Molecular Biology Laboratory

Lab 4: DNA extraction

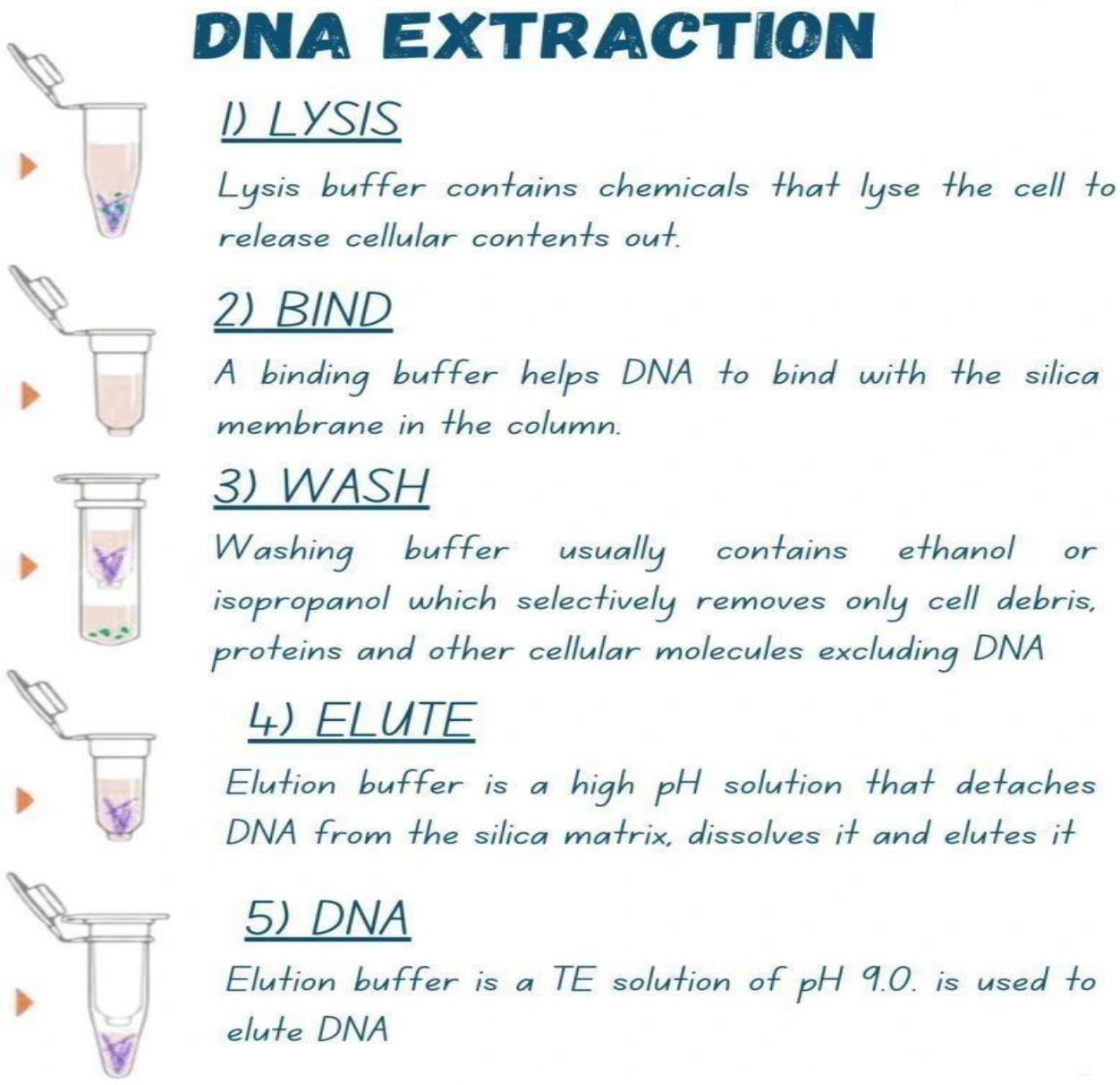
Since DNA is the blueprint for life, everything living contains DNA.DNA isolation is one of the most basic and essential techniques in the study of DNA. The extraction of DNA from cells and its purification are primary importance to the field of forensics and biotechnology. Nucleic acid isolation and purification is a set of molecular biology techniques used for the extraction of DNA and RNA for use in downstream applications, such as sequencing, cloning, and polymerase chain reaction. These downstream applications are important parts of life science research, molecular diagnostics, forensics, and genetic engineering. The process of extracting DNA from operations necessary to obtain a sample of DNA and whatever the source extraction (bacteria, eukaryotic cells), the extraction process also provides remove impurities (الشوائب) associated with proteins and fats model and others.

Most DNA extraction protocols consist of two parts

1. A technique to lyse the cells gently and solubilize the DNA

2. Enzymatic or chemical methods to remove contaminating proteins, RNA, or macromolecules

While In plants, the nucleus is protected within a nuclear membrane which is surrounded by a cell membrane and a cell wall. Four steps are used to remove and purify the DNA from the rest of the cell: Lysis, Precipitation, Wash, Resuspension.



There are nucleic acids in living cells are interconnected with proteins as they appear in the cell is a Nucleoprotein complex, so begin extraction first process of cracking walls or cell membranes are careful to allow the exit of DNA and other cellular components without exposing them to significant damage, then place the process of separating and extracting DNA from these complexes (proteins) through the process of removing proteins (Deproteinization process) involving three transactions:

1) **Enzymatic Treatments**: These transactions involve the use of proteiolytic enzymes such as proteinase K & Pronase that lead to cracking molecule of protein to short peptide chains to facilitate removed in subsequent transactions.

2) **Chemical Treatments**: which divided in three treatments:

- **Chelating agents** such as EDTA (Ethylene Diamine Tetra Acetic Acid), It has the ability to pull and remove ions bilateral parity Ca++ & Mg++ That contribute to maintaining the stability of protein complex and the stability of nuclear and cellular membranes, in addition to being catalysts for the effectiveness of enzymes Nuclease.
- **Detergents agents**: such as SDS (Sodium dodecyl sulfate) of ionic detergents and Sarkosyl you prefer to use on the SDS because the latter works on cracking small pieces of DNA either Sarkosyl be few crackers.

On the other detergents Tritonx-100 which is non-ionic detergents. Detergents considered highly effective crash factors acting on breaking peptide bonds and thus disengagement amino acids.

- **Treatment organic solvents**: such substance Isoamyl alcohol, Chloroform, Phenol: acting with Two- Phase system, when treatment Lyset cell or cell lysis, these solvents or a mixture of them in addition to helping denaturation (مسخ)and remove the fat, they lead to the formation of several phases due to their inability to mixing with the water fully and when used the DNA is pulled to the aqueous phase which formed.

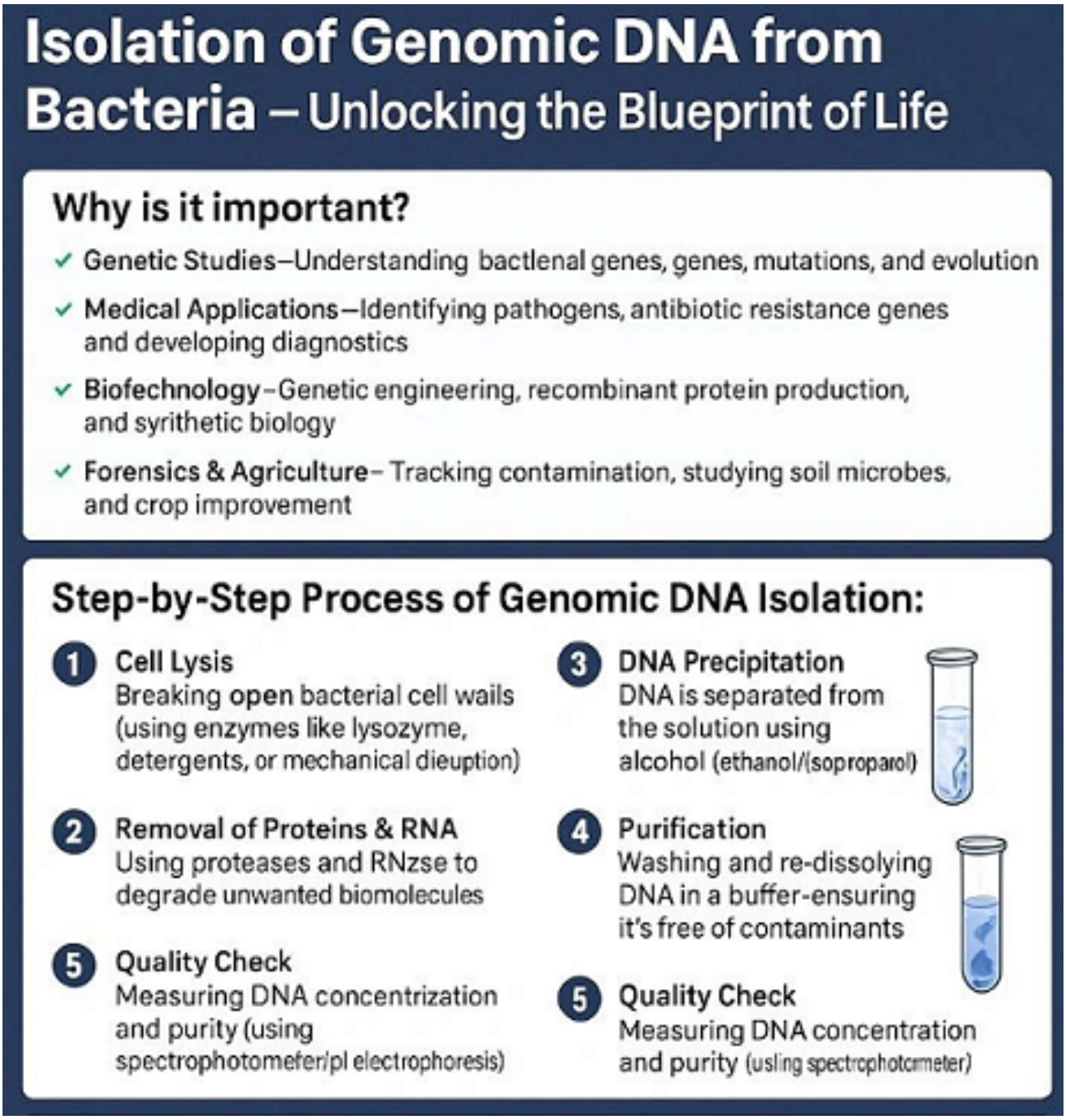
Is sometimes used ether saturated with water as it works to dissolve the phenol and chloroform before deposition of DNA aqueous phase as using cold alcohol, the presence of salt, such as sodium acetate for the purpose of DNA deposition. As the **salt works** on:

1 - Keep the secondary structure of DNA without denaturation.

2 - inhibits the growth of microorganisms.

3 - equivalent to the base of the SDS

3) **Mechanical Treatments**: This processes by using Centrifuge. The type of treatments used in extraction depends on the type of tissue you want to isolate the DNA from it, and in general, the extraction of DNA from animal tissue is much easier than the isolation of plant tissue for hardness cellular walls plants add to oppose (تعارض) the presence of sugars and other metabolic product with purification processes.



Applications of Bacterial Genomic DNA Isolation

PCR & Sequencing
Detecting and characterizing genees

Microbiome Studics
Exploring microbial communities in soil water and the human gut

Cloning & Recombinant DNA
Producing insulin, vaccines, enzymes, and biofuets

Agricultural Biotechnology
Enhancing crop resistance and sail fertility