**AIM: Isolation of Genomic DNA from *E. coli* DH5α cells**

**Introduction**

Deoxyribose Nucleic Acid (DNA). is very long and contains the genetic code needed to direct a cell’s activities. DNA was first isolated by Friedrich Meischer in 1869. He extracted a substance from the cells’ nuclei that he called nuclein and later on nucleic acid since the substance isolated was acidic in nature. This discovery of DNA paved the way for many scientists whose work contributed to the understanding of DNA.In 1920s P.A. Levene found that DNA molecule contain three main components:

* 1. Five carbon sugar

1. Deoxy ribose in DNA
2. Ribose in RNA
   1. Phosphate group
   2. Nitrogenous base
3. Purines: Adenine and Guanine
4. Pyrimidines: Cytosine ,Thiamine and Uracil

He concluded that DNA molecule is a polymer and each unit is made up of above mentioned components which he called nucleotides. Later on Chargaff showed that four nucleotides are not present in equal proportions in DNA but there is always an equal proportion of purines and pyrimidines. In 1953 Rosalind Franklin carried out x rays diffraction of DNA molecule which suggested that DNA molecule either helical or corkscrew in shape. Finally Watson and Crick gave the most acceptable model of DNA structure According to this model DNA does not exist as a single strand but as two chains of nucleotides spiralling tightly around an imaginary axis to form a double helix. Deoxyribose sugar-phosphate backbones are on the outside of the helix, and the four different nitrogen bases are paired in the interior of the helix. Double strands are held together by hydrogen bonds between the complementary bases. There is a crucial relationship between the two strands: where there is adenine (A) on one strand, it can only pair with thymine (T) on the other strand, and guanine (G) always pairs with cytosine (C). There is a complementarity between the two strands.

DNA is found both in prokaryotes and eukaryotes. In prokaryotes, DNA is double stranded and circular and is found throughout the cytoplasm and is called nucleoid. In eukaryotes, DNA is located in the nucleus and in mitochondria or chloroplasts. The DNA in the nucleus is double stranded and linear, whereas the DNA in mitochondria and chloroplasts is like prokaryotic DNA, double stranded and circular. The DNA in prokaryotes is relatively free of associated protein, but the DNA in the nucleus of eukaryotes is associated with basic proteins, called histones. Now that the structure of DNA has been studied for over 100 years and has basically been accepted, procedures have been devised to isolate almost pure DNA from its other components.

The isolation of DNA is one of the most commonly used procedures in many areas of bacterial genetics, molecular biology and biochemistry. Purified DNA is required for many applications such as studying DNA structure and chemistry, examining DNA-protein interactions, carrying out DNA hybridizations, sequencing or PCR, performing various genetic studies or gene cloning. The isolation of DNA from bacteria is a relatively simple process. The organism to be used should be grown in a favorable medium at an optimal temperature, and should be harvested in late log to early stationary phase for maximum yield.

**REQUIREMENTS:**

**Biological**

Overnight grown culture of *E. coli*.

**Chemicals**

**A. Luria Broth medium to grow *E. coli*:**

Tryptone - 1.0g

Yeast extract - 0.5g

NaCl - 0.5g

Glucose - 0.1g

Dissolve the components in 75ml of distilled water, adjust the pH to 7.6 and finally make the volume to 100ml. Sterilize by autoclaving

**B. GTE mix:**

50mM Glucose

50mM Tris-HCl

10mM EDTA

Sterilize by autoclaving

**C. 10mM NaCl**

Sterilize by autoclaving

D. **10% SDS**

Dissolve 10g of SDS in 80 ml of distilled water adjust pH to 7.2. Raise the volume to 100ml.

**Do not Autoclave**.

**Glassware/Plastic ware**

All glassware and plastic ware used should be sterilized

* Conical flasks 100 ml
* Petri plates
* Eppendroffs
* Micro tips
* Pipettes

**Instruments/ equipment**

* Orbital shaker
* pH meter
* Weighing balance
* Centrifuge
* Micropipettes

**Principle:**

The isolation of genomic DNA is important as it serves as one of the important starting materials of many further experiments, such as PCR, restriction digestion and whole genome sequencing, etc. There are several basic steps in DNA extraction. The five steps are as follows:

1. **Homogenization or disruption of cells**: The cell must first be lysed (broken open) to release the nucleus in eukaryotes or nuleoid in prokaryotes. Cells are broken by grinding, tissue homogenization, or treatment with Iysozyme
2. **Inhibition of DNAase**: At this point the DNA must be protected from enzymes that will degrade it, causing shearing. Many of the nucleases present in cells can digest nucleic acids. When the cell is disrupted, the nucleases can cause extensive hydrolysis. Nucleases apparently present on human fingertips are notorious for causing spurious degradation of nucleic acids during purification. Chelating agents are added to remove metal ions required for nuclease activity.
3. **Dissociation of nucleoprotein complexes:** DNA-protein interactions are disrupted with SDS, phenol, or broad spectrum proteolytic enzymes as pronase or proteinase K. Alkaline pH and high concentration of salts improve the efficiency of the process.
4. **Removal of contaminating materials;** contaminating molecules especially proteins are removed by treatment with phenol or chloroform-isoamyl alcohol or phenol chloroform. Proteins can also be removed by salting out proteins by sodium acetate.
5. **Precipitation of DNA:** Once the DNA is released, it must be precipitated in alcohol (with salt). The DNA in the aqueous phase is precipitated with cold (0oC) ethanol. The precipitate is usually redissolved in buffer and treated with phenol or organic solvent to remove the last traces of protein, followed by reprecipitation with cold ethanol. RNA is removed by limited treatment with deoxyribonuclease-free ribonuclease.
6. **Washing of DNA:** Final step is the washing of DNA with 70% ethanol to remove the traces of salt used in precipitation step. This is followed by drying and dissolving in double distilled water or TE buffer.

**Role of Chemicals:**

1. **GTE Mix:** It is mainly used for resuspending the cell pellet prior to lysing them and extracting the DNA. Besides GTE also provides a stable environment for the DNA. 50 mM glucose is added because it maintains osmolarity and it being a non-electrolyte doesn’t interfere with the buffering properties of the other components. Tris pH 8.0 is the pH buffer and it maintains the near physiological pH which doesn’t harm the components inside the cell. EDTA chelates the metal ions such as zinc, magnesium and calcium and thus prevent the degradation of the DNA.
2. **Sodium Dodecyl Sulfate 1%:** SDS is a strong anionic agent and it removes the negative ions from the proteins and destroys its structure. This also aids in destroying the proteins of the cell membrane and destroys them, as a result of which cell lysis takes place. It thus exposes the chromosomal DNA. SDS also frees the DNA from the histones and other DNA binding proteins attached to the DNA by denaturing them.
3. **Sodium Chloride (NaCl) and Isopropanol:** It is used to precipitate solubilized high molecular weight DNA from the phenol (inorganic layer).
4. **Ethanol 70%:** It is used to wash/purify the DNA once it is isolated. Ethanol 70% maintains the osmolarity of the DNA.

**PROCEDURE**

* Take 1.5 ml of overnight grown culture of *E. coli* in microfuge tube.
* Centrifuge at 10,000 rpm for 5 minutes to obtain pellet
* Discard the supernatant and add 200 µl of GTE mix. Break the pellet.
* Incubate at room temperature for 5 minutes.
* Add 400 µl of 1% SDS and at room temperature for 5 minutes.
* Add 60 µl of 10mM NaCl and 700 ul of isopropanol in ice.
* Invert mix.
* Centrifuge at 10,000 rpm for 20 minutes.
* Decant the alcohol and air dry.
* Dissolve in 50 µl of TE buffer/ddH2O.
* Add the loading buffer and subject it to gel electrophoresis.

**RESULT**

**(PASTE THE WELL LABELLED GEL PHOTOGRPAPH)**

**DISCUSSION**

Nucleic acids are the most polar of the biopolymers and are therefore soluble in polar solvents and precipitated by nonpolar solvents. It is also imperative to mention here, that the source of DNA isolation is an important consideration for the protocol to be followed and the quality as well as the purity of the product obtained. The extracted DNA was dissolved in TE buffer. The quality of DNA was judged by the electrophoresis. On electrophoresis one band was observed near the well which clearly indicate that the band is of high molecular weight and thus it’s a genomic DNA. Since there only one band is seen on the gel, so no shearing of DNA has taken place.

**PRECAUTIONS**

1. Use caution when operating the centrifuge.
2. The laboratory surfaces should be very clean during all procedures used in this activity.
3. Use thoroughly clean instruments and glassware. Rinse all equipment with isopropyl alcohol or acetone.
4. Ethanol is highly flammable; use caution.