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Polymerase Chain Reaction (PCR)

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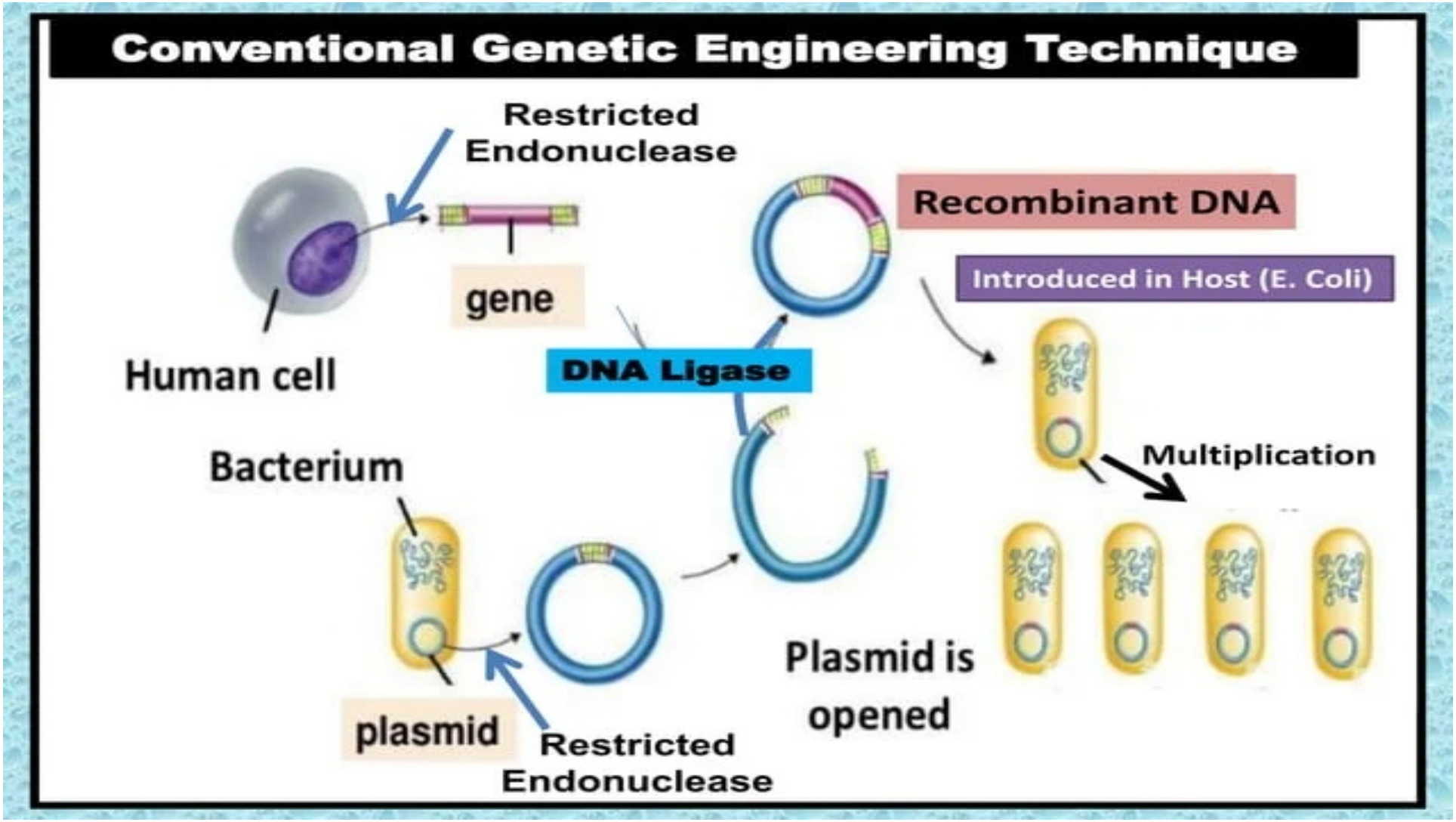
What is PCR

- Polymerase chain reaction (PCR) is a method used widely in molecular biology to make millions to billions of copies of a specific DNA sample rapidly, allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail.
- PCR was invented in 1983 by the American biochemist Kary Mullis.

- It is fundamental to much of genetic testing including analysis of ancient samples of DNA and identification of infectious agents. Using PCR, copies of very small amounts of DNA sequences are eponentially amplified in a series or cycles of temperature changes. PCR is now a common and often indispensable technique used in medical laboratory and clinical laboratory research for a broad variety of applications including biomedical research and criminal forensics.


Conventional Genetic Engineering Technique
Restricted Endonuclease
gene
Human cell
Recombinant DNA
Introduced in Host (E. Coli)
DNA Ligase
Multiplication
Bacterium
plasmid
Restricted Endonuclease
Plasmid is opened

Components of PCR set ups

- A basic PCR set-up requires several components and reagents including:

1. ***DNA template*** that contains the DNA target region to amplify.

2. ***DNA polymerase***; an enzyme that polymerizes new DNA strands; ***heat-resistant Taq polymerase*** is especially common, as it is more likely to remain intact during the high-temperature DNA denaturation process.

Components of PCR set ups

3. Two DNA *primers* that are complementary to the 3' (three prime) ends.
DNA polymerase can only bind to and elongate from a double-stranded region of DNA; without primers there is no double-stranded initiation site at which the polymerase can bind.

- **4. Nucleotides** containing triphosphate groups also called building blocks from which the DNA polymerase synthesizes a new DNA strand.

Components of PCR set ups

- 5. **A buffer solution** providing a suitable chemical environment for optimum activity and stability of the DNA polymerase Containing mutagen like magnesium (Mg) or manganese (Mn) ions; potassium (K) ions

Thermal Cycler of PCR & PCR tubes

Procedure

- The individual steps common to most PCR methods are as follows:

Initialization:

- This step is only required for DNA polymerases that require heat activation by hot-start PCR.
- It consists of heating the reaction chamber to a temperature of ***94–96 °C,*** or sometimes ***98*** °C if extremely thermostable polymerases are used.

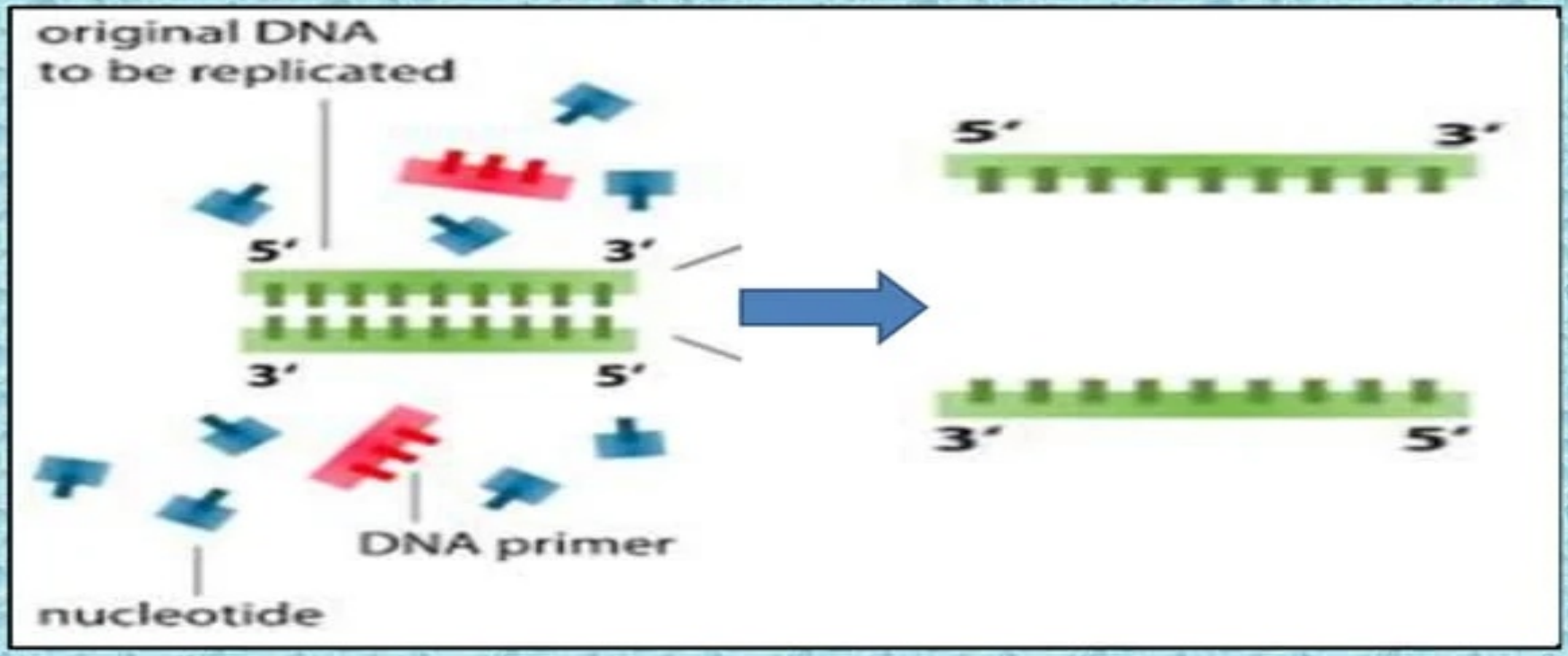
Procedure

Denaturation

- It consists of heating the reaction chamber to *94–98 °C* for 20–30 seconds.
- This causes ***DNA melting***, or ***denaturation,*** of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.

Initiation
Temp. *94–96 °C*

Denaturation
Temp. *94–98 °C*



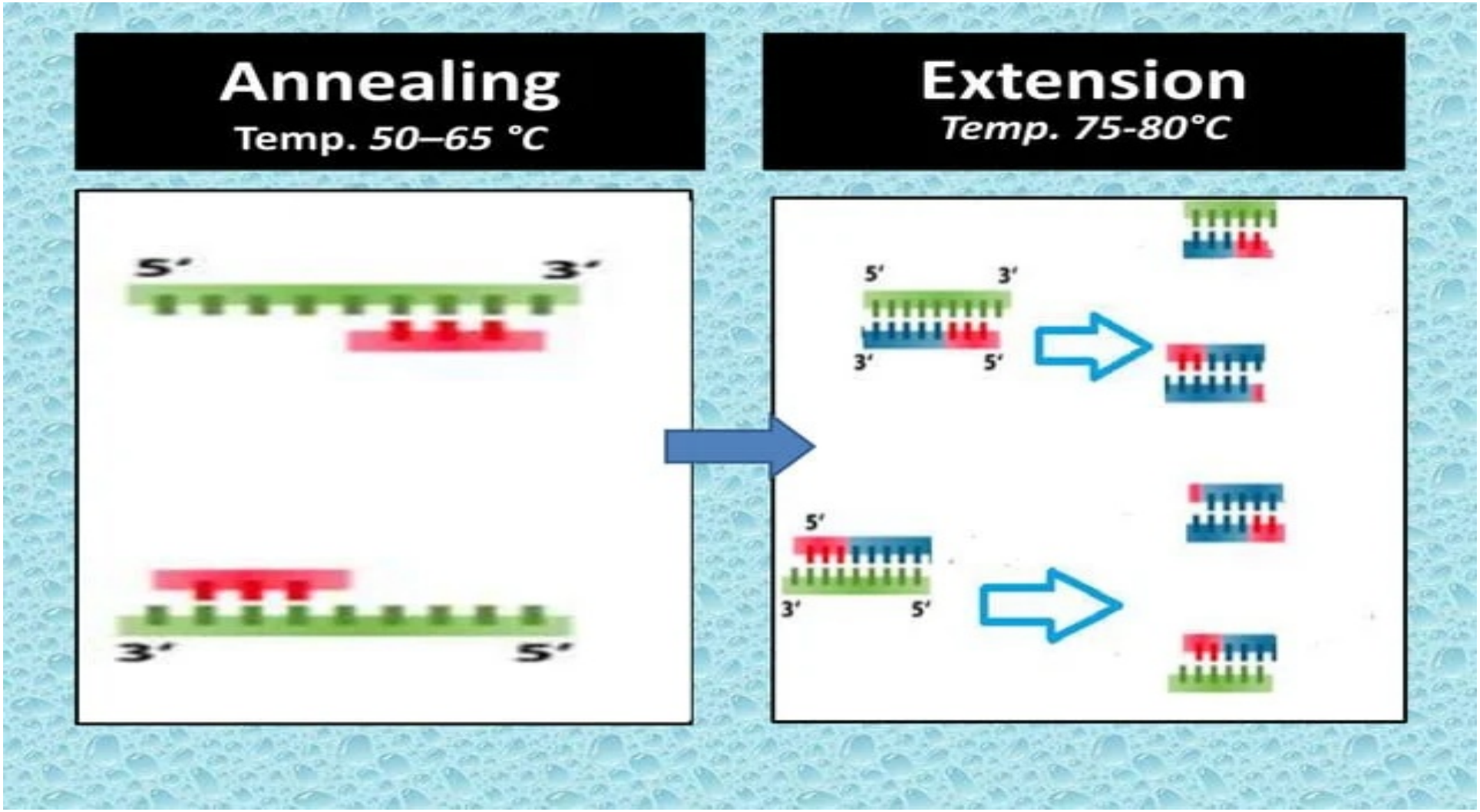
Procedure

Annealing

- In this step, the reaction temperature is lowered to ***50–65 °C*** for 20–40 seconds, allowing annealing of the primers to each of the single-stranded DNA templates.
- Two different primers are typically included in the reaction mixture.
- One for each of the two single-stranded complements containing the target region.
- The primers are single-stranded sequences themselves, but are much shorter than the length of the target region.

Procedure

- ***Extension/elongation***
- In this step the reaction temperature is kept ***75-80°C*** for Taq polymerase enzyme though a temperature of 72 °C is commonly used with this enzyme.
- In this step, the DNA polymerase synthesizes a ***new DNA strand*** complementary to the DNA template strand by adding free nucleotides.
- The precise time required for elongation depends both on the DNA polymerase used and on the length of the DNA target region to amplify.


Annealing
Temp. 50–65 °C
Extension
Temp. 75-80°C
5′
3′
3′
5′
5′
3′
3′
5′
5′
3′
3′
5′