University of Al-Maarif

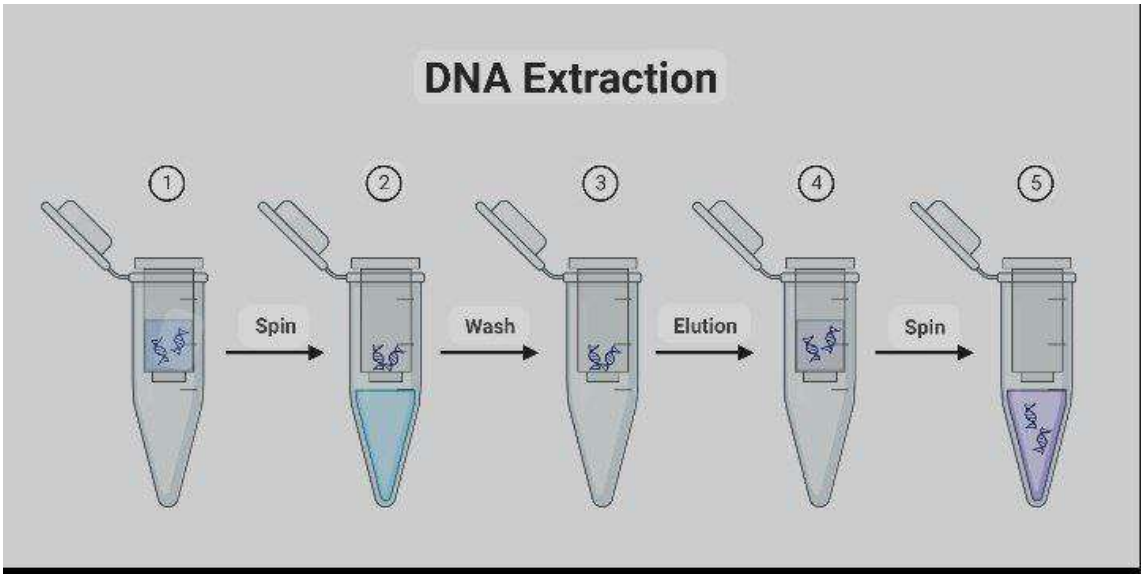
Medical Laboratory Technique Department.

2nd. Stage

Practical Molecular Biology

DNA Extraction

The first isolation of deoxyribonucleic acid (DNA) was done in 1869 by Friedrich Miescher . **DNA extraction** is the process of isolating DNA from the cells of an organism isolated from a sample, typically a biological sample



DNA extraction is used to isolate:

- **Mitochondrial DNA**
- **Genomic DNA**
- **Plasmid DNA (Bacteria)**

Purpose of DNA Extraction

For **studying genetic causes** of various diseases and development of drugs and diagnostics. Additionally, **it is important for conducting forensic science.** Detecting viruses and bacteria within the environment, detecting paternity and sequencing genomes.

DNA extraction involves the following **four steps**:

1- Cell disruption

There are many methods called **Mechanical methods** that **breaking down the walls** and **cell membranes** and the exit of the internal components of the cell without harming it. These methods include grinding, blending, and high pressure.

- Plant cells, **breaking down the walls** done using a **Mortar and Pestle**, where liquid nitrogen is added at a temperature of 176 degrees below zero.
- Bacterial cells by enzyme.

**Detergents work to remove lipids from the cell membrane in addition to having an inhibitory effect on DNases enzymes, which breaks down DNA.

Cell membranes are dissolved by an extraction solution as **EDTA** and **SDS**. EDTA (ethylenediaminetetraacetic acid) **works to remove magnesium ions** that maintain the plasma membrane, while SDS (sodium dodecyl sulfate), it works to **destroy cell** membranes by **decomposing lipids**.

Q: How can be overcome the enzyme of cells after breaking the cell wall during DNA Extraction??

2-Purification of DNA from cell extract

The cell extract contains **proteins, enzyme and RNA.** The presence of these materials impedes the extraction of DNA in a pure form, so these materials must be disposed of.

Removal of protein:

Proteins can be removed from the cell extract by different methods and processes called deproteinization process where specific proteins and enzymes are used to break down proteins.

There are **two ways to remove proteins from a solution**:

A- Deproteinization using organic solvents:

Most of the methods used to remove proteins depend on the solubility of these substances in water. Nucleic acids are hydrophilic and dissolve easily in water layers, while proteins contain hydrophobic radicals, so they dissolve in organic solutions such as phenol and chloroform.

B- Deproteinization using enzymes:

It is possible to remove proteins from the cell extract **using enzymes** such as **proteinase** k or **pronase**. These enzymes are extracted from fungi or manufactured chemically. It is known that enzymes act on the substrate, so with the passage of time for the reaction the substrate decreases and this is one of the **problems of removing proteins with enzymes**, so it is possible to use some negative detergents during extraction.

Removal of RNA:

By add of enzyme(RNase)

** Two enzyme (RNase) used are:

Ribonuclease T and Ribonuclease A**.**

***After** using **organic solvents or enzymes** to remove proteins and RNA from the extraction solution, centrifugation is used to precipitate these deformed particles at the bottom.

3- Precipitation of the DNA

Precipitation of DNA with alcohol depends mainly on reducing the solubility of DNA in water. Therefore, some addition of alcohol to the aqueous

solution makes the DNA strands aggregate, which facilitates their precipitation with centrifugation.

3- Determination of the purity and concentration of DNA

This is the final stage of the DNA extraction process. This is done using a spectrophotometer system at a wavelength of 260 nm. The optical density is used at 260 nm because DNA has the highest absorbance at this optical density, While the lowest absorbance of DNA occurs at a light intensity of 230 nm.

Questions and answers about this Lab.

What is the function of a DNA extraction kit?

A DNA extraction kit provides pre-packaged reagents and materials optimized for efficient and reliable DNA extraction.

What is the purpose of a lysis buffer in DNA extraction?

A lysis buffer is used to break down the cell membrane and nuclear envelope, releasing DNA from cells.

What is the role of a protease enzyme in DNA extraction?

Protease enzymes are used to degrade proteins that may interfere with DNA extraction by binding to or degrading DNA.

What is the function of a chelating agent in DNA extraction?

A: Chelating agents are used to bind and inactivate metal ions that can degrade DNA.

What is the role of a centrifuge in DNA extraction?

A centrifuge is used to spin samples at high speeds, separating the DNA-containing supernatant from other cellular components.

What is the purpose of an ethanol wash in DNA extraction?

An ethanol wash is used to remove impurities and residual salts from the DNA sample.

What is the function of a DNA binding column in DNA extraction?

DNA binding columns contain materials that selectively bind DNA while allowing other contaminants to pass through.

Why is RNase used during DNA extraction?

RNase is used to degrade RNA molecules that may be present in the sample, ensuring that only DNA remains.

What is the purpose of a Elution buffer in DNA extraction?

A DNA elution buffer is used to recover DNA from the DNA binding column (Spin coulum).

Why is phenol-chloroform extraction used in some DNA extraction protocols?

Phenol-chloroform extraction is used to remove proteins and lipids from the DNA sample, improving DNA purity.

Why is liquid nitrogen used in some DNA extraction procedures?

Liquid nitrogen is used to rapidly freeze and disrupt cells, aiding in the release of DNA.

What is the purpose of a buffer in DNA extraction?

Buffers are used to maintain the pH and ionic strength of the DNA extraction reaction, optimizing DNA yield and stability.

What is the role of a vortex mixer in DNA extraction?

A vortex mixer is used to mix reagents and samples thoroughly, promoting efficient DNA extraction.