Recombinant DNA Technology Laboratory Report.

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Title: DNA isolation and gel electrophoresis.

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Introduction:

A plasmid is a small circular DNA molecule found in bacteria and some other microscopic organisms. Plasmids are physically separate from chromosomal DNA and replicate independently. They typically have a small number of genes — notably, some associated with antibiotic resistance — and can be passed from one cell to another. Scientists use recombinant DNA methods to splice genes that they want to study into a plasmid. When the plasmid copies itself, it also makes copies of the inserted gene.

Recombinant DNA technology involves using enzymes and various laboratory techniques to manipulate and isolate DNA segments of interest. This method can be used to combine (or splice) DNA from different species or to create genes with new functions. The resulting copies are often referred to as recombinant DNA.

The isolation of plasmid DNA from bacteria is a crucial technique in molecular biology and is an essential step in many procedures such as cloning, DNA sequencing, transfection, and gene therapy. These manipulations require the isolation of high purity plasmid DNA. The purified plasmid DNA can be used for immediate use in all molecular biology procedures such as digestion with restriction enzymes, cloning, PCR, transfection, in vitro translation, blotting and sequencing.

Purification of plasmid DNA from bacterial DNA using is based on the differential denaturation of chromosomal and plasmid DNA using alkaline lysis in order to separate the two. The basic steps of plamid isolation are disruption of the cellular structure to create a lysate, separation of the plasmid from the chromosomal DNA, cell debris and other insoluble material. Bacteria are lysed with a lysis buffer solution containing sodium dodecyl sulfate (SDS) and sodium hydroxide. During this step disruption of most cells is done, chromosomal as well as plasmid DNA are denatured

and the resulting lysate is cleared by centrifugation, filtration or magnetic clearing. Subsequent neutralization with potassium acetate allows only the covalently closed plasmid DNA to reanneal and to stay solubilized. Most of the chromosomal DNA and proteins precipitate in a complex formed with potassium and SDS, which is removed by centrifugation.

Aim of the experiment:

Mapping of plasmids extracted from bacterial cells for various uses.

Materials and methods :

Materials :

- RNase and cell lysis containing SDS detergent (Sodium Dodecylsulfate).
- Potassium acetate.
- Isopropanol.
- Ethanol.
- EDTA Buffer.
- Concentrated Gel Loading.
- Electrophoresis Buffer.
- Blue Tracking Dye.
- Centrifuge.
- Micropipette.
- Microcentrifuge Tubes.
- Casting Tray.
- InstaStain Blue.
- Flask.
- Distilled Water.
- Microwave.
- Agarose Powder.

Methods:

- A labeled E.coli microcentrifuge is collected and placed in a test tube.

- At speed of 14000 rpm, the sample is centrifuged for 2 minutes duration at room temperature.
- Supernatant is removed and 200 microliter was added to the pellet followed by pipetting up and down.
- 5 microliter of RNase solution is added to the cell suspension.
- The sample is incubated at room temperature for 5 minutes.
- 350 microliters of freshly prepared Lysis buffer was added ti the cell pellet.
- 200 microliters of Potassium Acetate solution is added and mixed until white precipitate is performed.
- The sample is incubated for 5 minutes for ice without shaking it.
- At full speed for 5minutes, the sample is centrifuged at room temperature.
- In a clean 1.5ml microcentrifuge tube is added the sample by discarding the tube containing the pellet.
- 0.6 volume of 100% isopropanol is added to the supernatant following by inverting the tubes 4 to 6 times.
- The sample is incubated for 5 minutes at room temperature.
- The sample is centrifuged for5 minutes at full speed until a small plasmid DNA is formed with white pellet at the bottom.
- The supernatant is removed gently.
- DNA pellet is added by the addition of 350 microliters of ice-cold Ethanol.
- At full speed for 3 minutes, the sample is centrifuged.
- The supernatant is obtained after centrifugation and removed after air drying the pellet for 5-10minutes for ethanol removal.
- The pellet is suspended in a 60 microliters of EDTA Buffer, followed by centrifugation.
- Purified DNA is obtained for electrophoresis.
- 40 microliters of **concentrated** DNA is transferred to a fresh centrifuge tube and 5microliters of 10x gel loading solution is added by labeling and placing it.
- Agarose Gel is performed and placed into the electrophoresis chamber by covering it with electrophoresis buffer which the whole gel is submerged.
- 40 microliters of the sample is loaded into the well.

- Power source is connected and electrophoresis is performed.
- Gel is removed and casting tray is stained into the agarose gel.
- The agarose gel and casting tray in the following steps are removed into a small, clean gel-staining tray.
- With 1x FlashBlue, the gel is covered and stained for 5 minutes.
- Results are visualized after gel is removed from destaining liquid.
- InstaStain Blue is used to for Agarose gel staining.

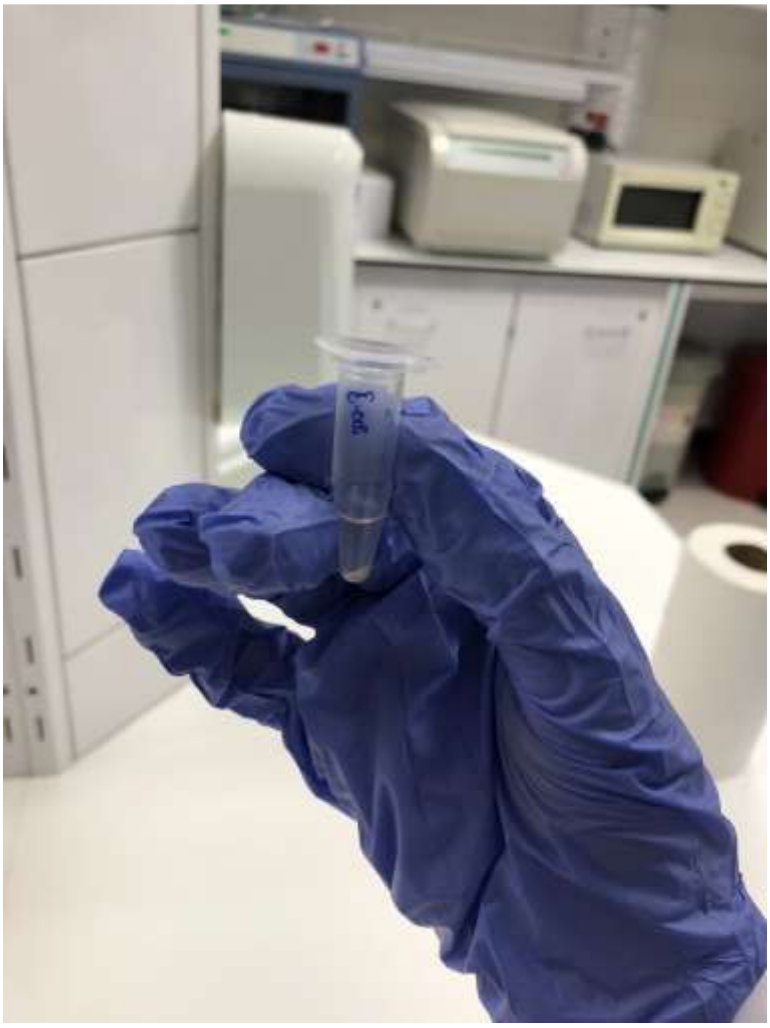

Figure 1: Sample of E.coli in microCentrifuge Tube

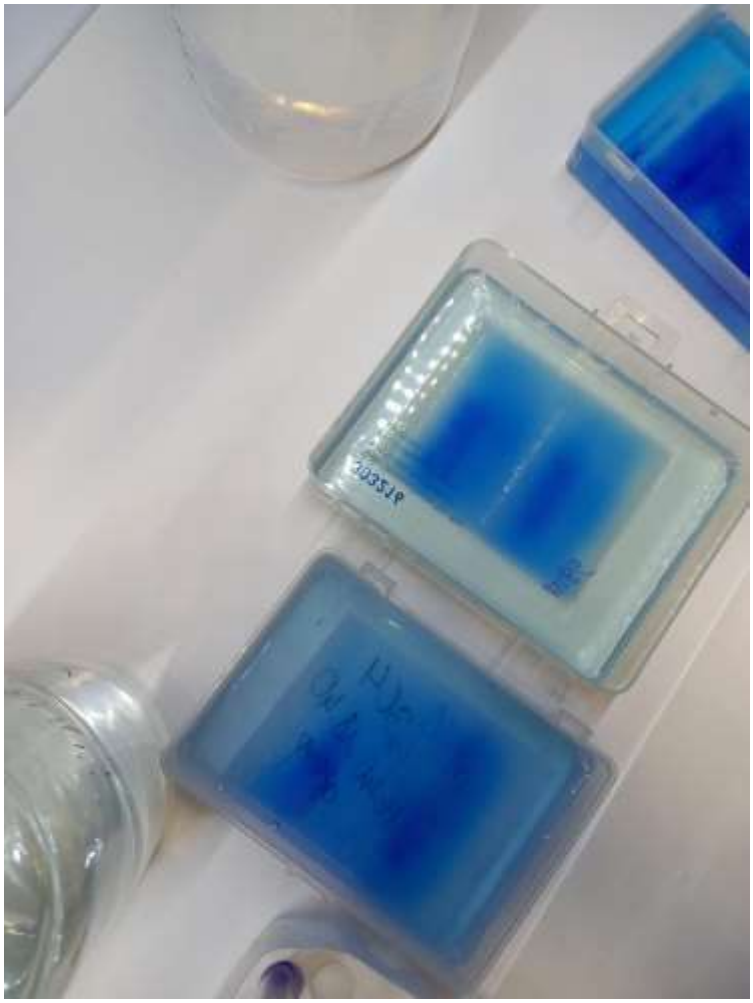

Figure 2 : FlashBlue DNA Stain.

Figure 3 : Electrophoresis Chambers connected to power supply.

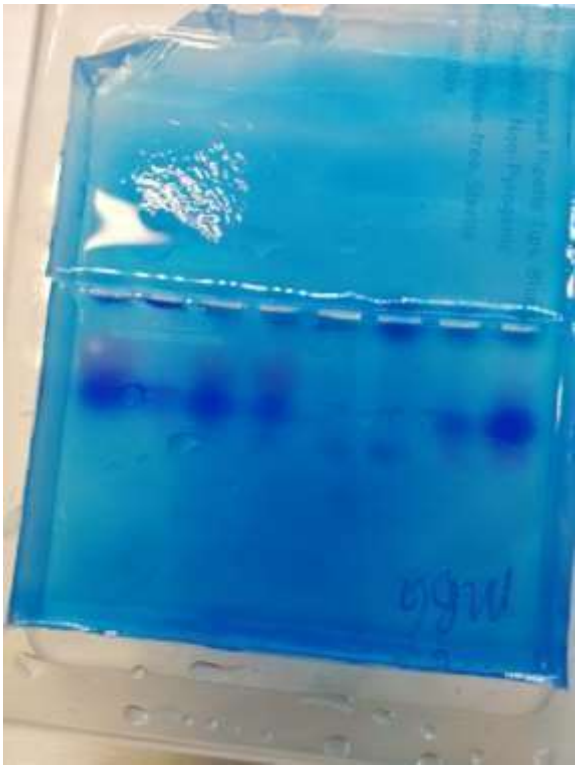

Figure 4 : Results of dark blue DNA on a white light visualization system.

Results:

After adding several chemical components during plasmid isolation, high quality purified DNA was successively generated and extracted to be performed in a gel electrophoresis containing an electric field, provided from a power supply source in a electrophoresis chamber safety, cover with loading different sample obtained in a consecutive wells. Otherwise, the DNA fragments purified migrated to the positive terminal due to their overall negative charge. Larger fragments remained up, closer to the point of origin, while small fragments down, travel farther down the gel.

Some of the DNA fragments did not migrate even under the influence of the voltage, which was the unexpected results during the experiment.

Discussion :

-During the first procedures of plasmid extraction, several steps were included in order to perform the plasmid needed, the RNase and cell lysis were added to suspend bacterial cells and isolate the plasmid. The solution containing cell lysis contains SDS which is called Sodium Dodecylulfate is an alkaline solution of pH greater than 12, it is used to denature the proteins and dissolve the cell membrane. Because of highly pH solution, Buffer was added to neutralize the solution 'Potassium Acetate' and Isopropanol to precipitate the remaining DNA and plasmid. Plasmid DNA also usually requires subsequent column purification steps, probably because of SDS contamination. The experiment was further continued with gel electrophoresis to separate different fragments of DNA under influence of electrical field,. The migration is not only based on size and shape, but also on type of electrophoresis buffer, gel concentration, and applied voltage. The concentration of gel loading solution should be denser than of the Buffer so as the sample will be submerged in the wells and Blue tracking dye is added to monitor the electrophoresis, causing the negatively charged nucleic acids to move toward the positive electrode. Shorter DNA fragments will travel more rapidly, whereas the longest fragments will remain closest to the origin of the gel.

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