DNA EXTRACTION

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Definition

DNA Extraction = DNA isolation

It is a process used for purification (Deoxyribonucleic acid) DNA from sample using combination of physical and chemical methods.
Types of samples

Samples
Blood (citrate, EDTA or heparin)
Blood spotted on filter paper
Insects
Plant
Tissue
Procedure of DNA extraction
Step I

Cell Lysis

Breaking the cell to expose the DNA.
This is commonly achieved by:
1- chemical method.
2- physical methods like grinding, blending or sonication the sample. and
Grinding of the samples
Blending of the samples
Blending of the samples with Silica beads
sonication of the sample

It means subject (a biological sample) to ultrasonic vibration so as to fragment the cells, macromolecules, and membranes.
Cell lysis
Procedure of DNA extraction
Step II

Removing membrane lipids, proteins and RNA by adding detergent, surfactants, protease and Rnase.

DNA purification

Ethanol precipitation: by ice cold ethanol or isopropanol. *The DNA is insoluble in these alcohols*, so it will aggregate together, giving a pellet upon centrifugation.

Minicolumn purification: DNA may bind (adsorption) to the solid phase (silica or other) depending on the pH and the salt content of the buffer.
**Procedure of DNA extraction**

**Step III**

**DNA purification:**

**Phenol–chloroform extraction**

In which phenol denatures proteins in the sample. After centrifugation of the sample denaturated proteins stay in organic phase while aqueous phase containing nucleic acid is mixed with the chloroform that removes phenol residues from solution.

Mg$^{2+}$ and Ca$^{2+}$, which prevents enzymes like Dnase from degrading the DNA.
DNA Hydration:

After isolation, the DNA is dissolved in slightly alkaline buffer, usually in the hydration or elusion buffer or in ultra-pure water.
DNA Extraction
DNA Extraction Kit
DNA Extraction steps

Sample Preparation → DNA Binding → Wash → Elution
Exterachromosomal DNA
Extraction of the Extrachromosomal DNA

Extrachromosomal DNA is generally easy to isolate.

**Plasmids** may be easily isolated by cell lysis followed by precipitation of proteins, which traps chromosomal DNA in insoluble fraction and after centrifugation, plasmid DNA can be purified from soluble fraction.
Alkaline liquid, Centrifuge -> Cell Lysate -> Supernatant (Retain) -> Pellet (Discard)

Acidic liquid, Centrifuge -> Chromosomal DNA -> Plasmid DNA

Supernatant from Lysate -> Centrifuge and Wash

Plasmid DNA

Retained in Column

Chromosomal DNA

Flows Through Column

Discard

Elution Liquid and Centrifuge

Plasmid DNA

Flows Through Column

Collect
DNA Detecting

By using Spectrophotometer:

Measuring the intensity of absorbance of the DNA solution at wavelengths 260 nm and 280 nm is used as a measure of DNA purity.

DNA absorbs **UV** light at 260 and 280 nanometres, and aromatic proteins absorb UV light at 280 nm; a pure sample of DNA has a ratio of 1.8 at 260/280 and is relatively free from protein contamination.

DNA preparation that is contaminated with protein will have a 260/280 ratio lower than 1.8.
Gel Electrophoresis:
Running it on an agarose gel, staining with ethidium bromide and comparing the intensity of the DNA with a DNA marker of known concentration.
“Change is never easy, you fight to hold on, and you fight to let go.”
many Thanks!

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