POLYMERASE CHAIN REACTION (PCR)

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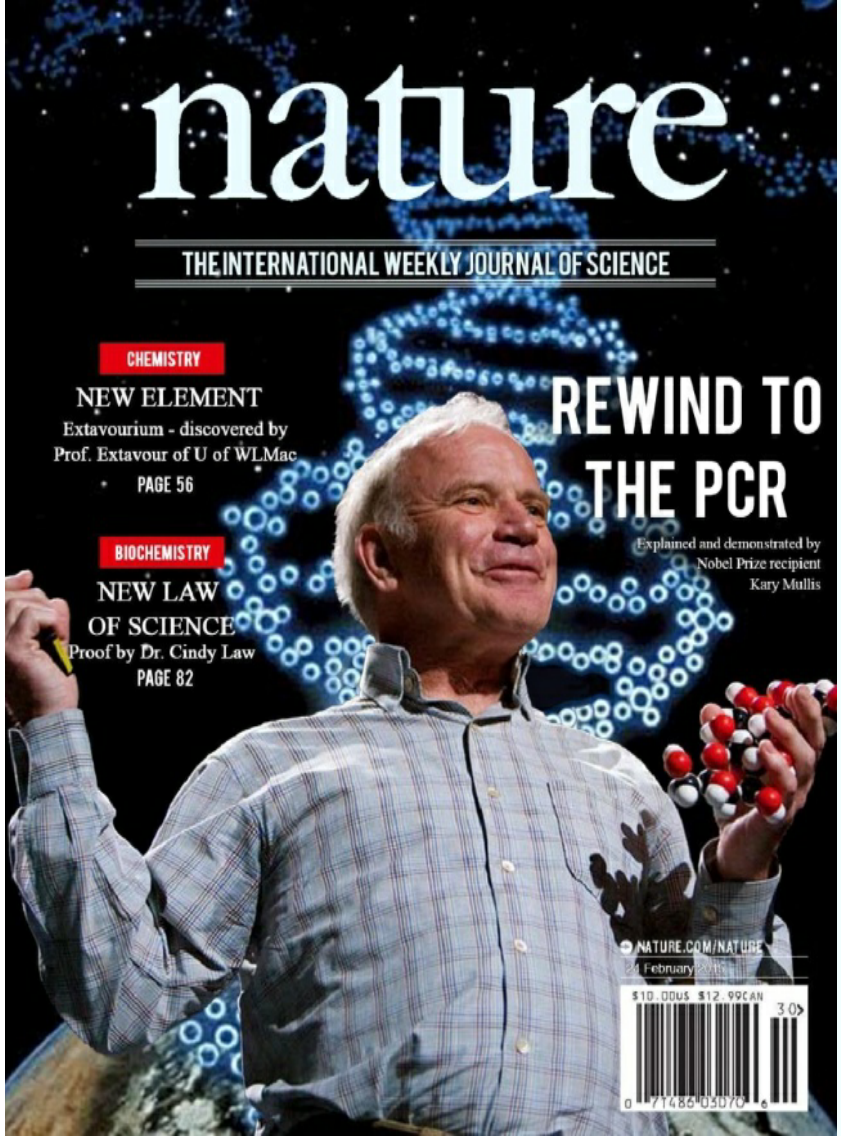
Learning outcomes

After studying this unit you will be able to understand

- The purpose of PCR
- Components of PCR
- Steps of PCR
- PCR cycling parameters
- Applications of PCR

Kary Banks Mullis(1944-2019)

- **PCR (Polymerase Chain Reaction)** is a revolutionary method developed by Kary Mullis in the 1983.
- Kary Banks Mullis(1944-2019) was an American biochemist. In recognition of his invention of the polymerase chain reaction technique, he shared the 1993 Nobel Prize in Chemistry with Michael Smith.



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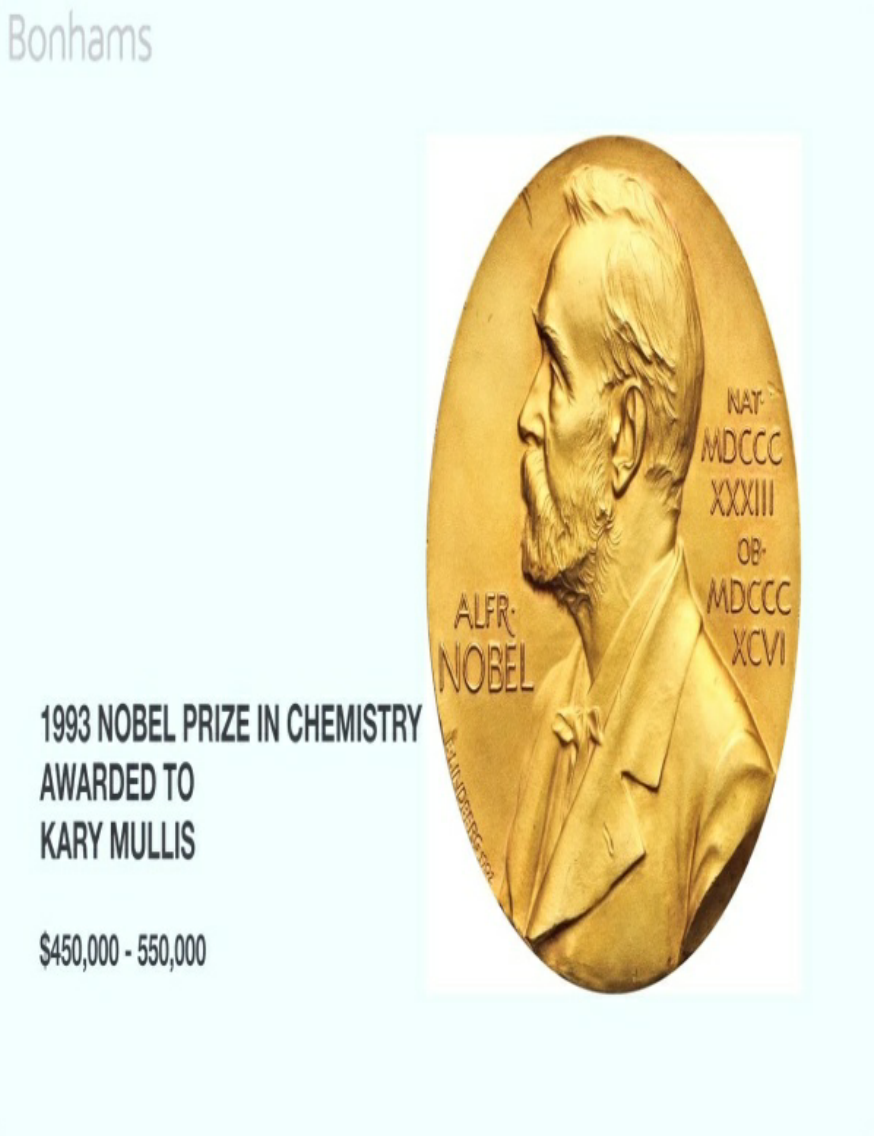

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PCR (Polymerase Chain Reaction

- PCR is a DNA amplification technique in which millions of DNA copies are made using a single DNA segment.

- It is a widely used method in molecular biology for genetic testing including analysis of samples of DNA ,in criminal forensics and identification of infectious agents (such as SARS CoV II virus for COVID)

Principle of PCR

- PCR is based on using the ability of DNA polymerase enzyme to synthesize new strand of DNA complementary to the template strand.

- At the end of the PCR reaction, the specific sequence will be accumulated in billions of copies (amplicons).

Components of PCR

1. DNA template
2. DNA Polymerase
3. Primers
4. Deoxynucleotide triphosphates or dNTPs
5. Buffer solution
6. Bivalent cations

Components of PCR

1. DNA template

The sample DNA that contains the target sequence.

2. DNA polymerase

A type of enzyme that synthesizes new strands of DNA complementary to the target sequence.

*Taq*DNA polymerase - from *Thermus aquaticus*

Pfu DNA polymerase -from *Pyrococcus furiosus*

These two enzymes are heat resistant and

Components of PCR

3. Primers

Primers are short single-stranded DNA fragments known as oligonucleotides that are a complementary sequence to the target DNA region.

Two DNA primers (forward and reverse primers) that are complementary to the 3’ (three prime) ends of each of the sense and antisense strands of target DNA are used.

Components of PCR

4. **Deoxynucleotide triphosphates or dNTPs**

Four types of dNTPs – dATP, dGTP, dCTP, dTTP are the "building blocks" from which the DNA polymerase synthesizes a new DNA strand.

Components of PCR

5. **Buffer solution** provides a suitable chemical environment for optimum activity and stability of DNA polymerase.

6. **Bivalent cations -** Magnesium ions Mg^{++} are required for the activity of DNA polymerases. Manganese ions Mn^{++} can be used for PCR-mediated DNA mutagenesis, as a higher Mn^{++} concentration increase the error rate during DNA synthesis.

PCR is carried out in thermal cycler

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PCR is carried out in thermal cycler

- PCR reaction is carried out in small reaction tubes (0.2-0.5 ml volumes) in a thermal cycler.

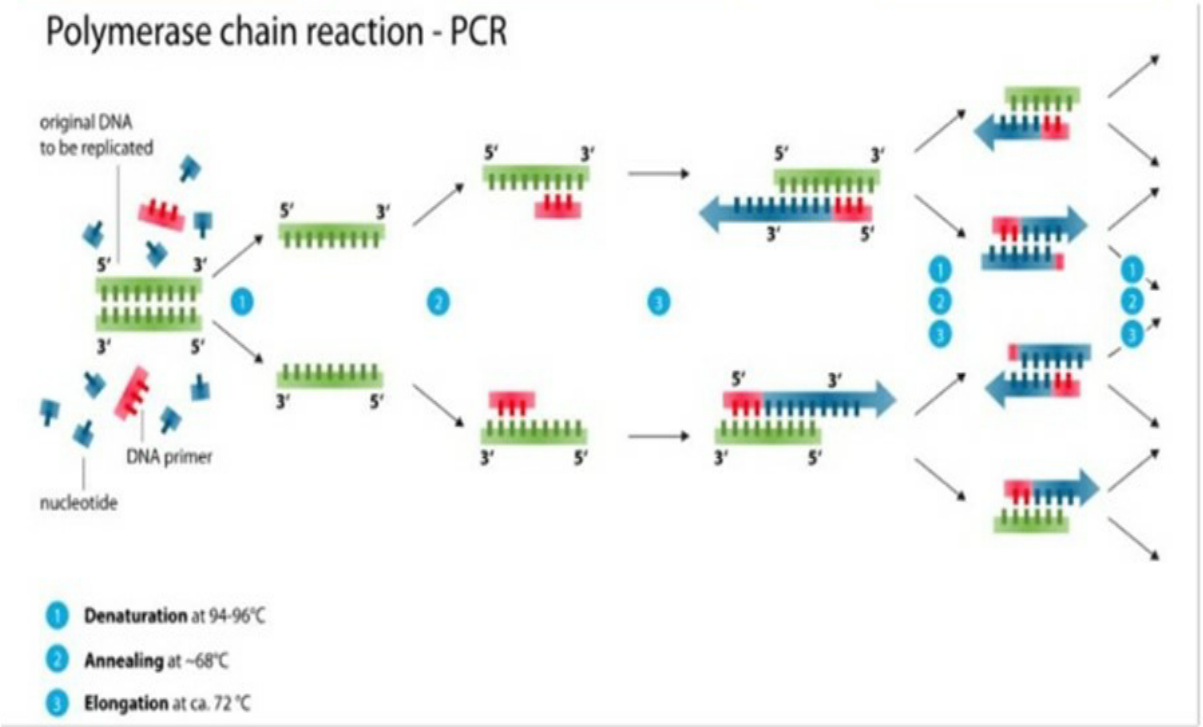
- Thermal cycling exposes reactants to repeated cycles of heating and cooling to permit different temperature dependent reactions.

The three steps of PCR are:

I. Denaturation or DNA melting
II. Annealing
III. Extension/ Elongation

Polymerase Chain Reaction (PCR)

STAGES



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Steps of PCR: I. Denaturation or DNA melting

- The initial denaturation step is commonly performed at 94–98°C for 1–3 minutes.

- The two strands of DNA double helix are separated by breaking of hydrogen bonds between complementary bases at a high temperature in a process called nucleic acid denaturation.

- DNA with high GC content (e.g., >65%) requires higher temperature for

Steps of PCR: II. Annealing

- In the second step, the temperature is lowered to 50-65°C for 20-40 seconds and the primers bind/ anneal to the complementary sequences of single stranded DNA templates.
- The annealing temperature is determined by calculating the melting temperature (T_m) of the selected primers
- Annealing temperature is 3–5°C lower than the lowest T_m of the primers.

Melting temperature (T_m) of the selected primers

- T_m is defined as the temperature at which 50% of the primer and its complementary sequence form a duplex.

- T_m can be estimated using the formula:

$$\mathbf{T_m = 4\,(G + C) + 2\,(A + T)}$$

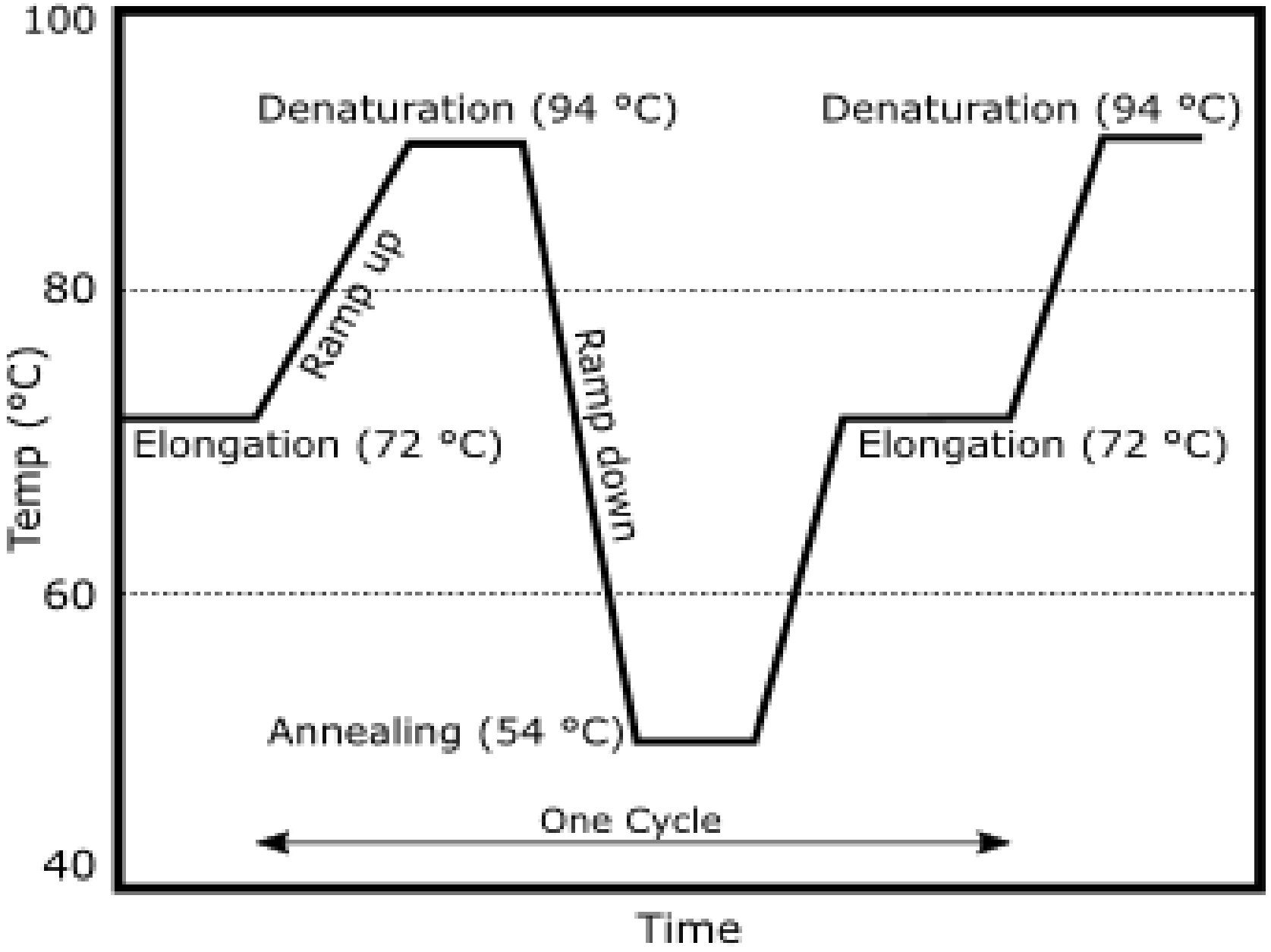
Steps of PCR: 3. Extension/ Elongation:

- In this step DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free dNTPs.

- The temperature in this step depends on the DNA polymerase used . For Taq polymerase it is 72°C.

Typical Cycling Parameters

- The process of denaturation, annealing and elongation constitute a single cycle.
- Multiple cycles are required to amplify the DNA target to millions of copies.
- The formula used to calculate the number of DNA copies formed after a given number of cycles is 2^n, where n is the number of cycles.
- 25-30 cycles of amplification are recommended.



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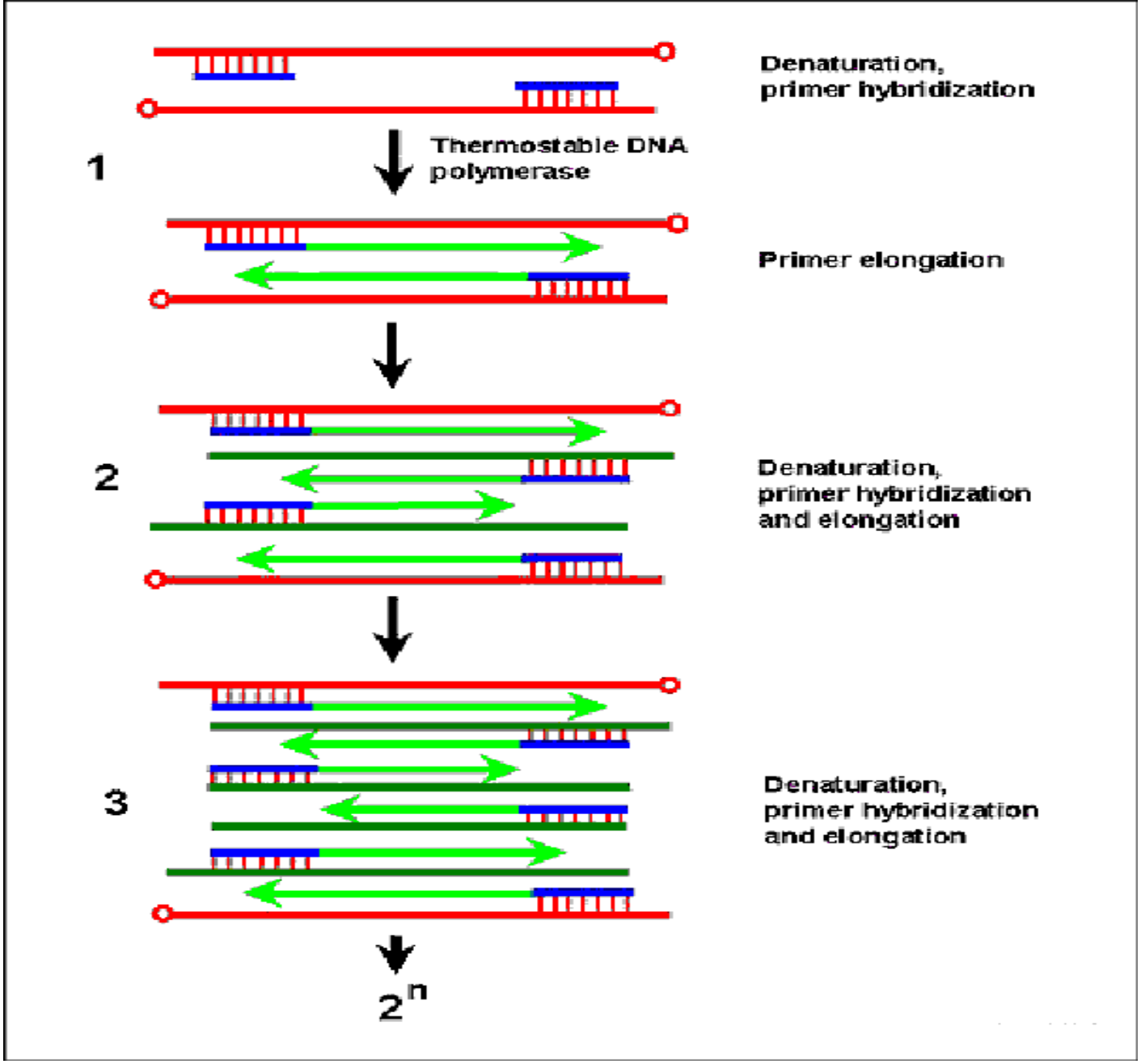

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Applications of PCR

Cancer detection

- Circulating tumor cells produce unique mRNA transcripts depending on the type of cancer.
- Expression levels of mRNA transcripts that serve as the best biomarkers for a particular cancer cell type are analyzed by RT-PCR (reverse transcription PCR).

Studying the genomes of viruses

- RT-PCR is commonly used in studying the genomes of viruses whose genomes are composed of RNA, such as Influenza virus A, retroviruses like HIV and Sars-Cov-2 .

Gene expression studies

- Variations in gene expression among cell types, tissues, and organisms at a specific time point are commonly examined by RT-PCR.
- In this process, RNA is isolated from samples of interest and the messenger RNA (mRNA) is reverse-transcribed into complementary DNA (cDNA).
- The original levels of mRNA can then be determined from the quantity of cDNA amplified in PCR.
- This process is also known as **reverse**

Genotyping

- PCR can be used to detect sequence variations in alleles in specific cells or organisms. The primer sets are designed to flank regions of interest and assess genetic variations based on the presence or absence of an amplicon.
- Genotyping by PCR is also a fundamental aspect of **genetic analyses of mutations** in cancer and heredity.

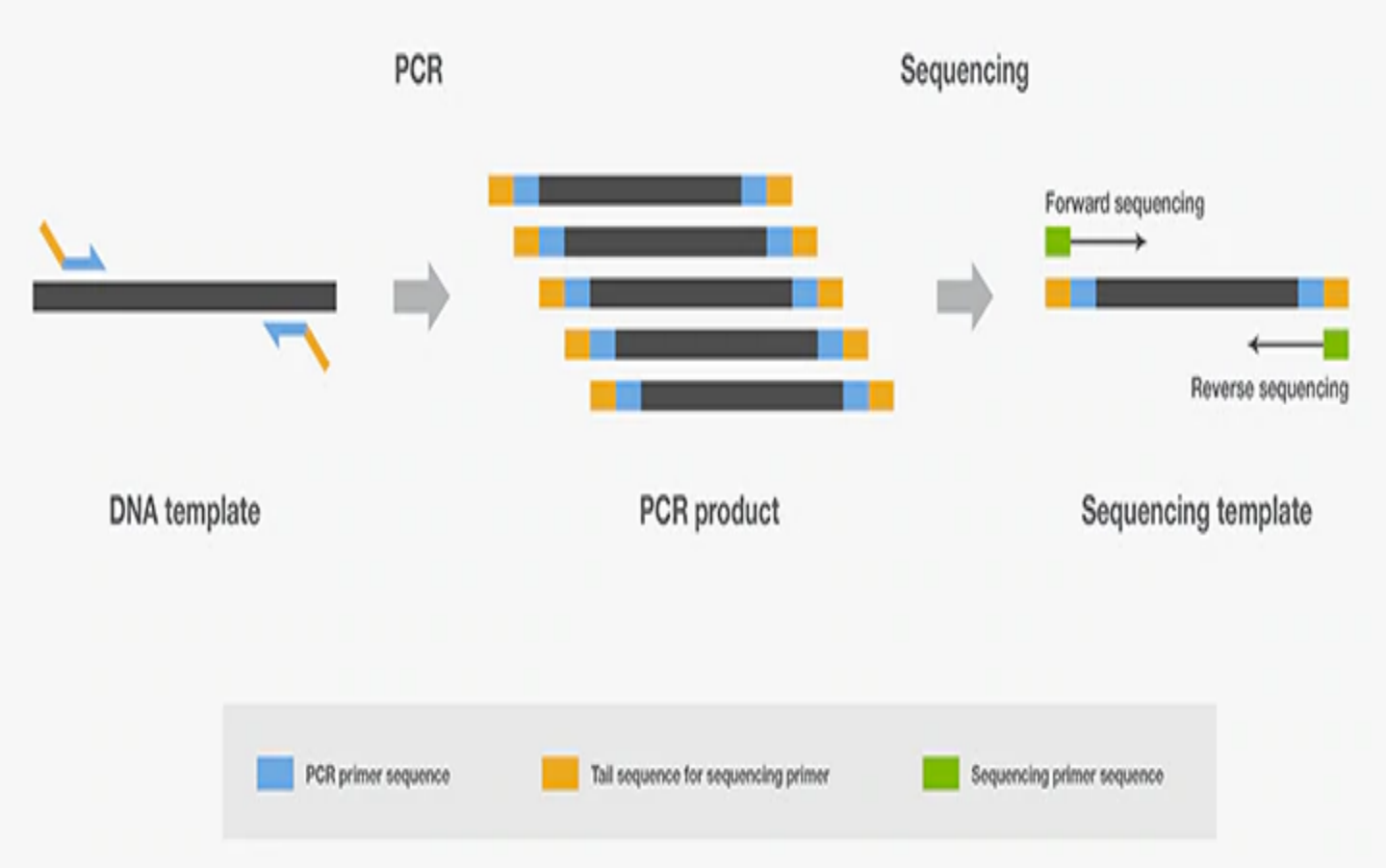
Cloning

- PCR is widely used in cloning DNA fragments of interest, in a technique known as PCR cloning.
- In **direct PCR cloning**, the desired region of a DNA source (e.g., gDNA, cDNA, plasmid DNA) is amplified and inserted into specially designed compatible vectors.
- PCR is a useful method after cloning to screen for whether colonies carry the desired insert

Mutagenesis

- One of the benefits of PCR cloning is the ability to introduce desired mutations into the gene of interest via cloning, for mutagenesis studies.
- In **site-directed mutagenesis**, PCR primers are designed to incorporate base substitutions, deletions, or insertions within a specific sequence.
- The PCR product, containing the introduced mutation, is then self-ligated to regenerate a circular plasmid and used to transform competent cells.

In **Sanger sequencing**, PCR-amplified fragments are purified and subjected to the sequencing reactions.



Medical, forensic, and applied sciences

- Examples of **molecular diagnostics** include genetic testing, detection of oncogenic mutations, and testing for infectious diseases.
- In **forensics**, human identification by PCR relies on amplification of unique short tandem repeats (STRs) on gDNA to differentiate individuals.
- In **agriculture**, PCR plays an integral role in food pathogen detection, plant genotyping for breeding, and GMO testing.

Let's revise

Q.1 Describe the principle and steps of PCR.

Q.2 Why Taq polymerase is used in PCR?

Q.3 What is RT PCR?

Q.4 What are the different applications of PCR technique?

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