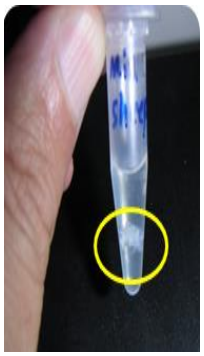
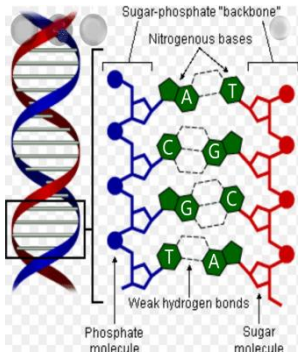
Lab 5 DNA Extraction

DNA (deoxy ribonucleic acid): is a double stranded biological macromolecule that carries genetic information in many organisms. DNA is necessary for the production of proteins, cell growth, regulation, metabolism, and cell reproduction.

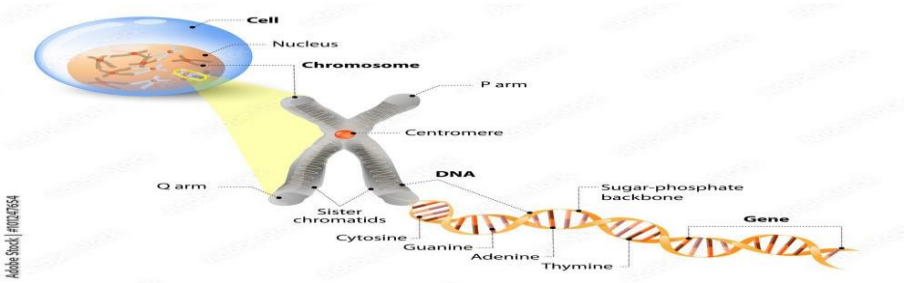




DNA Extraction: is the technique used to isolation and purification of DNA in a biological sample.

Purpose of DNA Extraction

- Used in many techniques such as:
  - PCR (polymerase chain reaction)
  - RFLP (restriction fragment length polymorphism)
  - Southern Blotting
- DNA Analysis:
  - Revealing how organisms are related
  - Locate mutations in DNA



The principles of Isolation and extraction DNA from organisms are one and include the following four steps:

Step 1: Preparation of cell extract (Cells breakage).

Step 2: Purification of DNA from cell extract .

Step 3: Precipitation of the DNA .

Step 4: Determination of the purity and concentration of DNA .

Preparation of cell extract (Cells breakage)

There are many techniques that are used to break down cells in order to extract existing vital molecules such as DNA and RNA.

Mechanical Techniques

1. Automated Milling Technique
2. French Press Technique

Physical Methods

1. Sonication
2. Freezing and Thawing

Chemical Techniques

1. Use Detergents
2. Use of Acid or Basic Treatments

Biological Methods

1. Use of hydrolysis enzymes
2. Phages(bacteriophage)
3. Antibiotics

Purification of DNA from Cell Extract

DNA contains amounts of proteins and RNA . These contaminants should be removed to obtain pure DNA.

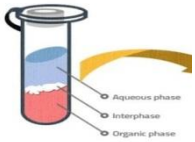
1. Removal of Protein

There are two ways to remove the proteins from the solution:

a. Removal of proteins using organic solvents:

*** The most common organic solvents used in the removal of proteins are phenol and chloroform with a small amount of isoamyl alcohol .

Chloroform: An organic solvent that has a polarizing characteristic. The solvent that works on distribution of cellular contents between two phases organic and water.



b. Removal of proteins using enzymes

The proteins can be removed from the extract using enzymes, which are the most abundant and commonly used as proteinase K and pronase, both enzymes are extract from fungi and is characterized by being free of DNase enzymes. But, it known to be expensive

2. Removal of RNA

*** The best enzymes available for this purpose are ribonuclease A and ribonuclease T1 which can break the RNA molecule, especially at the base of Cytosine and Uracil .

Precipitation of the DNA

usually ice-cold 100%ethanol or isopropanol is used for Precipitation of the DNA, in the presence of moderate concentrations of monovalent cations (salt, such as Na^{+}). Since DNA is insoluble in these alcohols, it will aggregate together, giving a pellet upon centrifugation.

*** The **salts** interrupt the hydrogen bonds between the water and DNA molecules.

* The precipitate containing the DNA is washed with 70 % ethanol in order to remove the salt

***Dissolve DNA in final buffer**

Precipitated DNA is dissolve by addition TE buffer (Tris, EDTA) solution and leaving it overnight to dissolve the DNA pellet completely.

Determination of the purity and concentration of DNA

The concentration and purity of the DNA samples are measured by **Spectrophotometer** and **Nano drop** .

Rapid extraction of high quality DNA from whole blood

Solutions used for DNA extraction (Standard chemicals):

1. **EDTA (0.5 M) pH 8.0**

Function: (It hydrolyzes the cell wall by with drawing the positive magnesium ions Mg^{++} that maintain the stability of the walls and membranes of the cells).

2. **Tris-HCl (1 M) pH 7.6**

Function: (It maintains the stability of the pH of the solution)

3. Red blood cell lysis buffer (RBC lysis buffer)

This solution consists of :
Tris-HCl (0.01M)
Sucrose (320 mM)
$MgCl_2$ (5 mM)
Triton -X 100 (1%)

Function: (It is used to analyze red blood cells and isolate white blood cells to extract DNA from them)

4. Nucleic lysis buffer

This solution consists of :
Tris-HCl (0.01M)
sodium citrate (11.4 Mm)
EDTA (1 mM)
sodium dodecyl sulphate (SDS) (1%)

Function: (It is used to break down the wall of white blood cells during DNA extraction).

5. **TE Buffer , pH 8.0**
6. **Cloroform prechilled to (4° C)**
7. **NaCl (5M)**
8. **Ethanol (100%) prechilled to (-20° C)**
9. **Ethanol (70%)**

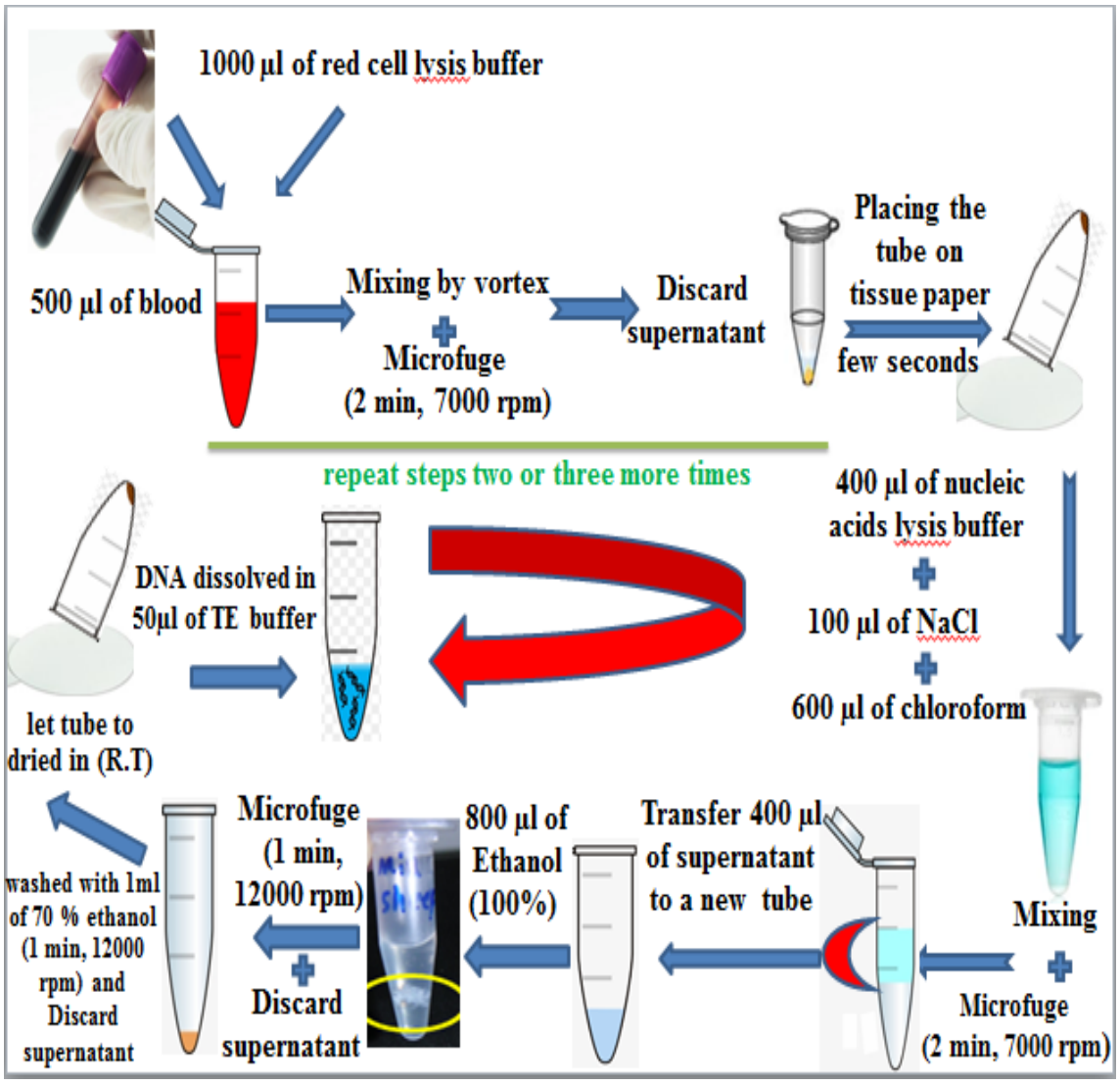
Procedure of DNA Extraction

Blood collection:

Blood should be collected in EDTA tubes. Blood samples can be stored at room temperature for DNA extraction within the same working day or at refrigerator for later uses.

1-Pour (500) µl of blood into a 1.5 ml eppendorf tube then add 1000 µl of red cell lysis buffer. Mixing by vortex, then centrifuge it using microfuge for 2 min (up to homogenizing), at 7000 rpm.
2-Discard supernatant and repeat steps two or three more times to remove hemoglobin. It is important to breakdown the pellet by vortexing and rinses it well in red blood cell lysis buffer in order to clean the white blood cells from residual of hemoglobin.
3-Placing the tube on tissue paper for few seconds.

4-Add 400 µl of nucleic acids lysis buffer to eppendrof tube and mix in vortex.
5-Add 100 µl of saturated 5M NaCl and 600 µl of ice-cold chloroform to the eppendrof tube and mix then spin it for 2 minutes at 7000 rpm.
6-Transfer 400 µl of supernatant to a new 1.5 ml eppendrof tube.
7-Add 800 µl of cold (-20°C) absolute ethanol and mix carefully. DNA should appear as a mucus-like strand in the solution phase.
8-Spin the the microfuge tube for one minute at 12000 rpm to precipitate, then discard supernatant carefully.
9-The precipitate washed with 1ml of 70 % ethanol (for one minute at 12000 rpm), then discard supernatant and let tube be completely dried in room temperature (place eppendrof tube upside down on the tissue paper).
10-Add 50µl of TE to the DNA .keep Eppendrof tube of DNA in 4°C or (-20°)C for later uses.



Extraction of DNA from whole blood