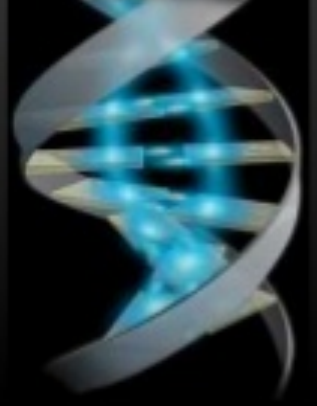
DNA EXTRACTION: THEORY AND PRACTICAL ASPECT

Presented by:
Dr. Sonia Sharma
Assistant Professor
Department of Biotechnology(ccs)

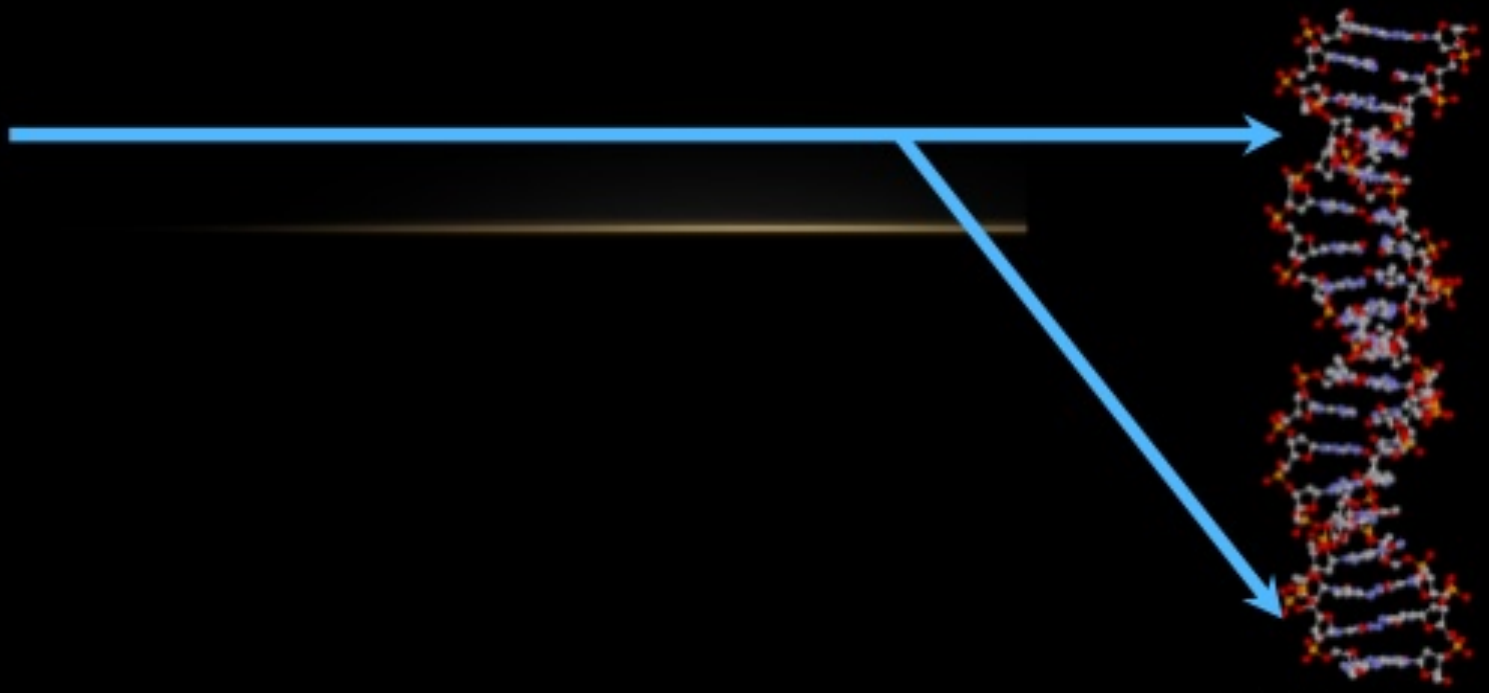
Contents

- Structure of DNA
- LOCATION OF DNA
- DNA Extraction
- Most commonly used DNA extraction methods
- Video demonstration of DNA extraction from blood using kit

DNA (DEOXYRIBONUCLEIC ACID)



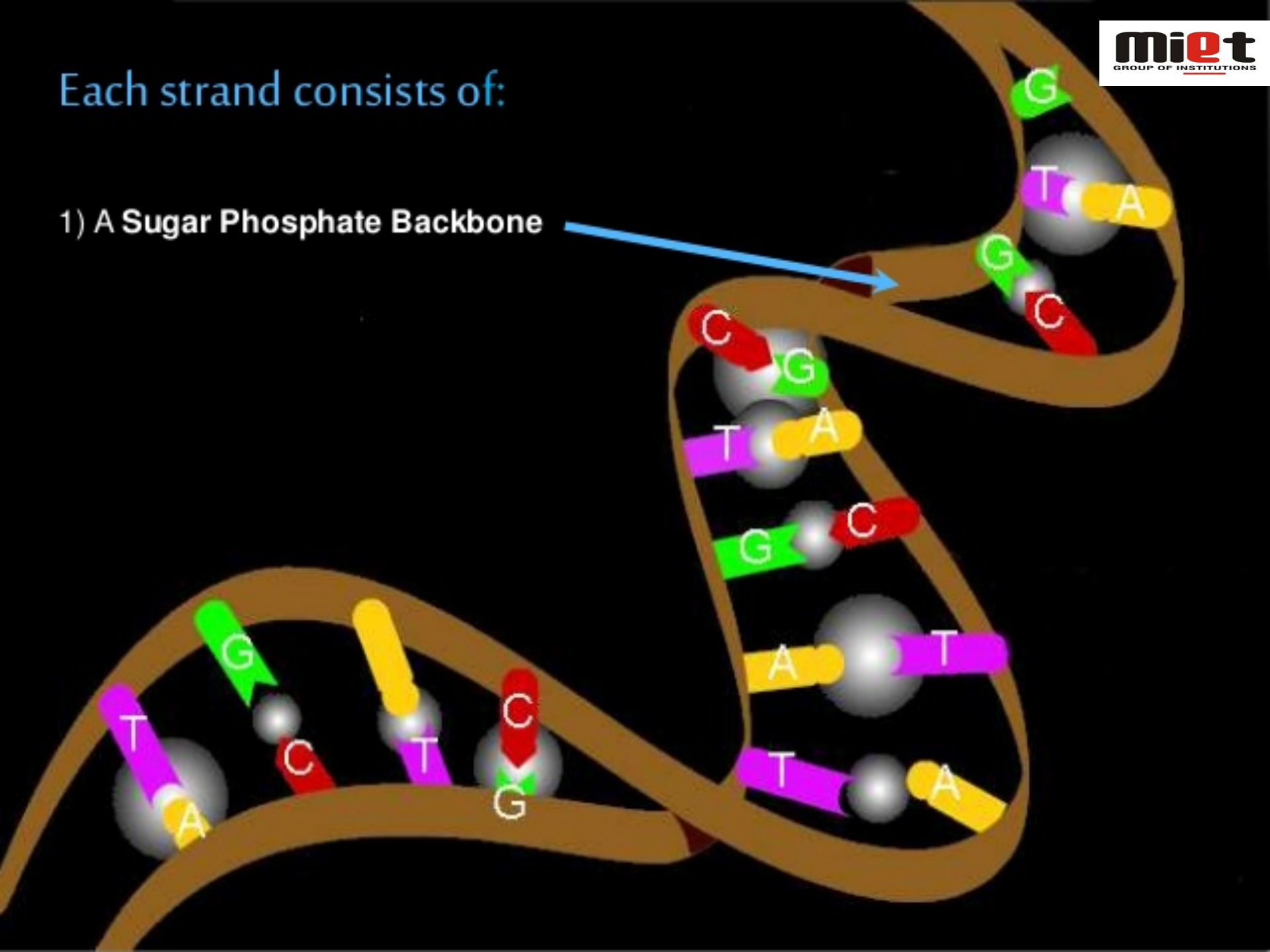
DNA is a double stranded molecule that is twisted into a **Helix** (Spiraling Staircase)



DNA Double Helix

Each strand consists of:

1) A **Sugar Phosphate Backbone**



STRUCTURE

Basic structure of DNA is a sugar-phosphate backbone with 4 variable nitrogenous bases. This structure is called a nucleotide.

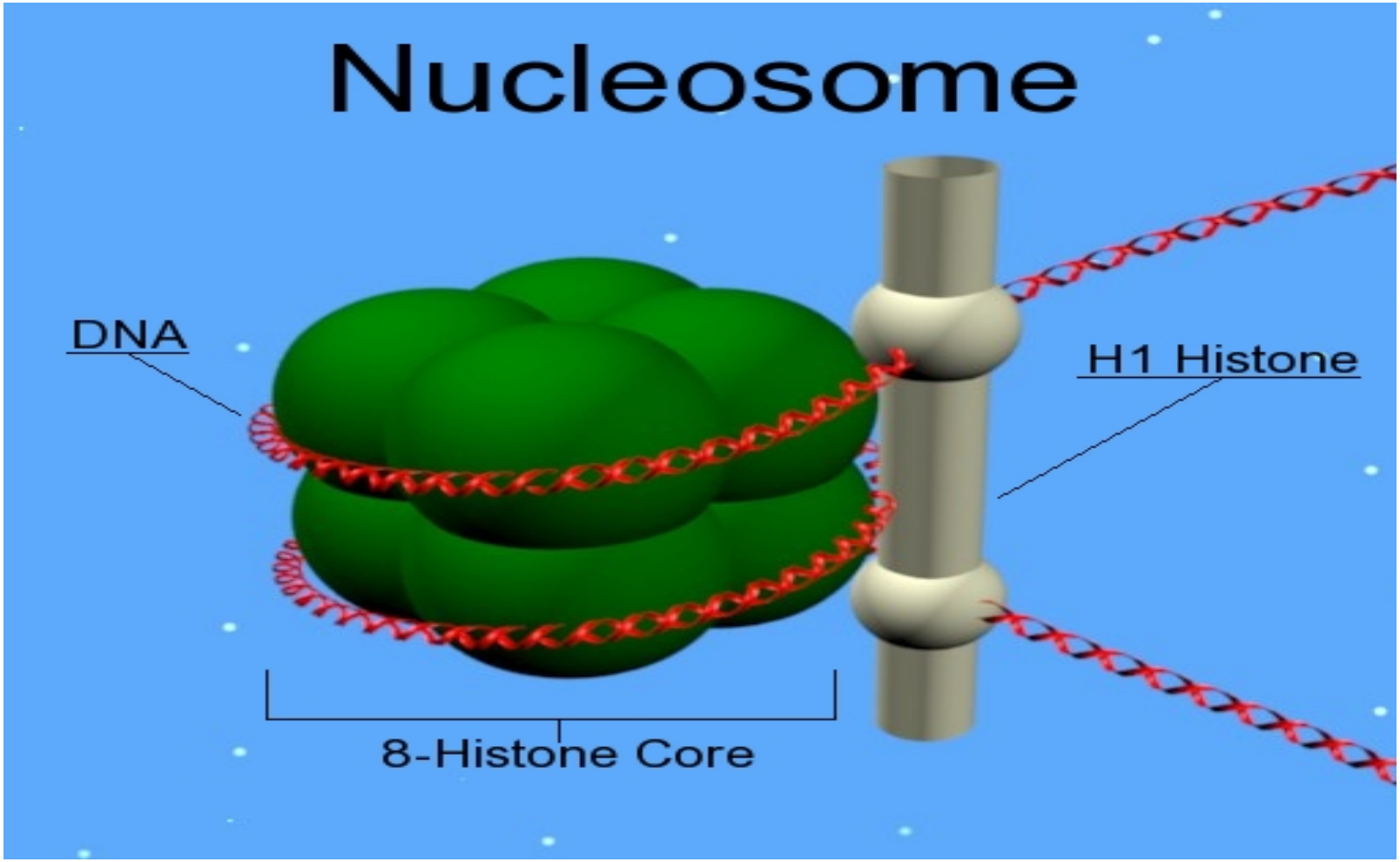
CONTD……

- When the covalent bonds are formed between nucleotides they attach in the direction of 5’ ->3 ’
- The 5’ end of one nucleotide attaches to the 3’ end of the previous nucleotide

DNA STRUCTURE

- In eukaryotes DNA is associated with proteins
- In prokaryotes the DNA is naked
- Nucleosomes is the basic beadlike unit of DNA packing
- It is made of a segment of DNA wound around a protein core that is composed of 2 copies of each of 4 types of histones

STRUCTURE OF NUCLEOSOME



CONTD……

- Nucleosomes have 8 histones in the core
- DNA wrapped twice around the core
- One histone holding the nucleosome together
- A DNA ‘linker’ continuing towards the next nucleosome

CONTD…..

- The DNA has a negatively charged backbone (because of the phosphate groups)
- The proteins (the histones) are positively charged
- The DNA and proteins are electromagnetically attracted to each other to form chromatin

DNA LOCATION IN CELL

- Nearly every cell in a person's body has the same DNA.
- Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA)

DNA EXTRACTION

- In order to study DNA, you first have to get it out of the cell. In eukaryotic cells, such as human and plant cells, DNA is organized as chromosomes in an organelle called the nucleus. Bacterial cells have no nucleus. Their DNA is organized in rings or circular plasmids, which are in the cytoplasm. The DNA extraction process frees DNA from the cell and then separates it from cellular fluid and proteins so you are left with pure DNA.

DNA EXTRACTION

- DNA extraction is a procedure used to isolate DNA from cells.
- Purpose of DNA Extraction To obtain DNA in a relatively purified form which can be used for further investigations, i.e. PCR, sequencing, etc

PCR: Polymerized chain reaction

DNA EXTRACTION

- What does DNA extraction involve?

Step 1. Breaking cells open to release the DNA

Step 2. Separating DNA from proteins and other cellular debris.

Step 3. Precipitating the DNA with an alcohol

Step 4. Cleaning the DNA

Step 5. Confirming the presence and quality of the DNA

BASIC STEPS

- The cells in a sample are separated from each other, often by a physical means such as grinding or vortexing, treating the sample with lysis buffer and put into a solution containing salt. The positively charged sodium ions in the salt help protect the negatively charged phosphate groups that run along the backbone of the DNA.

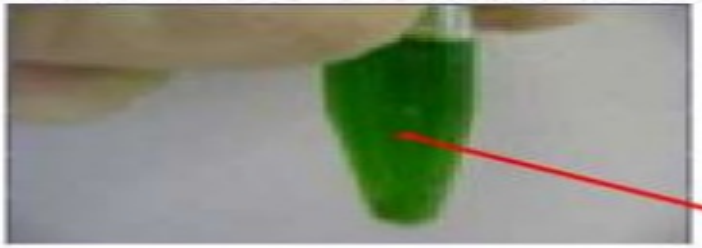
- Purposes of the Extraction Buffer
- 1. Dissolve cellular membranes
- 2. Assist in the removal of contaminants

contd

Step 1: Disruption of cell walls by grinding

Grind sample into a fine powder to shear cell walls and membranes

Step 2: Lysis of cells in extraction buffer



Mix thoroughly with extraction buffer to dissolve cell membranes and inhibit nuclease activity

CONTD….

- Removing membrane lipids by adding a detergent. The detergent breaks down the lipids in the cell membrane and nuclei. DNA is released as these membranes are disrupted.

- Removing proteins by adding a protease (optional but almost always done).
- Precipitating the DNA with an alcohol — usually ice-cold ethanol or isopropanol.
- Since DNA is insoluble in these alcohols, it will aggregate together, giving a pellet upon centrifugation. This step also removes alcohol-soluble salt.

Breaking cells open to release the DNA

- In this step, the cell and the nucleus are broken open to release the DNA inside and there are two ways to do this.
- First, mechanical disruption breaks open the cells. This can be done with a tissue homogenizer (like a small blender), with a mortar and pestle, or by cutting the tissue into small pieces.
- Mechanical disruption is particularly important when using plant cells because they have a tough cell wall.

- Second, lysis uses detergents and enzymes such as Proteinase K to free the DNA and dissolve cellular proteins.

Separating DNA from proteins and other cellular debris

- To get a clean sample of DNA, it's necessary to remove as much of the cellular debris as possible.
- This can be done by a variety of methods. Often a protease (protein enzyme) is added to degrade DNA-associated proteins and other cellular proteins.
- Alternatively, some of the cellular debris can be removed by filtering the sample.

Precipitation

- When you complete the lysis step, the DNA has been freed from the nucleus, but it is now mixed with mashed up cell parts. Precipitation separates DNA from this cellular debris. First, Na+ ions (sodium) neutralize the negative charges on the DNA molecules, which makes them more stable and less water soluble. Next, alcohol (such as ethanol or isopropanol) is added and causes the DNA to precipitate out of the aqueous solution because it is not soluble in alcohol.

Precipitating the DNA with an alcohol

- Finally, ice-cold alcohol (either ethanol or isopropanol) is carefully added to the DNA sample.
- DNA is soluble in water but insoluble in the presence of salt and alcohol.
- By gently stirring the alcohol layer with a sterile pipette, a precipitate becomes visible and can be spooled out. If there is lots of DNA, you may see a stringy, white precipitate.

Purification

- Now that DNA has been separated from the aqueous phase, it can be rinsed with alcohol to remove any remaining unwanted material and cellular debris. At this point the purified DNA is usually re-dissolved in water for easy handling and storage.

Cleaning the DNA

- The DNA sample can now be further purified (cleaned). It is then resuspended in a slightly alkaline buffer and ready to use.

Confirming the presence and quality of the DNA

- For further lab work, it is important to know the concentration and quality of the DNA.
- Optical density readings taken by a spectrophotometer can be used to determine the concentration and purity of DNA in a sample.
- Alternatively, gel electrophoresis can be used to show the presence of DNA in your sample and give an indication of its quality.
- What can this DNA be used for?
- Once extracted, DNA can be used for molecular analyses including PCR, electrophoresis, sequencing, fingerprinting and cloning.

Most Commonly used DNA Extraction Procedures

- Organic (Phenol-Chloroform) Extraction
- Inorganic (Proteinase K and Salting out)
- NCM / Nylon membrane i.e. kit method(Collection, Storage, and Isolation)

ORGANIC EXTRACTION REAGENTS

- • Cell Lysis Buffer - Non-ionic detergent , Salt, Buffer, EDTA designed to lyse outer cell membrane, but will not break down nuclear membrane.
- • EDTA (Ethylenediaminetetraacetic disodium salt) is a chelating agent of divalent cations such as Mg2+. Mg2+is a cofactor for DNase nucleases. If the Mg2+is bound up by EDTA, nucleases are inactivated.
- • Proteinase K - it is usual to remove most of the protein by digesting with proteolytic enzymes such as proteinase K, which are active against a broad spectrum of native proteins, before extracting with organic solvents. Proteinase K is approximately 10 fold more active on denatured protein. Proteins can be denatured by SDS or by heat.

Contd…..

- Phenol/Chloroform- remove proteinaceous material.
- TE Buffer - Tris-EDTA Buffer: TE buffer is often used to store DNA and RNA.
- EDTA in TE chelates Mg2+ and other divalent metals ions necessary for most causes of DNA and RNA degradation, suppressing these processes.
- Tris is a buffering agent to keep the solution at a defined pH. • Ethanol or isopropanol: which help in precipitation of DNA.

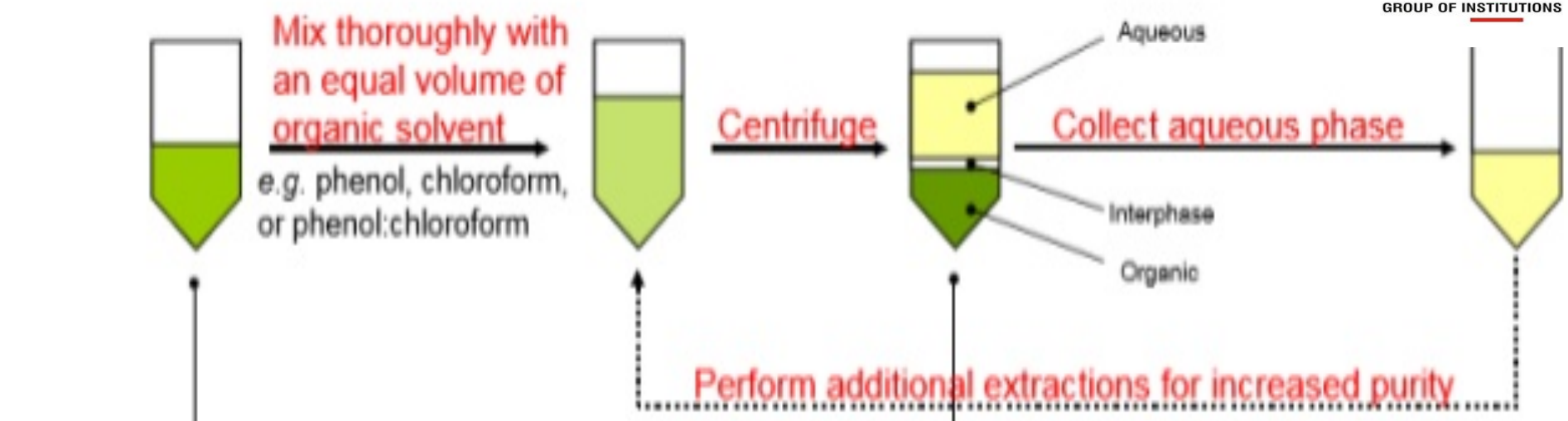
ORGANIC EXTRACTION PROCEDURE

1. Cell Lysis Buffer – This buffer will lyse cell membrane, nuclei are intact, pellet nuclei.
2. The nuclei is Resuspended in a buffer containing Sodium Dodecyl Sulfate (SDS) and Proteinase K. This will Lyse nuclear membrane and digest protein.

ORGANIC EXTRACTION PROCEDURE

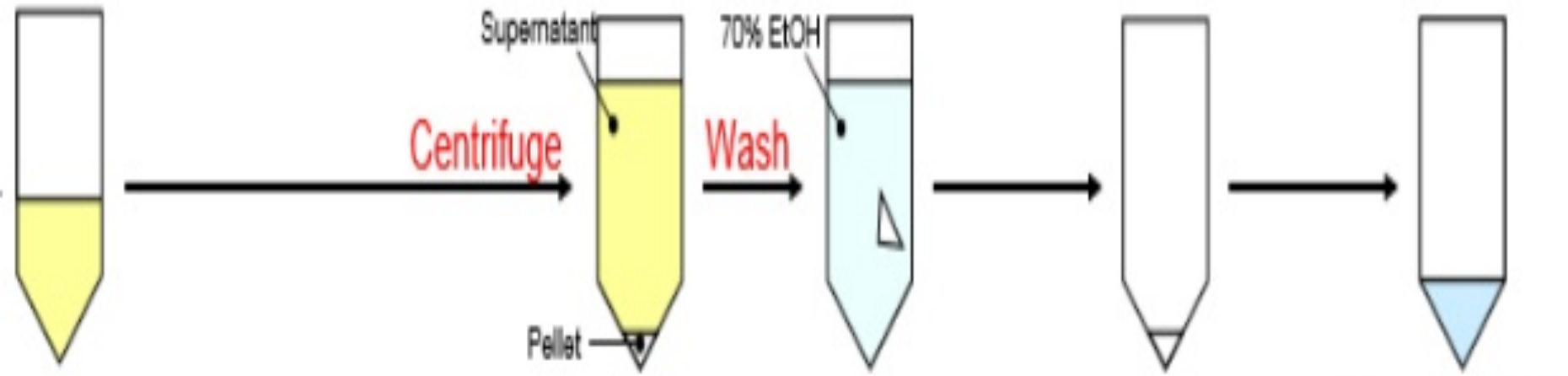
- 3. DNA released into solution is extracted with phenol- chloroform to remove proteinaceous material.
- 4. DNA is precipitated from the aqueous layer by the addition of ice cold 95% ethanol and salt.
- 5. Precipitated DNA is washed with 70% ethanol, dried under vacuum and resuspended in TE buffer.

Step 3: Organic extraction



Crude lysate containing nucleic acids and other cell constituents

The **aqueous phase** contains water-soluble molecules, including nucleic acids. Proteins and lipids become trapped in the **organic phase**, and are thus separated away. Insoluble plant debris become trapped in the **interphase** between the two layers



Add alcohol and salt to precipitate nucleic acids from the aqueous fraction

- Pellet down nucleic acids.
- Wash pellet with 70% ethanol to remove residual salts and other contaminants.
- Discard ethanol and allow pellet to dry.

INORGANIC DNA EXTRACTION

- Does not use organic reagents such as phenol or chloroform.
- Digested proteins are removed by salting out with high concentrations of LiCl. However, if salts are not adequately removed, problems could occur with the procedure due to alteration of DNA mobility (band shifting)

INORGANIC DNA EXTRACTION Procedure

- 1. Cell Lysis Buffer Containing Proteinase - lyse cell membrane, lyse nuclear membrane and digest protein at high temperature e.g. 65℃ for 2 hours. Temperature helps denature proteins, and Proteinase K auto digests itself
- 2. To remove proteinaceous material, LiCl is added to a final concentration of 2.5 M, and incubated on ice. Proteins precipitate out and are pelleted by centrifugation.
- 3. DNA remains in solution. Transfer supernatant to a new tube, care must be taken not to take any of protein pellet.
- 4. DNA is precipitated by the addition of room temperature isopropanol. LiCl will not precipitate with DNA.
- 5. Precipitated DNA is washed with 70% ethanol, dried under vacuum and resuspended in TE buffer.

Membrane based method(i.e. kit method)

- • A spin column using a silica-based extraction method is used. This does not require the use of hazardous chemicals. Nucleic acids are attracted to the silica bead under high chaotropic salt concentrations.
- STEPS • The stages of the method are: • Lyse – The cells of a sample are broken open with a lysis procedure. • Bind – A buffer solution is then added to the sample along with ethanol or isopropanol. This forms the binding solution. The binding solution is transferred to a spin column and the column is put in a centrifuge. The centrifuge forces the binding solution through a silica gel membrane that is inside the spin column. If the pH and salt concentration of the binding solution are optimal, the nucleic acid will bind to the silica gel membrane as the solution passes through.

Contd….

- • Wash – The flow-through is removed and a wash buffer is added to the column. The column is put in a centrifuge again, forcing the wash buffer through the membrane. This removes any remaining impurities from the membrane, leaving only the nucleic acid bound to the silica gel. • Elute – The wash buffer is removed and an elution buffer (or simply water) is added to the column. The column is put in a centrifuge again, forcing the elution buffer through the membrane. The elution buffer removes the nucleic acid from the membrane and the nucleic acid is collected from the bottom of the column.

CONT……

- The lysate is combined with alcohol and placed into the spin column, which is inserted into a tube.
- The removal of proteins and divalent cations is accomplished using multiple buffer washes and centrifugation steps.
- Removal of cations, such as Mg2+, prevents nucleases from further degrading the DNA.
- Pure DNA is eluted from the membrane into sterile water or TE buffer.


Spin
Spin
Spin
Spin
Add Binding buffer
DNA Binding
Washing
Dry
Add Elution buffer
Purified fragment DNA

Practical Demonstration

- https://www.youtube.com/watch?v=gmNw6CWtN5k