Djilali Bounaama University – Khemis Miliana **Level: L3 – Microbiology**
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Course: Molecular Biology and Genetic Engineering (Tutorial Session)

Review on the Preparation and Separation of Nucleic Acids

1. Preparation of Nucleic Acids: Extraction and Purification

The extraction and purification of nucleic acids are crucial steps for obtaining high-quality DNA or RNA suitable for analysis, cloning, or sequencing. The main stages are as follows:

• Cell Lysis:

- This step involves breaking open the cells to release nucleic acids. Physical methods (such as freeze–thaw cycles, sonication, or grinding) or chemical methods (using detergents like SDS) can be applied.
- Enzymes such as **proteinase K** (for DNA extraction) or **RNase** (for RNA extraction) are often added to degrade proteins and contaminants that could interfere with nucleic acid analysis.

• Removal of Proteins and Other Contaminants:

- After cell lysis, the mixture contains proteins, lipids, salts, and other impurities. Common techniques for their removal include **salt precipitation**, **centrifugation**, or the use of **organic solvents** (e.g., phenol–chloroform extraction) to separate nucleic acids from proteins.
- These techniques make it possible to recover an **aqueous phase** containing purified DNA or RNA.

• DNA/RNA Precipitation:

- Nucleic acids can be precipitated to recover them in solid form. This step involves adding **ethanol** or **isopropanol**, sometimes with salts such as **sodium acetate** to facilitate precipitation.
- The mixture is then centrifuged to collect the DNA/RNA as a small **pellet** at the bottom of the tube.

• Final Purification:

- Commercial purification kits using **spin columns** or **magnetic beads** enable rapid isolation of high-purity nucleic acids.
- This purification step removes residual contaminants, ensuring that the extracted DNA or RNA is ready for molecular biology applications (such as **PCR**, **sequencing**, etc.).

2. Separation of Nucleic Acids: Electrophoresis

Electrophoresis is a key method used to visualize and separate DNA or RNA fragments. The principle is based on the fact that nucleic acids, being **negatively charged**, migrate toward the **anode (positive pole)** in an electric field.

• Agarose Gel Electrophoresis:

- An **agarose gel** is prepared by dissolving agarose in a buffer and pouring it into a mold. Once solidified, the gel forms a **porous matrix** through which nucleic acids can move.
- DNA fragments are loaded into **wells** at the top of the gel. When an electric current is applied, the fragments migrate through the gel: **smaller fragments** travel faster and farther than **larger ones**.
- Visualization of DNA fragments is performed using **staining agents** (such as **ethidium bromide** or safer dyes like **SYBR Safe**) that bind to DNA and allow detection under **UV light**.

• Pulsed-Field Gel Electrophoresis (PFGE):

- This technique is used to separate **very large DNA fragments**, such as whole chromosomes or large genomic fragments (**>50 kb**).
- Unlike standard gel electrophoresis, the electric field is applied **alternately in different directions** (with periodic changes in polarity or "pulses"). This allows large DNA molecules to reorient and migrate through the gel more efficiently.
- PFGE thus improves the resolution of large DNA fragments that would otherwise migrate very slowly or remain trapped in a standard agarose gel.