DNA extraction (DNA isolation): is a process of purification of DNA from sample using a combination of physical and chemical methods.

The basic procedure of DNA extraction:

There are three basic and two optional steps in a DNA extraction.

1) Cell lysis: Breaking the cell membranes open to expose the DNA along with the cytoplasm within, This is commonly achieved by chemical (ex: lysozyme enzyme , NaOH, etc) and physical methods(ex: grinding, sonication, beads beats, etc) .

2) Remove lipids from the cell membrane and the nucleus are broken down with detergents and surfactants (contain sodium dodecyl sulfate SDS). The solution is treated with concentrated salt solution to make debris such as broken proteins, lipids and RNA to clump together. Centrifugation of the solution, which separates the clumped cellular debris from the DNA 3) Removing protein by adding a protease K (optional).

4) Breaking RNA by adding an RNase (optional).

5) DNA purification from detergents, proteins, salts and reagents used during cell lysis step. The most commonly used procedures are:

   - **Ethanol precipitation** usually by ice-cold ethanol or isopropanol. Since DNA is insoluble in these alcohols, it will aggregate together, giving a *pellet* upon centrifugation. Precipitation of DNA is improved by increasing of ionic strength, usually by adding sodium acetate.
   - **Phenol–chloroform extraction** in which phenol denatures proteins in the sample. After centrifugation of the sample, denaturated proteins stay in the organic phase while aqueous phase containing nucleic acid is mixed with the chloroform that removes phenol residues from solution.
   - **Minicolumn purification** that relies on the fact that the nucleic acids may bind (adsorption) to the solid phase (silica or other) depending on the pH and the salt concentration of the buffer.

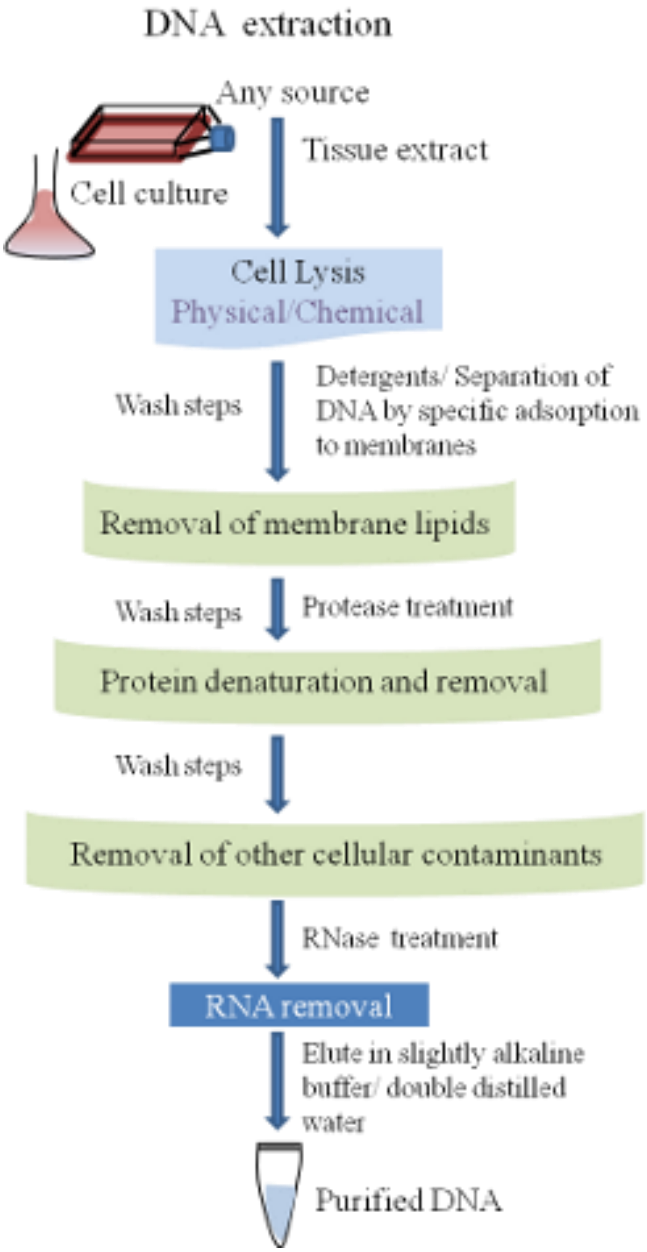

Figure 1. Basic steps involved in all DNA extraction methods

What can this DNA be used for?

Once extracted, DNA can be used for molecular analyses electrophoresis, sequencing, including PCR, fingerprinting and cloning

DNA Extraction process

DNA is an incredibly small molecule, but in large quantities, it can be seen. In this activity, we will extract DNA from banana or strawberry fruits. We are going to use fruits because they are soft and easy to pulverize.

There are three basic steps in DNA extraction. **First,** the cell must be broken open to release the nucleus. **Next,** the nucleus must also be opened to release the DNA. **Lastly,** once the DNA is released, it must be precipitated out of solution. The reagents we require to complete the extraction procedure are salt, detergent, and alcohol. Both the cell and nuclear membranes are composed primarily of lipids.

*In order for the cell to be broken open, the lipid walls must be broken down. The manual grinding and detergent solutions accomplish this. Soap molecules mix with fats or lipids, causing structures made of lipids to break apart.

*The addition of salt solution provides the DNA with a favorable environment by contributing positively charged atoms that neutralize the normal negative charge of the DNA, allowing the DNA to clump together.

*Ethyl alcohol is used to precipitate the DNA. In water, DNA is soluble. However, when it is in ethanol, it uncoils and precipitates.

Equipment

- 25 ml Ethyl Alcohol
- 5 ml detergent
- 5 ml NaCl
- Spatula
- 2.5 cm square piece of fruit in Zip bag
- 50 ml falcon tube
- 200 ml beaker
- Plastic pipette
- Glass rod
- Gloves

- ✦ Safety glasses

Procedure:

1) Measure the mass of the fruit with the scale. Record your result in the data table
2) Mash the fruit in a zip bag.
3) Add 5 ml detergent and a spatula of NaCl (salt) to falcon tube.
4) Add 45 ml tap water in the falcon tube and cover it.
5) Gently turn the falcon tube up and down.
6) Pour the solution in the falcon tube to the fruit mash in the zip bag.
7) Gently squash the bag and wait 2-3 minutes to allow mixing the solution and fruit mash.
8) Get another falcon tube and a funnel.
9) Fit a paper towel (by wrapping it) onto funnel and water it lightly.
10) Pour the mixture in the zip bag to funnel.
11) Wait for the filtered solution to build in the falcon (25 ml of solution would be enough).
12) Add 25 ml of alcohol in the falcon tube.
13) After a few minutes, bundles of isolated DNA will float on the alcohol solution.
14) Carefully collect the isolated DNA with a glass rod or a pipette and place it in a beaker of known mass.