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**Lab 2**

 **DNA extraction**

**DNA isolation** is a process of purification of DNA from sample using a

combination of physical and chemical methods.

\*The isolation method of choice is dependent upon:

1- The source of the DNA: blood, tissue, bacterial, virus etc.

2- The final application: PCR, restriction, sequencing, fingerprinting, library

construction etc.

3- The type of DNA: genomic or plasmid.

**Basic steps in a DNA extraction:**

1. Breaking the cell membranes open to expose the DNA along with the

cytoplasm within (cell lysis).

Lipids from the cell membrane and the nucleus are broken down

with detergents and surfactants.

Breaking proteins by adding a protease (cellular and histone proteins).

Breaking RNA by adding an RNase.

2. The solution is treated with concentrated salt solution to make debris such

as broken proteins, lipids and RNA to clump together.

3. DNA precipitation by centrifugation of the solution, which separates the

clumped cellular debris from the DNA.

**The most commonly used procedures for precipitation are**:

1- Ethanol precipitation usually by ice-cold ethanol or isopropanol. Ethanol is

added because DNA is soluble in water. The alcohol causes DNA to

precipitate, leaving behind all the cellular components that aren’t soluble in

alcohol.

2- Phenol-chloroform extraction in which phenol denatures proteins in the

sample. After centrifugation of the sample, denatured proteins stay in the

organic phase while aqueous phase containing N.A is mixed with the

chloroform that removes phenol residues from solution.

**Chromosomal DNA extraction:** There are many ways by which

chromosomal DNA is isolated from microorganism, fungi, as well as high

organisms and plant cells. These methods are:

1- Boiling method

2- Detergent method

3- Alkaline method.

4- Enzymatic method.

5- Mechanical method.

**Bacterial DNA extraction by boiling method**

1- Different strains were isolated from a variety of clinical specimen. Samples

were grown at 37 °C on infusion brain heart agar before extracting their

DNA.

2- Selected 3-4 colonies of types are separately suspended in 0.5 ml of

sterile distilled water in the eppendorof tube and mixed by vortex.

3- Boiling for 10 minutes in a water bath, and then centrifuged for 1-2

minutes at 1300 rpm.

4- Remove supernatant in a clean eppendorof tube.

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