Molecular Detection of *Acinetobacter* bacteria

Experiment 1

Isolation, quantification and purity determination of Bacterial genomic DNA

Objectives:-

- To isolate gDNA from Bacteria
- To obtain the purified form of DNA which can be further used for molecular analysis.
- To understand the principle and process of DNA extraction.

Theory:-

Deoxyribonucleic acid (DNA) is a complex nucleic acid containing the genetic code with the instructions for the development and functioning of all known living organisms, with the exception of some viruses. The DNA segments that carry this genetic information are called genes. DNA is transcribed into RNA which is then used as a template in the synthesis of proteins.

DNA isolation refers to the process of extracting DNA from a cell in a pure form. The extraction of DNA is an important preliminary step in which purified DNA is obtained from other cellular components such as proteins, RNA and lipids. DNA can be isolated from any nucleated cell from diverse sources, both living and dead, such as whole blood, hair, sperm, bones, nails, tissues, faeces, shed feathers, egg shells, saliva, epithelial cells, urine, bacteria, animal tissues or plants.

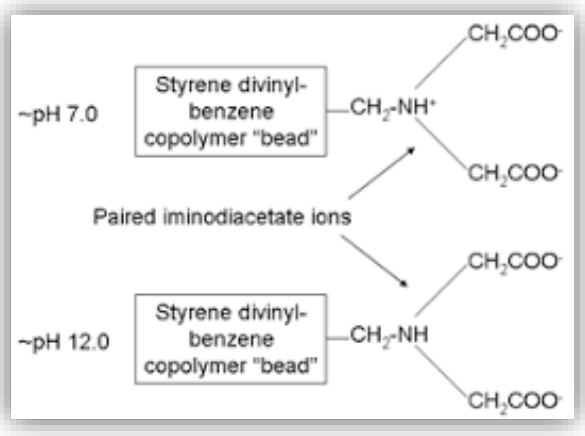
Isolation of DNA is required for a variety of applications in science, medicine and forensics. For example, scientists introduce DNA into the cells of animals or plants for SNP analysis, DNA methylation analysis, copy number variation (CNV) or comparative genomic hybridization (CGH) analysis, Southern Blotting, PCR etc. In medicine, DNA isolation is helpful for diagnostic purposes. Furthermore, DNA extraction is an important tool in forensic science for the identification of individuals (for example rapists, thieves, accident, or war victims), paternity determination, body identification etc.

There are a number of different procedures for the preparation of genomic DNA. They all start with some form of cell lysis, followed by de-proteination and recovery of DNA. The main differences between various approaches lie in the extent of de-proteination and in molecular weight of the DNA produced.

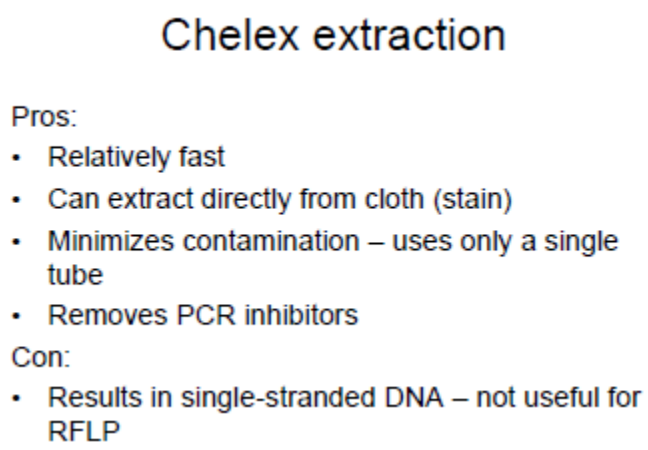
The presence of proteins, lipids, polysaccharides etc. during DNA preparation can interfere with DNA analysis methods by reducing the quality of DNA. The extraction methods to efficiently purify DNA from various sources have to be adapted depending on factors such as sample size, the freshness of the sample, and the biochemical content of the cells from which DNA is being extracted. The isolation method must vary depending on the size of sample. The freshness of the sample also affects the extraction technique. Extraction methods are also variable according to the biochemical content of the source cells. For example, in the case of bacteria, the main biochemicals present in a cell extract are protein, DNA and RNA. Therefore, phenol extraction or protease treatment, followed by removal of RNA with ribonuclease, leaves a pure DNA sample. These treatments may not be sufficient if the cells also contain significant quantities of other biochemicals.

Principle:-

Chelex 100, Molecular Biology Grade resin from BioRad is a highly pure, nuclease and ligase inhibitor-free chelating resin, certified not to interfere with downstream PCR . Chelex resin (Chelex 100) is a specialized resin that chelates metal ions as well as other contaminants (Chelex = Chelating Ion Exchange Resin). Chelex is composed of **styrene divinylbenzene copolymers** containing paired **iminodiacetate** ions that act as chelating groups in binding polyvalent metal ions such as magnesium (Mg^{2+}). By removing the Mg^{2+} from the reaction, nucleases are inactivated and the DNA is protected.



It can be used as a resin and added to mixtures, but is also usable in a column- based format depending on the application. For larger scale, the column-based format would be recommended as more material could be processed. For small scale or single-sample preparation, addition of the resin is preferred. Chelex is a stable resin over the entire pH range. It is functionally active over the pH range of 2-14. It is also a very economical choice for purification (i.e., cheap!) compared to other more hazardous extraction procedures such as phenol/chloroform/isoamyl extraction.



Materials:

1. Chelex 100 resin
2. Tris-EDTA (1X)
3. scale and weigh boat
4. small bottle for storage
5. 1.5 mL eppendorf tubes
6. tube racks
7. Marker for labeling
8. 100ºC heat block

Procedure:

1. Take the bacterial culture (1.5ml) into a microcentrifuge tube (MCT).
2. Centrifuge the tube at 10,000rpm for 10minutes.
3. Discard the supernatant.
4. Wash pellet with 750 µl of 1x PBS solution (or DI water) & centrifuge at 10,000rpm for 2minutes.
5. Discard the supernatant.
6. Dissolve the pellet with 500 µl of 5.0% chelex.
7. Keep the tube at 95°C for 20 minutes in heat block (600rpm).
8. Keep the tube at -20°C for 5 minutes.
9. Centrifuge the tube at 10,000rpm for 10minutes.
10. Collect the supernatant into a new microcentrifuge tube.

Quantitation of DNA:

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer/sterile diH_2O to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm.

For pure DNA the ratio of $A_{260/280}$ should fall on the range of **1.8-2.0**. For any DNA sample with A 260/280 ratio ***more*** than 1.8 indicates the *presence of RNA as contamination*. If the ratio is appreciably ***lower*** in either case, it may indicate the *presence of protein, phenol or other contaminants t*hat absorb strongly at or near 280 nm.

Concentration of DNA:

Ratio $A_{260/280}$: