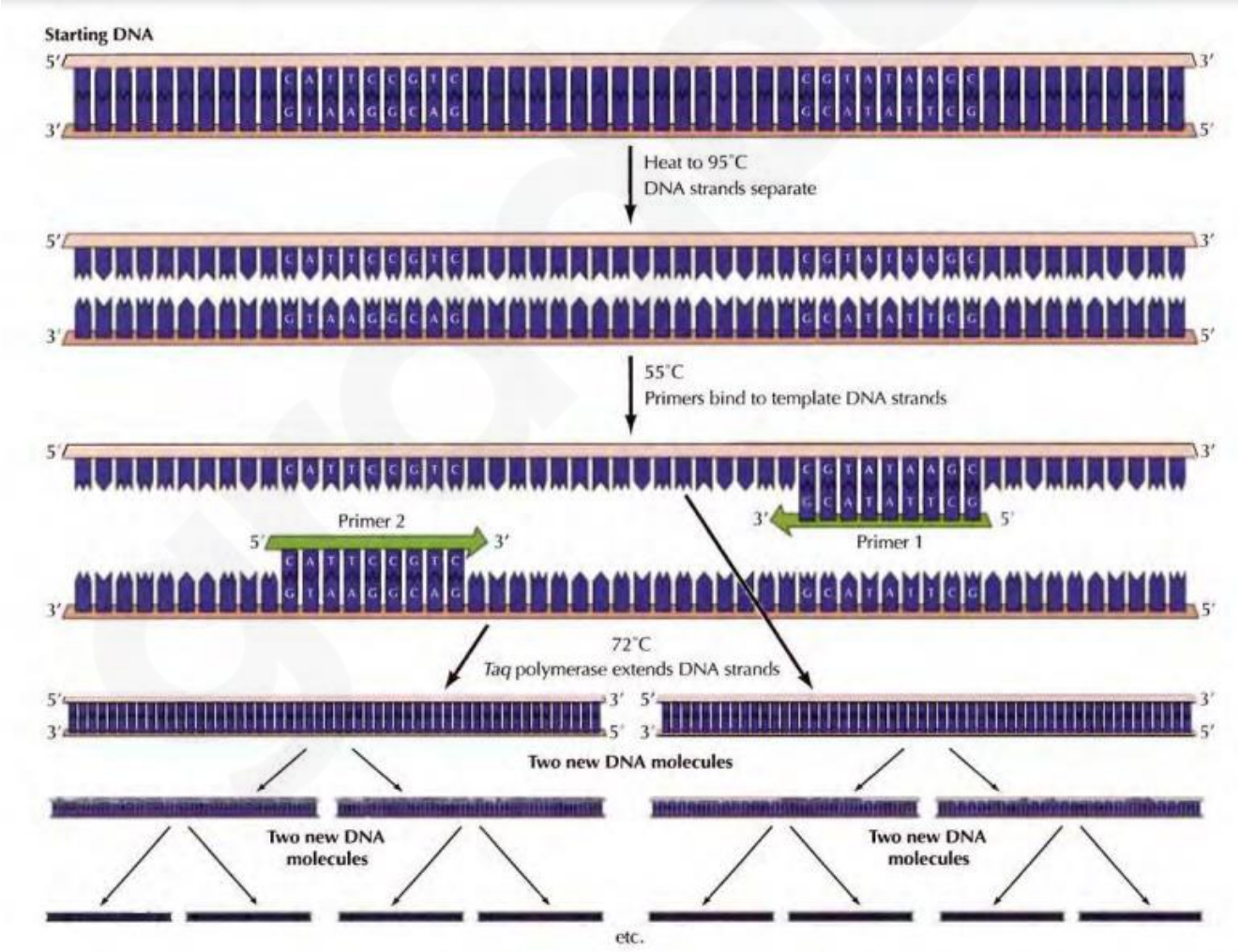
Polymerase Chain Reaction (PCR)

PCR is a breakthrough method invented by Kary Mullis in the 1980s. It is known as the thermocycler. PCR has the ability to synthesize new strands of DNA complementary to the template strand by DNA polymerase. Because DNA polymerase can only add a nucleotide to a 3'-OH group that already exists, it requires a primer to add the first nucleotide. The main components to carry out the PCR are 1) Template, 2) Taq polymerase, 3) dNTPs, 4) Buffer with Mg2+ and, 5) Sterile water.

Each cycle of PCR has three steps:

- Denaturation
- Annealing (or) Attachment of specific primers.
- Extension



PCR requirements:

- Template: DNA molecules, such as total DNA from human cells. A specific region of DNA amplified from such a mixture provided the nucleotide sequence surrounding the known DNA sequence. The primer is designed to initiate DNA synthesis at the desired point.
- This primer is usually a chemically synthesized oligonucleotide, containing 15 to 20 DNA bases. Two primers are used to initiate DNA synthesis in opposite directions of the complementary DNA strand.
- The primers depend on the primer length, melting point (Tm), specificity, GC content and complementary primer sequences.
- Taq polymerase is the DNA polymerase obtained from *Thermus aquaticus* that is stable at 95°C. Taq polymerase facilitates the stimulation, specificity, automation of the PCR process.
- Deoxynucleoside triphosphates (dNTPs) consist of four basic nucleotides—dATP, dCTP, dGTP, and dTTP—as building blocks of new DNA strands.
- PCR buffer consists of Magnesium chloride supplies Mg divalent cations required as a cofactor. The buffer provides a suitable chemical environment for the activity of DNA polymerase.

The steps involved in PCR:

- Denaturation occurs at 95°C temperature.
- Annealing (50-56°C)
- Extension (72°C)

The reaction begins by heating the template DNA to a high temperature (e.g., 95°C) and the two strands separate. The temperature is then reduced to allow the primers to pair with their complementary sequences on the template strands. DNA polymerase then utilizes the primers to synthesize a new strand complementary to each template.

Thus, in one cycle of amplification, two new DNA molecules are synthesized from one template molecule. The process can be repeated multiple times, with a two-fold increase in DNA molecules resulting from each round of replication. A single DNA molecule amplified via 30 replication cycles, for example, would theoretically create 230 (about 1 billion) progeny molecules.

Types of PCR

- Real-Time PCR (quantitative PCR or qPCR)
- Reverse-Transcriptase (RT-PCR)
- Multiplex PCR
- Nested PCR
- Hot Start PCR
- Arbitrary Primed PCR
- Touchdown PCR
- Inverse PCR
- Colony PCR

Role of PCR in Lifescience

- Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) can be used as the main technique for COVID-19 diagnosis.
- PCR is used for the identification of mutations, carriers of diseases like diabetes, obesity, and neurological disorders, cardiac, metabolic and congenital diseases.
- PCR can be used as a biomarker for the detection of several bacterial and viral diseases.
- PCR is used in DNA molecular markers techniques such as AFLP and RAPD and helps in identifying and differentiating plant pathogens and also producing a set of specific DNA fragments.
- DNA fingerprinting is the main application of PCR in forensic sciences.
- In molecular biology, the PCR technique is used in cloning the gene of interest into the specific DNA sequences.
- Genotyping uses the PCR for detection and characterization of normal as well as mutant alleles.

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