Djilali Bounaama University – Khemis Miliana **Level: L3 – Microbiology**
Faculty of SNV/ST **Academic Year: 2024/2025**
Department of Biology **Dr. Ghlis Dahmani B**
Course: Molecular Biology and Genetic Engineering (Tutorial Session)

Review on PCR (Polymerase Chain Reaction)

PCR is a molecular biology technique that allows the exponential amplification of a specific DNA sequence starting from a small initial quantity.

1. Principle of PCR

PCR relies on the use of a thermostable DNA polymerase (such as Taq polymerase) to synthesize new copies of a target fragment through successive cycles, in the presence of specific primers and free nucleotides (dNTPs).

2. Steps of PCR

2.1. Denaturation (94–98 °C)

Heating separates the two DNA strands by breaking hydrogen bonds between complementary bases. Result: single-stranded DNA.

2.2. Annealing (50–65 °C)

Lowering the temperature allows short oligonucleotide primers to bind to complementary sequences on the single-stranded DNA. The temperature depends on the melting temperature (Tm) of the primers.

2.3. Extension (68–72 °C)

DNA polymerase adds nucleotides to the primers, synthesizing new complementary strands. The duration depends on the length of the amplified fragment (approximately 1 kb/min).

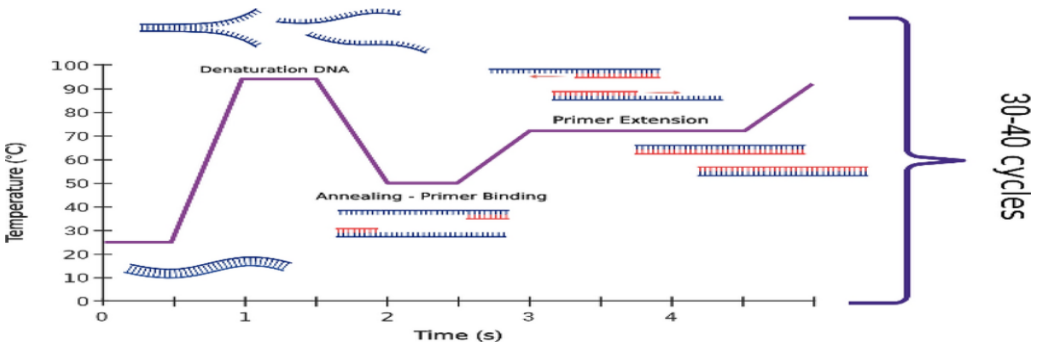

Figure 1. Temperature cycling profile of PCR.

3. Components of the PCR Reaction

• Template DNA: the target sequence to be amplified.

• Primers: oligonucleotides complementary to the ends of the target sequence.

• Thermostable DNA polymerase: commonly Taq polymerase.

• dNTPs: the four nucleotides required for DNA synthesis (dATP, dCTP, dGTP, dTTP).

• Buffer: maintains pH and provides Mg^{2+} ions essential for enzymatic activity.

• Water: used to adjust the final volume.

The reaction is carried out in a device called a thermocycler, which is a sophisticated water bath capable of rapidly changing temperatures according to programmed conditions.

Figure 3. Thermocycler used for PCR.

4. Melting Temperature (Tm) of Primers

The melting temperature is the temperature at which 50% of the primers are hybridized to their target sequences.

Approximate formula: $Tm = 4(G + C) + 2(A + T)$

5. Types of PCR

5.1. Standard (Conventional) PCR

Amplifies a specific DNA sequence using primers and Taq polymerase. Produces exponential amplification but does not quantify the product. Applications include mutation identification, gene cloning, and basic diagnostics.

5.2. Real-Time PCR (qPCR)

Monitors DNA amplification in real time using fluorescent probes or dyes (e.g., SYBR Green). Allows rapid and precise quantification with minimal contamination risk. Applications: diagnostics and gene expression analysis.

5.3. Multiplex PCR

Uses several primer pairs to amplify multiple targets simultaneously. Saves time and reagents but requires optimization. Applications: differential diagnosis and multi-mutation analysis.

5.4. Nested PCR

Two-step amplification: first with external primers, then with internal primers. Highly specific but time-consuming. Applications: detection of low-abundance DNA.

5.5. Reverse Transcription PCR (RT-PCR)

Combines reverse transcription of RNA into cDNA with conventional PCR. Used for RNA viruses and gene expression studies.

5.6. Digital PCR (dPCR)

Detects rare sequences, copy number variations, and mutations with high precision. More expensive than qPCR. Applications: quantification of rare mutations and pathogen detection.

5.7. PCR for DNA Walking

PCR-based DNA walking (genome walking) is a set of molecular techniques designed to identify and amplify unknown DNA regions adjacent to a known sequence. These methods are essential for gene discovery, mapping integration sites, and characterizing regulatory elements, especially when whole-genome sequencing is impractical.