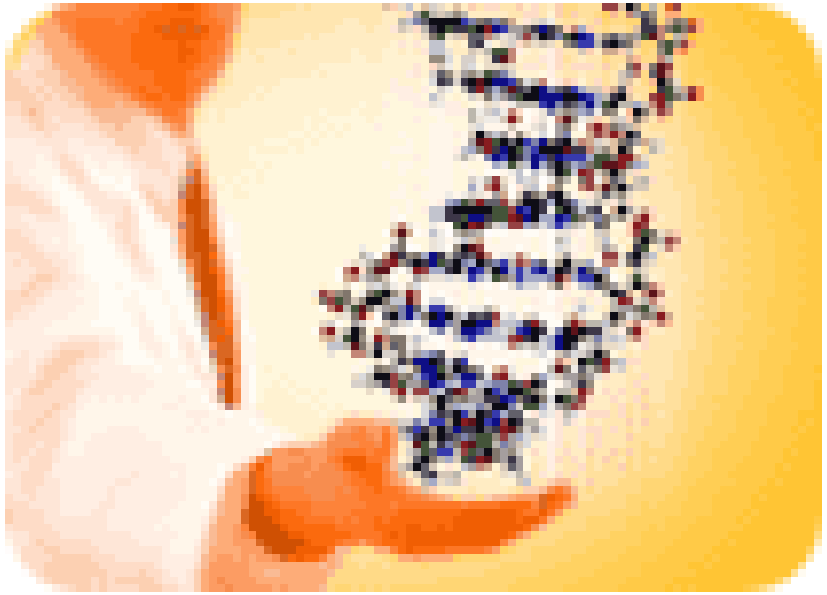
DNA EXRACTION



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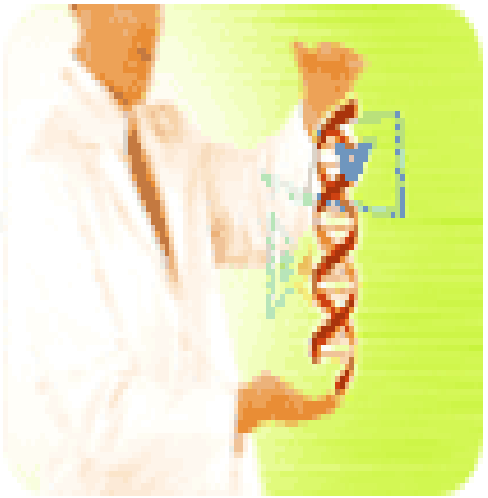
Basic DNA



- Located in the nucleus cell of all living organisms
- All cells except mature mammalian RBC(s) possess DNA
- Sources for human: tissue samples,skin,blood (except rbc),semen (spermatozoa),hair root, cells from saliva collected by swabbing the mouth (cheek and gums), etc

Purpose of DNA extraction

- To obtain DNA in a relatively purified form which can be used for further investigations, such as PCR, sequencing, etc

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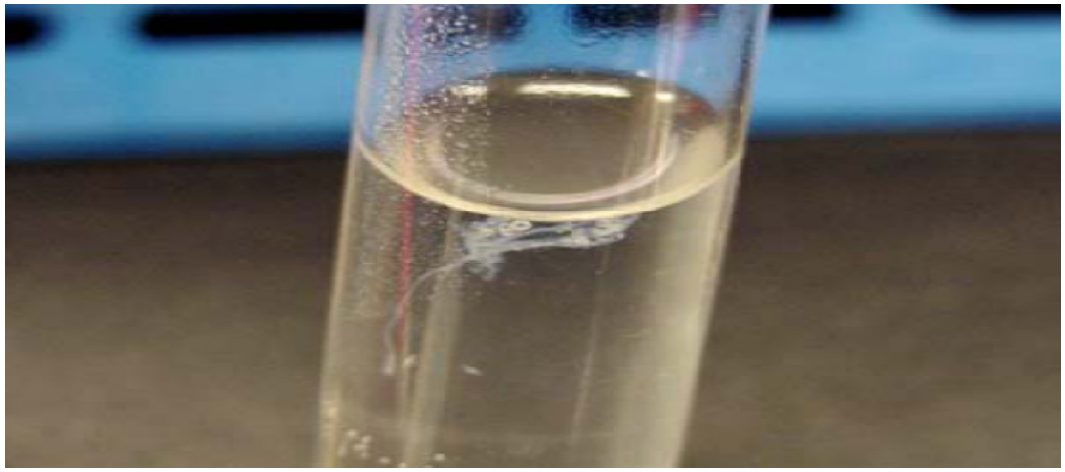
Basic Protocol

- Four steps are used to remove and purify the DNA from the rest of the cell.
  1. **Lysis**
  2. **Precipitation**
  3. **Wash**
  4. **Resuspension**

Conventional method

- Salting-out method (a quick, inexpensive, and safer method to extract DNA)
- Organic extraction method (Phenol, chloroform)



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Salting out vs Phenol-chloroform method

Phenol-chloroform

- ***Lysis***: use detergent & Proteinase K
- ***Precipitation*** : phenol/chloroform extraction to get rid of proteins
- ***Precipitation*** : addition of cold ethanol to force DNA out of solution
- ***Wash and resuspend:*** DNA is washed in ethanol, dried, and resuspended in H20 or TE buffer.

Salting out

- ***Lysis***: use detergent & Proteinase K
- ***Precipitation*** : addition of salts to denature proteins and lyse plasma membranes
- ***Precipitation*** : addition of cold ethanol to force DNA out of solution
- ***Wash and resuspend:*** DNA is washed in ethanol, dried, and resuspended in H20 or TE buffer.

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Why detergent?

- lyses the plasma membranes
  - Detergent dissolves the lipids in the cell membranes and nuclear envelope
  - Soap molecules and the lipids (fats) in cell membranes are made of two parts:
    - hydrophillic heads
    - hydrophobic tails
  - When detergent comes close to the cell, it captures the lipids and proteins (due to their similar structures)
  - The detergent molecules are able to pull apart the membranes

Why proteinase K?

- To get rid of any cell debris and remove proteins
- Overnight incubation (55°c) to activate proteinase K for complete digestion of protein

Why salt?

- To precipitate the proteins, lipid and membranes which are then removed by centrifugation
- The salts interrupt the hydrogen bonds between the water and DNA molecules
- Allows the **DNA** strands to clump together.

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Why phenol-chloroform?

- To remove the proteins from the DNA
  - Organic solvents, so lysed cell components that are hydrophobic will be trapped in these solvents, e.g. membrane lipids, etc.
  - Also powerful denaturants, so proteins will be denatured, leaving hydrophobic segments to interact with organic solvent and hydrophilic segment to interact with aqueous.
  - At the end, it leaves only hydrophilic entities in the aqueous solution, e.g. nucleic acids, sugar, salt, etc.

Why cold alcohol?

- In the presence of cations, ethanol induces a structural change in DNA molecules that causes them to aggregate and precipitate out of solution.
- Since cold alcohol is insoluble in high salt, it also precipitates the DNA.

Why cold alcohol (cont'd)

- Since water soluble in ethanol, water dissolved into ethanol-Effectively removes the water from around the **DNA**—causing the **DNA** to precipitate.

- The DNA is pelleted by spinning with a centrifuge and the supernatant removed

Washing and Resuspension

Washing:

The precipitated DNA is loaded with salts. It is “washed” with a 70% ethanol solution to remove salts and other water soluble

Resuspension:

The clean DNA is now resuspended in a buffer to ensure stability and long term storage.

The most commonly used TE buffer

How to check purity?

- Assessed by spectrophotometry
- by calculating A260/A280 ratio
- Range DNA purity: 1.80-2.00
- DNA purity:
  - < 1.8 → contaminated with protein. Solution: adding proteinase K for complete digestion
  - >2.0→ Contamination by an extraction solvent such as salt

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Can we extract DNA at home?

- Using a few household items
- E.g.. DNA extraction from banana
  - Add buffer solution to frozen banana and grind
  - Filter with coffee filter, then add cold alcohol
  - DNA precipitate-white mucous at the interface between the alcohol and banana liquid.
  - Remove the DNA by wooden stir stick

- Do not fulfill all the requirements (minimize the risk of DNA contamination, facilitating high-quality results)

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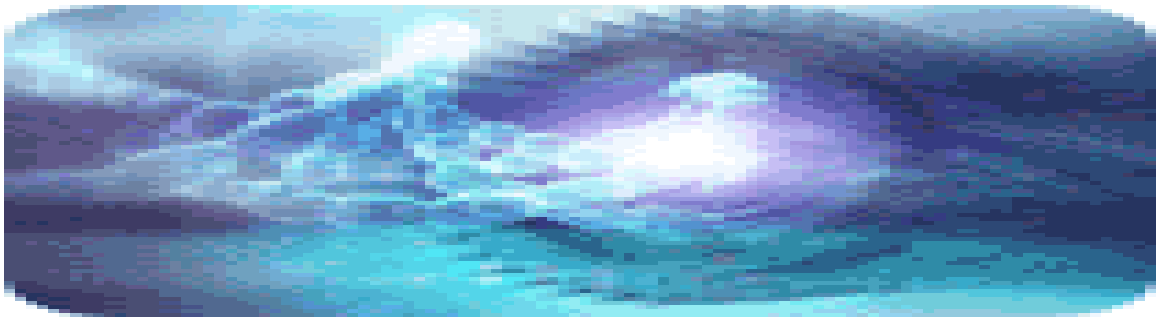
THANK YOU FOR LISTENING



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