In the process of DNA extraction, living cell wall and membranes are disrupted by some chemical substances and enzymes to release DNA. DNA Extraction is the isolation and purification of DNA. DNA isolation is one the important techniques in molecular biology.

The purpose of DNA isolation isto separate DNA present in the nucleus of the cell from other cellular components such as proteins, lipids, RNA in order to be used for further clinical investigations, such as:

* DNA sequencing
* PCR

Hereditary material DNA is a biological molecule that is now used in medicine for diagnosis, treatment, as well as other units related to biology, for a variety of purposes including genetic engineering.

In prokaryotes and eukaryotes, the cell membranes must be disintegrated first in order to obtain the DNA in the cell. This process is called ”lysis’’.

DNA extraction procedure included 4 main steps:

1. Lysis of cell membranes

2. Removal of proteins

3. Removal of RNA

4. Precipitation of DNA

There are many techniques which are used for DNA isolation. The following is an abbreviated form of the ***salting out method*** currently used for isolation of DNA in the student laboratory.

1. Centrifuge at 2500 rpm for 3 min to precipitate the cells in the medium,
2. Discard the upper phase (supernatant) and add 3ml STE to the remaining pellet,
3. 200 µl of 10% SDS is added and mixed for 30 sec to disrupt the cell membrane integrity and nucleic acids releasing,
4. Add 20µl proteinase K (20mg/ml) and incubate at 55C for 2h (but here we will continue without incubation),
5. After this process, 1 ml of 5.6 M NaCl (saturated salt) is added and 15 sec is inverted to precipitate the proteins and neutralize the DNA.
6. After addition of saturated saline solution, centrifuge at 2500 rpm for 5 min and transfer the supernatant to another tube.
7. 6 ml of 96% EtOH is added to the supernatant transferred to the falcon tube and shake it slowly to precipitated DNA, and makes DNA visible.
8. Visible DNA samples in falcon tubes are transferred to ependorf tubes and centrifuged at 13000rpm for 10 min.
9. After centrifugation, the supernatant is discarded and the underlying DNA pellet is allowed to dry.
10. After the drying process is completed, it is diluted according to the pellet amount and spectrophotometrically determined.
11. After determination of DNA quantity, DNA samples can be stored at +40C for short period, for long-term storage at -200C.

* **EDTA** is responsible for chelation of divalent ions. **It** stops the action of DNases found in cytoplasm of cells. These DNases, **DNA** cutting enzymes, can destroy the genomic **DNA** and reduce the yield of gDNA considerably. Mg+2 is an important factor for activity of DNases. EDTA chelates Mg ions and deactivates DNase enzymes. It also bind to Ca+2 and prevents blood clotting.
* **STE** (0.1 M NaCl, 10 mM Tris-HCl pH: 8.0, 1 mM EDTA) provides hypoosmolar, chelated solvent buffer media for blood cells.
* **SDS** (Sodium Dodecyl Sulfate) used in nucleic acid extraction procedures for the disruption of cell walls and dissociation of nucleic acid:protein complexes.
* **Proteinase K -** It is usual to remove most of the protein by digesting with proteolytic enzymes such as Pronase or proteinase K, which are active against a broad spectrum of native proteins, before extracting with organic solvents. Protienase K is approximately 10 times more active on denatured protein.
* **NaCl (sodium chloride)**: phosphate of DNA molecule repel one molecule from others. Na+ ions form an ionic bond with phosphates and neutralized the negative charge allowing DNA molecules grouping.

**DNA Quantification**

* Sample absorbances are determined on the spectrophotometer at 260nm and 280nm.
* Nucleic acid (DNA, RNA, nucleotides) absorb light at 260nm, whereas protein absorbs light at 280 nm.
* A260/A280 ratio is a measure of DNA purity
* The absorbance wavelength is directly proportional to the concentration of nucleic acid
* **Formula   
  Concentration = 260 Reading \* Absorbance unit \* Dilution factor**
* One optical density or absorbance unit at 260 nm is equal to 50 ug/mL for DNA or 40 ug/mL for RNA.

Spectrophotometer is used to measure DNA concentration. DNA/RNA ratio is a significant marker for quality of DNA.

Results can be altered by contaminants (phenol, proteins)

* If 260/280 = 1.8 means stable DNA amount
* 260/280 > 1.8 (contamination with RNA)
* 260/280 < 1.8 (contamination with protein)