

DNA Extraction



General Aim

To extract cellular DNA using phenol/chloroform method.

Method

Phenol/chloroform method.

Learning Objectives (ILOs)

- Demonstrate proficiency with the protocol involved in DNA extraction.
- Identify the role of specific reagents and equipment in the extraction of DNA.
- Practice basic laboratory techniques.
- Conclude downstream applications of DNA extraction.

Theoretical Background/Context

DNA (deoxyribonucleic acid) is a complex molecule that contains the genetic material of a cell. It defines the structure and the functions of a living organism. DNA is also responsible for heredity.

DNA is composed of a series of nucleotides linked together. These nucleotides are basically made of a nitrogenous base, a molecule of sugar (deoxyribose) and a phosphate group. The sequence of nucleotides in the DNA molecule determines the hereditary traits of a cell. Nucleotides are packed to fit inside the cell. DNA takes the shape of a double helix, which, in addition to the DNA molecule contains bound proteins. DNA double helix is enclosed inside a cell membrane in case the cell doesn't have a nucleus like in prokaryotes, and inside the nuclear membrane in cells with nuclei like in eukaryotes.

Theoretical Background/Context (Cont')

DNA has been the target of a lot of research. Investigation of the DNA structure and sequence in relation to diseases helped in finding out the molecular basis and cure for various diseases. DNA study also allowed the production of many vaccines, hormones and enzymes. DNA study was also very beneficial in the forensic/medico legal entities. To study DNA it must be extracted out of the cell, Hence; DNA extraction technique is widely used in research labs.

The isolation of the DNA is done in stages:

1. Cell dissolution: In this stage the cell and the nucleus are broken to extract a DNA sample using a lysis buffer.
2. Precipitation: In this stage, proteins and impurities are removed from the sample.
3. Purification: This stage involves isolating the DNA completely from the other substances, for a final eluted pure DNA sample preserved in a buffer.

Principle of Work

The extraction of DNA involves lysis of cellular and nuclear membranes to extract DNA from within. This is followed by separation of DNA from impurities, proteins, and other substances. DNA extraction usually proceeds through three stages:

1. Cell dissolution: where the cell and the nucleus are broken to extract DNA into the buffer.
2. Precipitation: where proteins and impurities are removed from the sample.
3. Purification: This final stage involves further purification of DNA to get a completely pure DNA sample ready to be used for downstream applications.