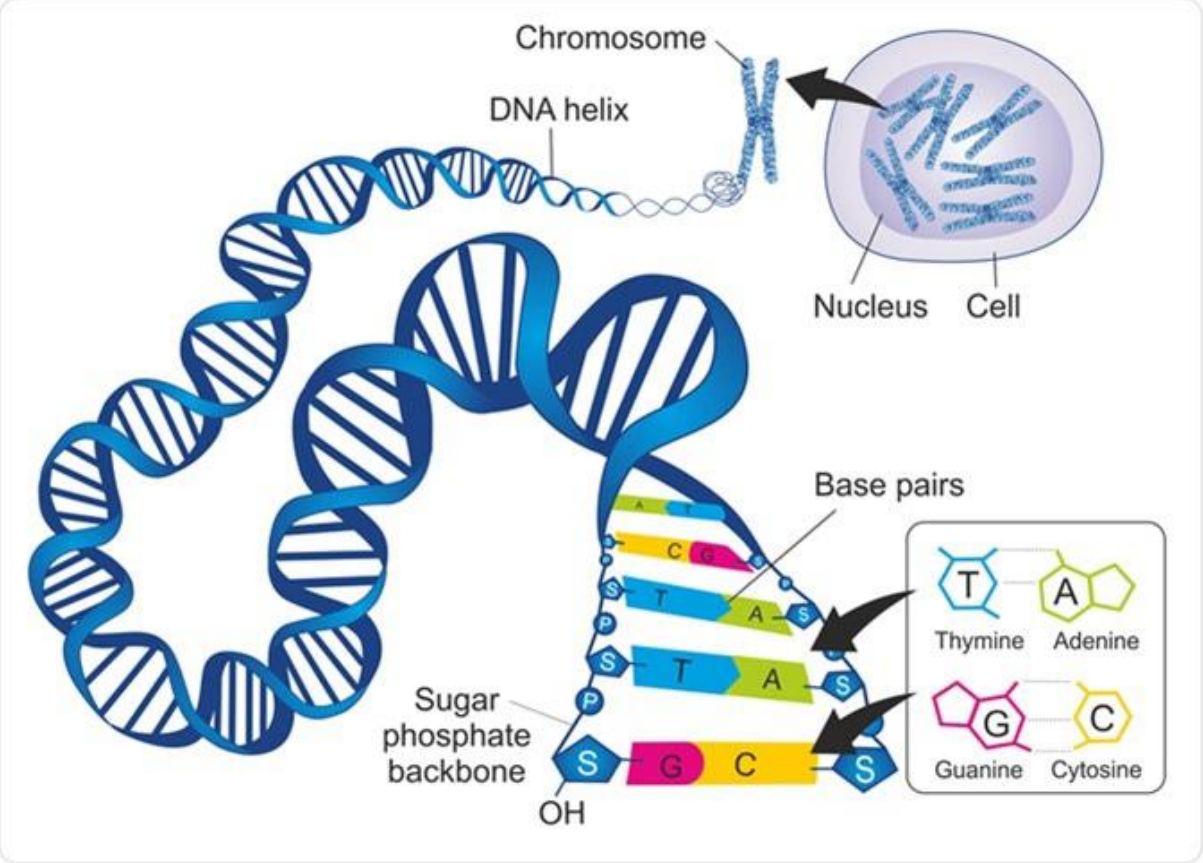
Assist lect. Anas Razak Mohsen & Assist lect. Assaad mudhiy Sakab

Lab. 2 ,3

DNA isolation

DNA (Deoxyribonucleic acid) is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses, being responsible of its hereditary transmission . DNA is molecule of two strand coiled around each other to form a double helix . Each strand is composed by **Sugar, Phosphate , Base (adenine, thymine ,cytosine, guanine)**



Aim of DNA isolation

To separate DNA present in the nucleus of the cell from other cellular components .

Assist lect. Anas Razak Mohsen & Assist lect. Assaad mudhiy Sakab

DNA Extraction kits

Sample selection

Sources for DNA isolation are very diverse

1- Basically it can be isolated from any living or dead organism.

2- Common sources for DNA isolation include whole blood , hair, sperm , bones, nails, tissues, blood stains, saliva , cheek swabs, epithelial cells, urine, paper cards used for sample collection, bacteria, animal tissues, or plants.

3- Stored samples can come from archived tissue samples, frozen blood or tissue, exhumed bones or tissues, and ancient human, animal, or plant samples.

Basic steps for DNA extraction

• 1. Breaking the cells open, commonly referred to as cell disruption or cell lysis, to expose the DNA within. This is commonly achieved by grinding or treating the sample with lysis buffer .

• 2. Removing membrane lipids by adding a detergent.

• 3. Removing proteins by adding a protease (optional but almost always done).

• 4. Precipitating the DNA with an alcohol — usually ice-cold ethanol or isopropanol.

• DNA is insoluble in these alcohols, it will aggregate together, giving a pellet upon centrifugation. This step also removes alcohol-soluble salt .

PROCEDURE

Isolation of DNA basically consists of four major steps

1- Preparation of a cell extract.

2- Purification of DNA from cell extract.

3- Concentration of DNA samples.

4- Measurement of purity of DNA concentration .

1-Preparation of a cell extract

To extract DNA from a tissue/cells of interest, the cells have to be separated and the cell membranes have to be disrupted.

• • The "Extraction buffer" helps in carrying out these processes.

• • Chemicals such as EDTA (Ethylene Diamine Tetra Acetate) which removes Mg^{2+} ions that are essential for preserving the overall structure of the cell membrane, and SDS (Sodium Dodecyl Sulfate) which aids in disrupting the cell membranes by removing the lipids of the cell membranes are included in the extraction buffer.

• * The final step in the preparation of a cell extract is removal of insoluble cell debris. Cell debris and partially digested organelles etc. can be pelleted by centrifugation leaving the cell extract as a reasonably clear supernatant .

2 . Purification of DNA from cell extract.

In addition to DNA the cell extract will contain significant quantities of detergents, proteins, salts and reagents used during cell lysis step and RNA.

A variety of procedures can be used to remove these contaminants, leaving the DNA in a pure form.

• The most commonly used procedures are:

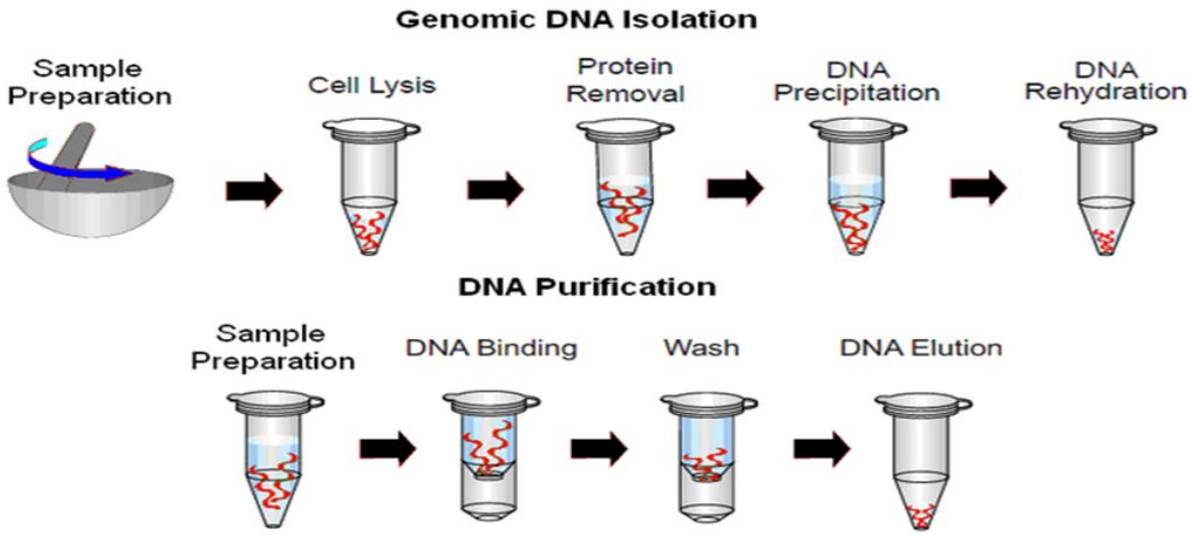
A - Ethanol precipitation.

B - Phenol–chloroform extraction.

C - Minicolumn purification.

3 . Concentration of DNA samples

• The most frequently used method of concentration is ethanol precipitation. In the presence of salt and at a temperature of -20°C or less, absolute ethanol will efficiently precipitate polymeric nucleic acid .

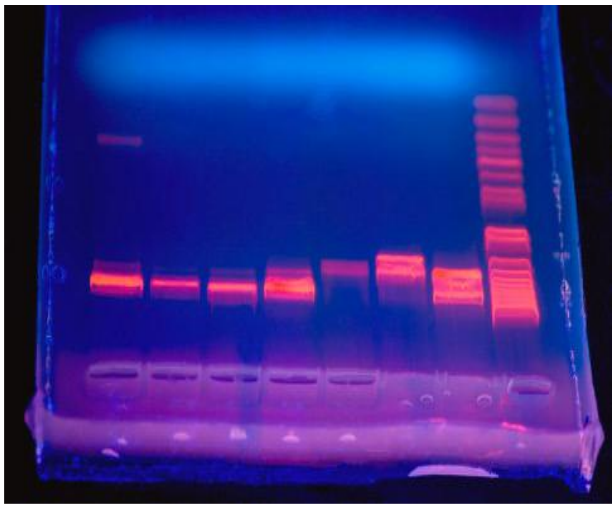


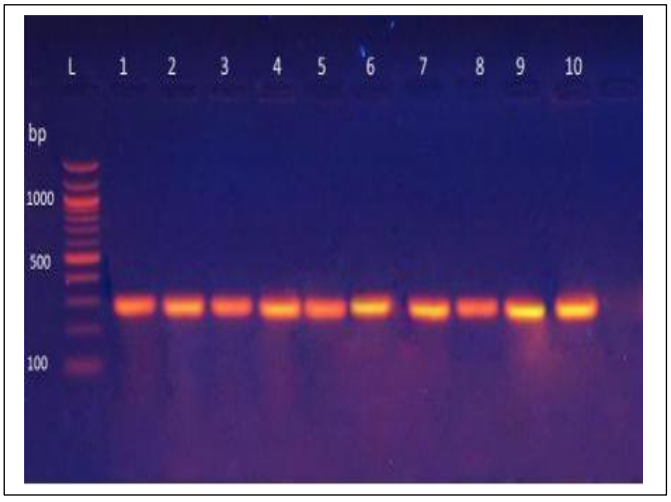
4. Measurement of purity of DNA concentration.

• a. Spectrophotometer / nanometer

b. Electrophoresis in agarose gel

b. Electrophoresis in agarose gel





Purposes of DNA isolation

• Isolation of DNA is needed for genetic analysis, which is used for scientific, medical, or forensic purposes

• * Scientists use DNA in a number of applications, such as introduction of DNA into cells and animals or plants, or for diagnostic purposes, in medicine the latter application is the most common.

• * On the other hand, forensic science needs to recover DNA for identification of individuals (for example rapists, petty thieves, accident, or war victims), paternity determination, and plant or animal identification.