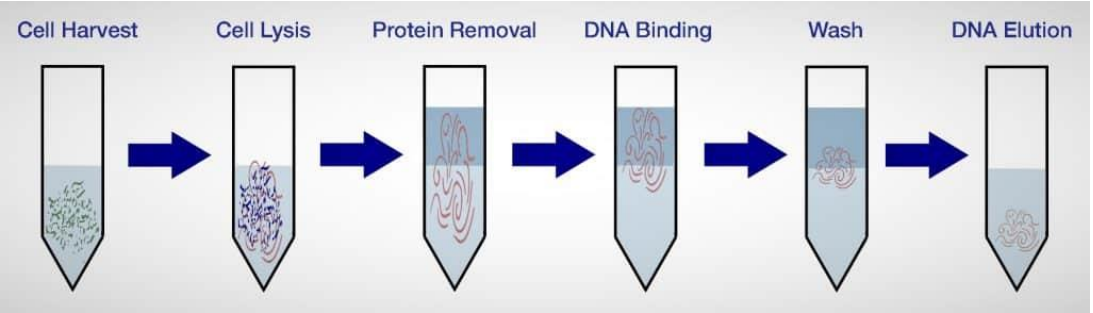
Genomic DNA Extraction



Dr. Nesrin Mwafi

Biochemistry & Molecular Biology Department

Faculty of Medicine, Mutah University

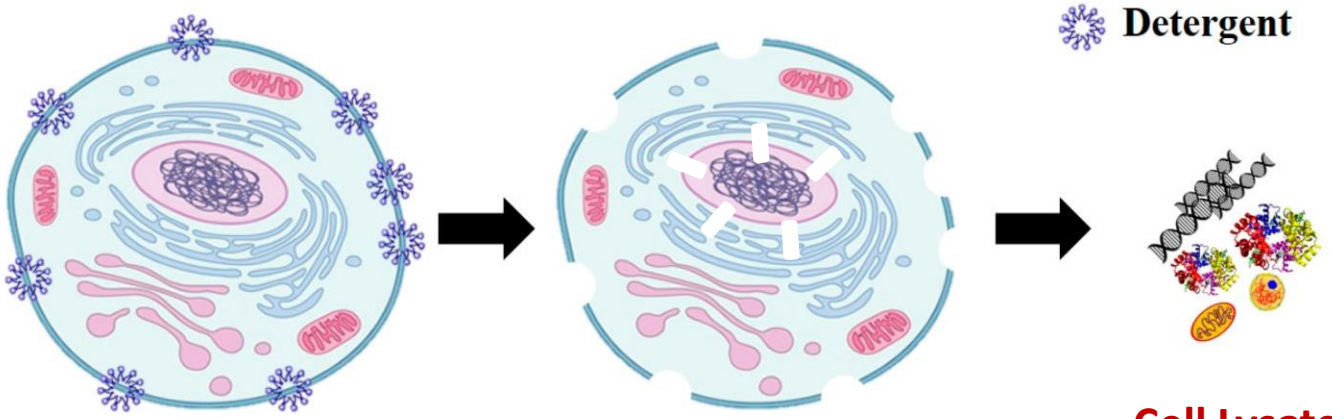
Part II

The Principle of DNA Extraction

The principle of DNA Extraction

- There are three basic steps in DNA extraction:

1. Cell lysis with digestive solution to expose the DNA. Lysis buffer contains detergents/surfactants such as SDS (sodium dodecyl sulphate) to disrupt both cellular and nuclear membranes (make holes in the membrane)



Detergent reacts with cell membrane

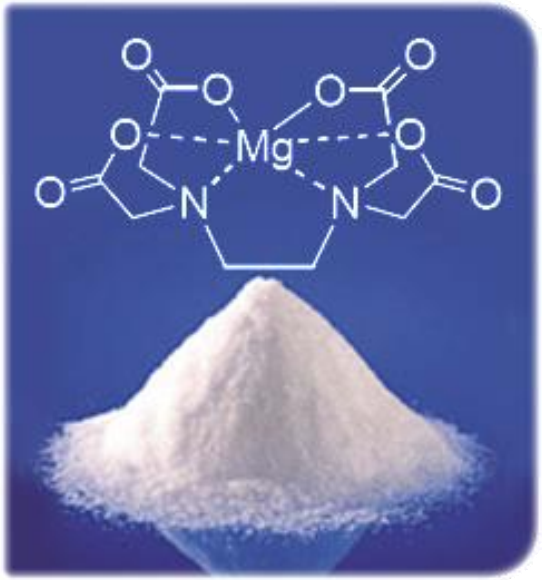
Detergent destroys the cell membrane and nuclear membrane

Intracellular components are released (lipids, proteins, nucleases, salts, minerals,....etc)

The principle of DNA Extraction

2. Inactivate endogenous nucleases like DNases. Actually, this is can be done by adding proteases like proteinase K

- Add also chelating agents (e.g. EDTA) which sequester Ca^{+2} and Mg^{+2} required for nuclease activity.
- On the other hand, RNases are usually added to the sample to get rid of RNA if we want to extract DNA
- To extract RNA, we add RNA guard to protect our RNA from endogenous and exogenous RNases



The principle of DNA Extraction

3. Purification of DNA from proteins, RNA, detergents, salts and reagents found in cell lysate:

    - Ethanol precipitation using ice-cold ethanol or isopropanol
    - Phenol/chloroform extraction
    - Minicolumn purification
    - Magnetic beads

The principle of DNA Extraction

1. Ethanol precipitation:

- DNA (polar molecule) is insoluble in absolute ethanol (99-100%) or isopropanol (anti-solvent) so it will aggregate together forming a pellet upon centrifugation

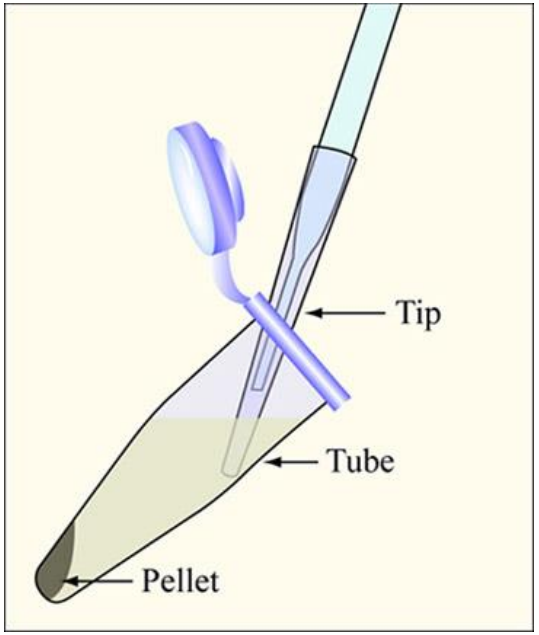
Centrifuge

Centrifuge

The principle of DNA Extraction

1. Ethanol precipitation:

- DNA is insoluble in absolute ethanol or isopropanol (anti-solvent) so it will aggregate together forming a pellet upon centrifugation
- After centrifugation, a pellet of crude DNA is formed

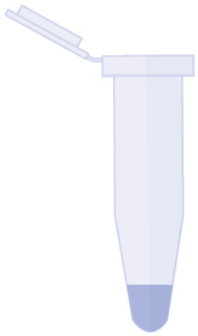


The principle of DNA Extraction

- To enhance precipitation of DNA in presence of 95-100% ethanol, the solution should contain positive ions such as sodium acetate
- The role of this salt is to neutralize the negative charge on DNA backbone so reduces its hydrophilicity and improves its precipitation
- (**use the right concentration !!!! 0.3M, pH = 5.2**)
- Too much sodium acetate, the salt will co-precipitate with DNA and too little will result in incomplete recovery of DNA

The principle of DNA Extraction

- The pellet is washed with 70% ethanol to remove some salts present in the left over supernatant and bound to DNA
- Air dry the pellet (5-10 min) then redissolve in ultrapure or Milli-Q, Millipore water (DNase/ RNase free water)

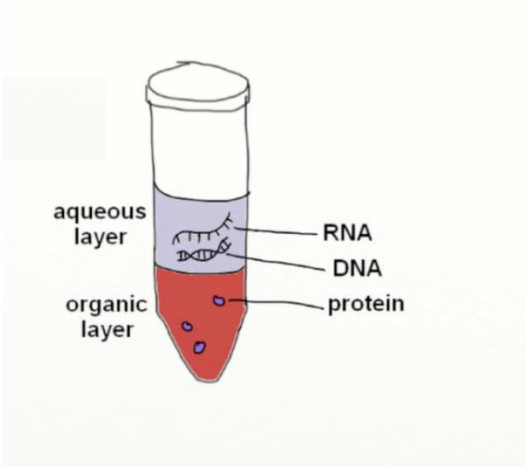


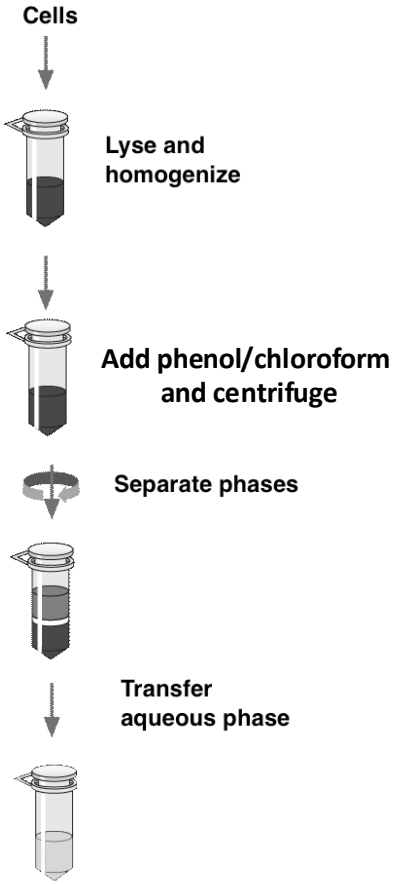
Store purified DNA in Eppendorf tube at -20C

The principle of DNA Extraction

2. Phenol/ chloroform extraction:

- Equal volume of phenol/chloroform added to an aqueous solution of lysed cells
- Centrifugation yields two phases: the upper aqueous phase (containing the nucleic acids DNA and RNA) and the lower organic phase (containing the lipids and denatured proteins)
- The upper layer is removed with pipette tip carefully

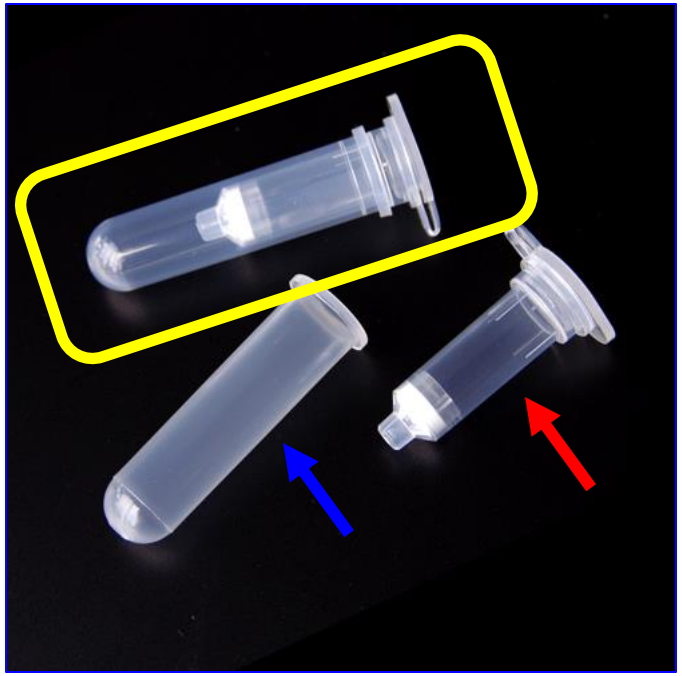


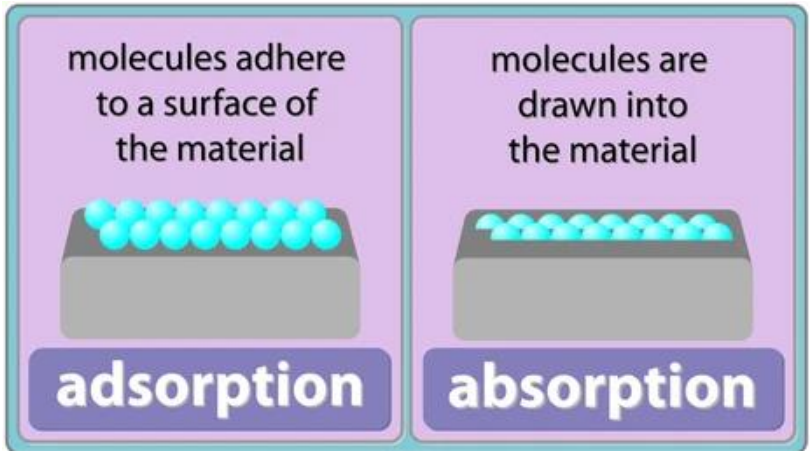


The principle of DNA Extraction

3. Minicolumn purification: (Spin-column based nucleic acid purification)

- which depends on the binding and adsorption of nucleic acids to a solid phase (e.g. silica, SiO_2)

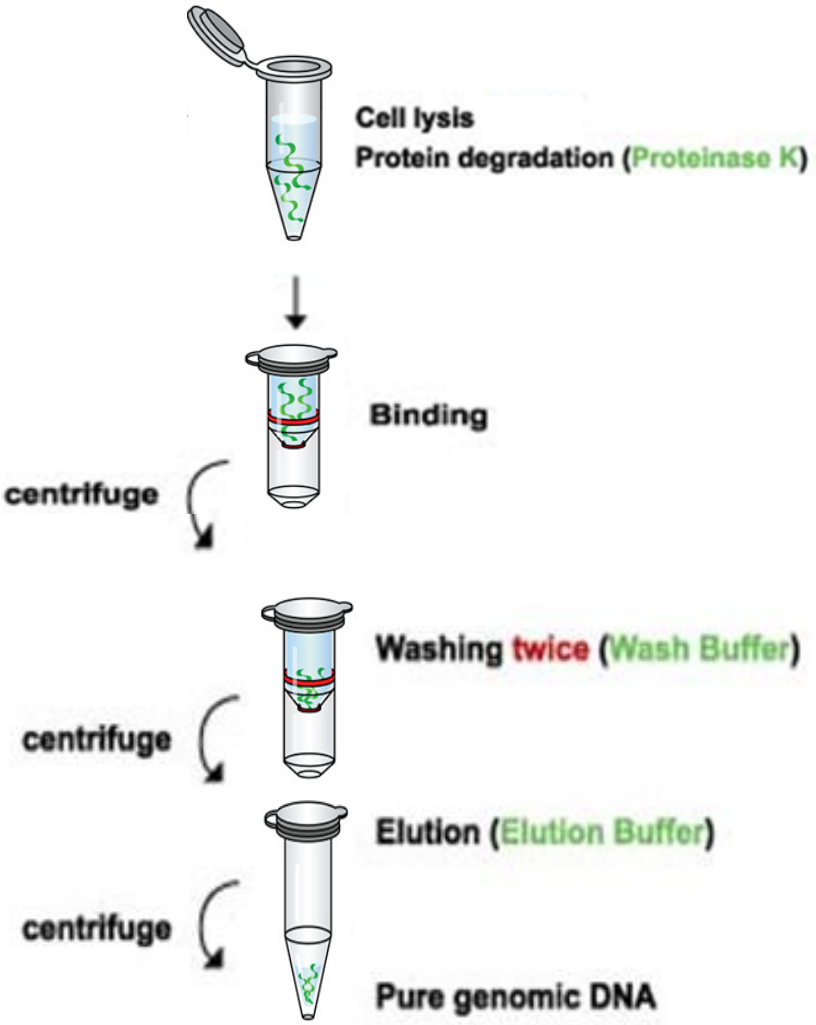




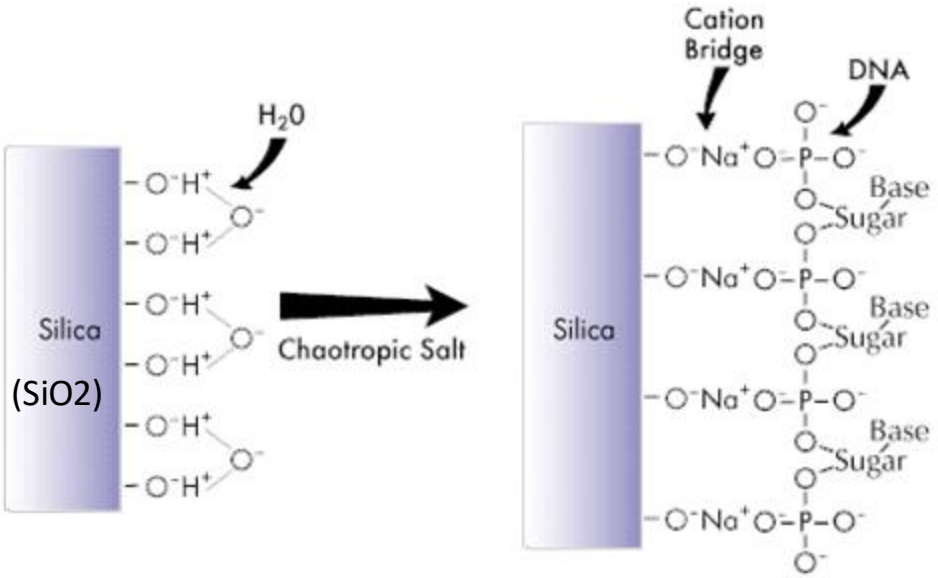
The principle of DNA Extraction

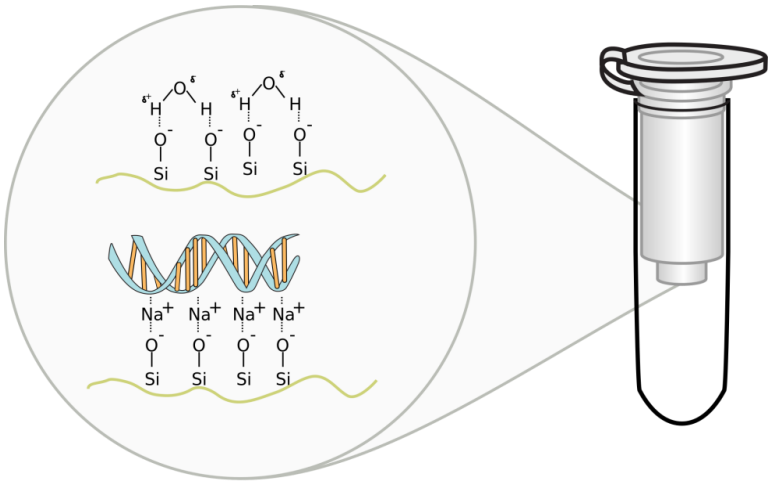
3. Minicolumn purification:

- After cell lysis, inactivate endogenous nucleases (e.g. DNases) with proteinase K enzyme and chelating agents (e.g. EDTA)
- Add binding solution to cell lysate, mix and centrifugate
- Washing and column elution(with DNase/ RNase free water)



Binding of DNA to Silica membrane





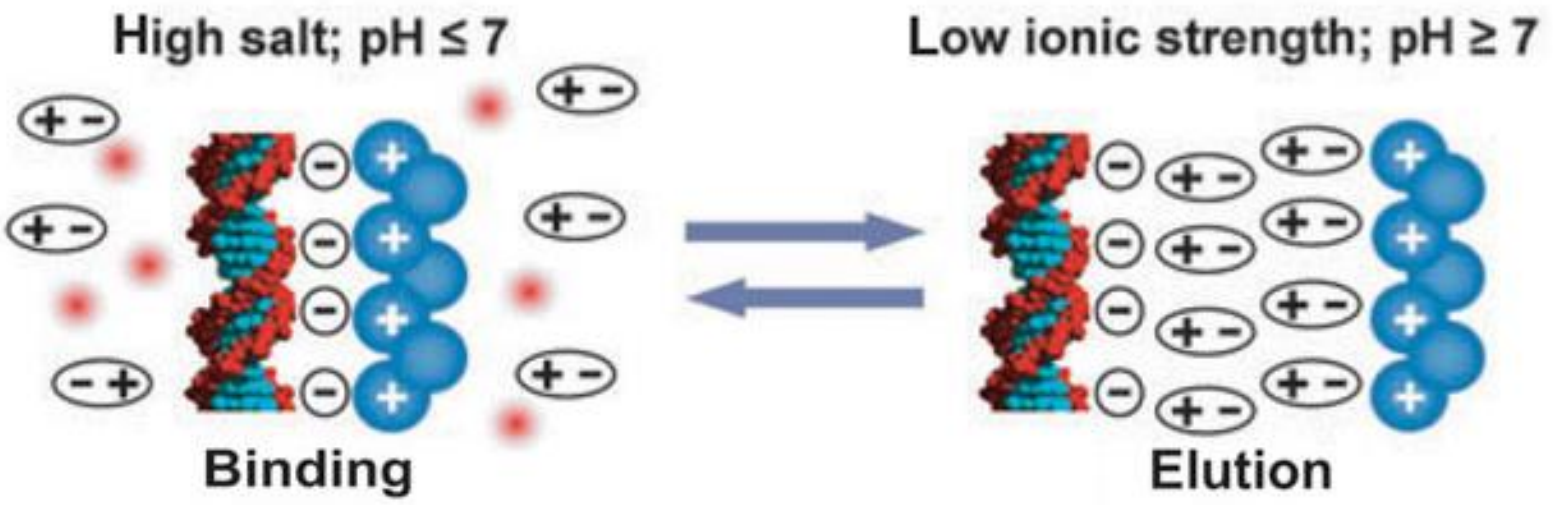
Spin column-based nucleic acid purification

Binding solution consists of :

1. Chaotropic salt like guanidinium hydrochloride

2. Sodium acetate salt (act as bridge)

Optimal Conditions For Binding and Elution



The principle of DNA Extraction

4. Magnetic Beads-based DNA/RNA extraction:

- Quick and efficient for direct separation of crude DNA or RNA from sample
- No need for centrifugation, separation by applying of magnetic field
- Various types of magnetic particles are commercially available working in manual or **automated mode** (save time and money in case of large numbers of samples and avoid the risk of cross-contamination during the traditional methods)