DNA Isolation

DNA EXTRACTION METHODS

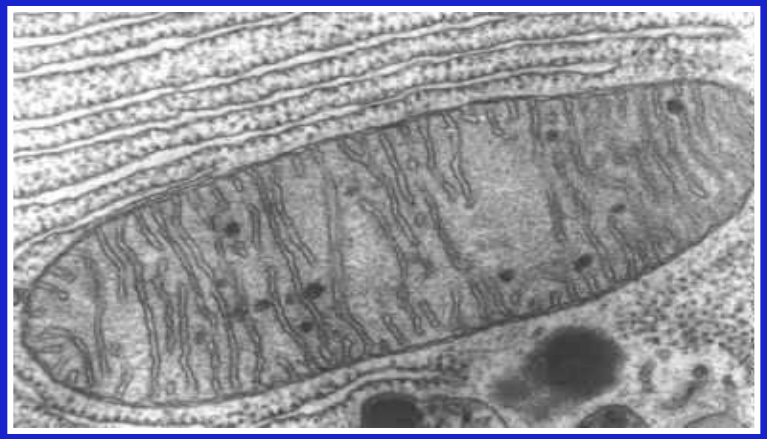
Definition

- DNA Extraction is the isolation and purification of DNA (deoxyribonucleic acid)

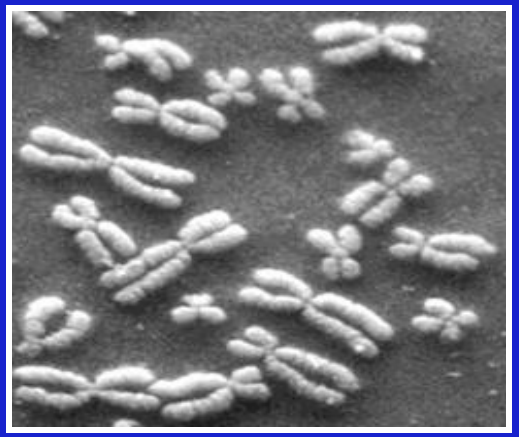
- DNA extraction is used to isolate…
  - Mitochondrial DNA
  - **Genomic DNA**

- DNA can be extracted from almost any intact cellular tissue

- Skin,
- blood,
- saliva,
- semen,
- mucus,
- muscle tissue,
- bone marrow, etc.



http://www.davidkfaux.org/shetlandislandsmtDNA.html



http://faculty.uca.edu/~benw/biol1400/pictures/

Purpose of DNA Extraction?

- To obtain DNA in a relatively purified form which can be used for further investigations such as:
  - PCR (polymerase chain reaction)
  - Southern Blotting

Basic Protocol

- Most DNA extraction protocols consist of two parts

1. A technique to lyse the cells gently and solubilize the DNA

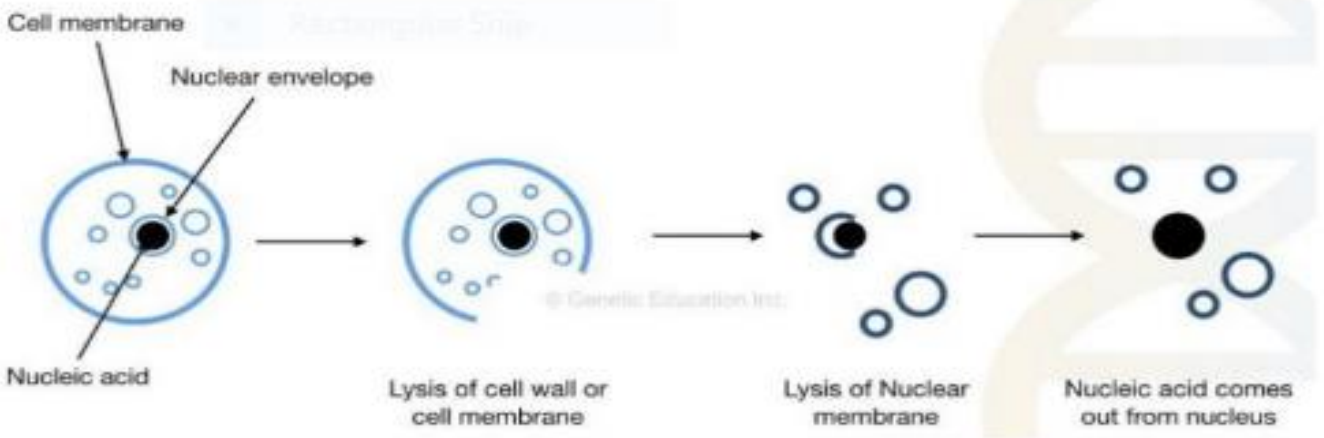
2. Enzymatic or chemical methods to remove contaminating proteins, RNA, or lipids

Nucleic Acid Extraction Requirements

1. Disruption of cell wall and membranes to liberate cellular components.

2. Inactivation of DNA- and RNA-degrading enzymes (DNases, RNases).

3. Separation of nucleic acids from other cellular components.



The image represents the general outline of how DNA extracted from the cell.

Basic step of DNA extraction

1. Lysis of cell wall or cell membrane.
2. Lysis of nuclear membrane.
3. Extracting the DNA from the other cell debris
4. DNA precipitation
5. Dissolving DNA.

What are the Most Commonly used DNA Extraction Procedures?

- Organic (Phenol-Chloroform) Extraction
- Non-Organic (Proteinase K and Salting out)
- Adsorption method (silica-gel membrane)

The method utilized may be sample dependant, technique dependant, or analyst preference

ORGANIC EXTRACTION

- The most basic of all procedures in molecular biology is the purification of DNA by organic extraction.

ORGANIC EXTRACTION PROCEDURE

Typical Procedure

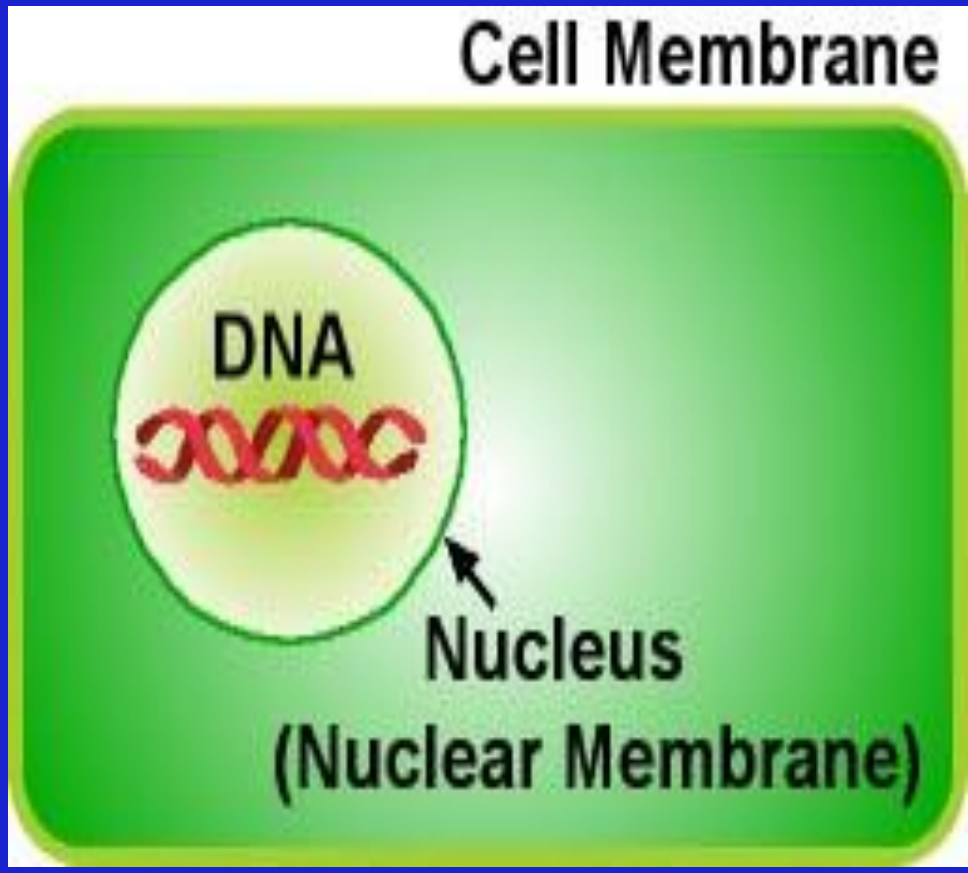
1. **Cell Lysis**
2. **Phenol Extraction**
3. **RNAse followed by proteinase K**
4. **Repeat Phenol Extraction**
5. **Ethanol Precipitation**

ORGANIC EXTRACTION REAGENTS

- Cell Lysis Buffer –
  - Non-ionic detergent
    - SDS (Sodium dodecyl sulfate), Tris-Cl, EDTA
  - Designed to lyse cell membrane and nuclear membrane.
- EDTA (Ethylenediaminetetraacetic)
  - is a chelating agent of cations such as Mg2+.
  - Mg2+is a cofactor for DNase nucleases.
  - If the Mg2+is bound up by EDTA, nucleases are inactivated.

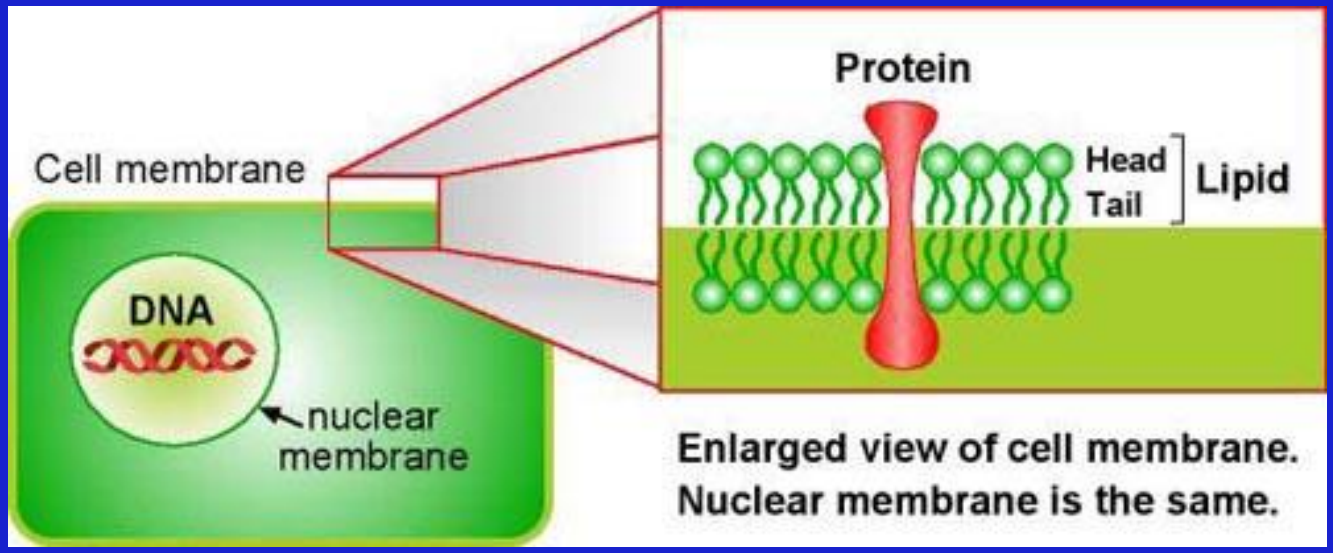
What does the detergent do?

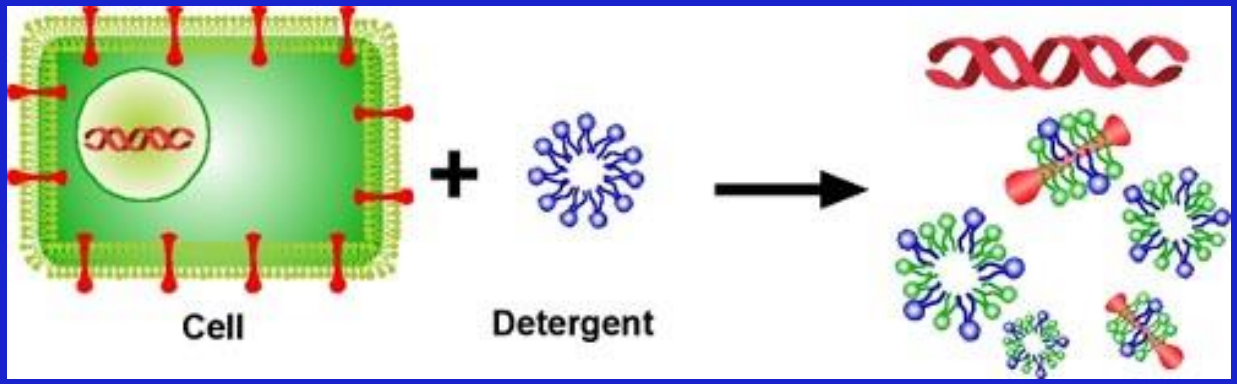
- Each cell is surrounded by a cell membrane
- DNA is in the nucleus of a cell, which is also surrounded by a membrane
- The membranes must be broken open in order to get the DNA out of the cell



Detergent:

- Breaks apart membranes by attaching to the lipids (fats) & proteins in the membranes





The cell and nuclear membranes have been broken apart,
as well as all of the organelle membranes,
such as those around the mitochondria and chloroplasts.

So what is left?

- Proteins
- Carbohydrates (sugars)
- DNA

ORGANIC EXTRACTION REAGENTS

- Proteinase K - it is usual to remove most of the protein by digesting with proteolytic enzymes such proteinase K

ORGANIC EXTRACTION REAGENTS

- Phenol/Chlorform –
- The standard way to remove proteins from nucleic acids solutions is to extract once with phenol, once with a 1:1 mixture of phenol and chloroform, and once with chloroform.
- Also, the final extraction with chloroform removes any remaining traces of phenol from the nucleic acid preparation.
  - Phenol is highly corrosive and can cause severe burns.

- Phenol denatures proteins and dissolves denatured proteins.

- Chloroform is also a protein denaturant

Concentrating DNA
Alcohol Precipitation

- The most widely used method for concentrating DNA is:
  - precipitation with ethanol.
- The technique is rapid and is quantitative even with Nano gram amounts of DNA.


Precipitated
DNA
Before
After
Adding Ethanol

Non-Organic DNA Extraction

- *Does not* use organic reagents such as phenol or chloroform.

- Digested proteins are removed by salting out with high concentrations of (NaCl).