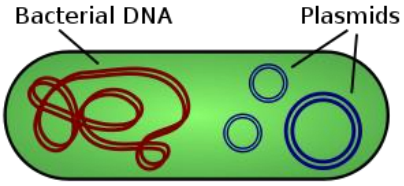
3. ISOLATION AND CHARACTERISATION OF DNA

a) isolation of plasmid DNA on columns (3.1)
b) measurement of DNA concentration by spectrophotometry (3.2)
c) electrophoretic separation of plasmid DNA on agarose gel (3.3)

3.1 ISOLATION OF PLASMID DNA

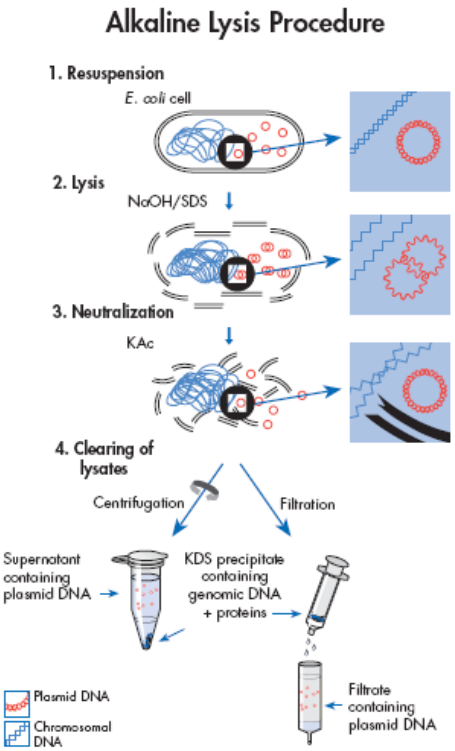
OBJECTIVE: ISOLATION OF PLASMID DNA FROM *E.COLI*

TOPICS:
DNA, chromatography, UV spectroscopy

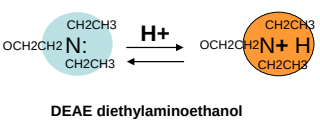


Plasmid DNA: In microbiology and genetics, a **plasmid** is a DNA molecule that is separate from, and can replicated independently of, the chromosomal DNA. They are double-stranded and, in many cases, circular. Plasmids usually occur naturally in bacteria, but are sometimes found in eukaryotic organisms (e.g., the *2-micrometre ring* in *Saccharomyces cerevisiae*). Plasmid sizes vary from 1 to over 1,000 kbp.

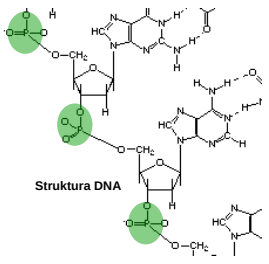
Isolation of plasmid DNA from *E. coli* is a common routine in research laboratories. You will perform a widely-practiced procedure that involves alkaline lysis of cells and chromatography on DEAE column. This protocol often referred to as a plasmid "mini-prep," yields fairly clean DNA quickly and easily.



Principe of chromatography separation of DNA on the columns



DEAE diethylaminoethanol



Protocol-at-a-glance

FastPlasmid™ Mini Kit quick protocol

1.5 ml Bacterial Culture: Centrifuge for 1 minute to pellet the cells

Decant the media

Add 400 µl of ICE-COLD Complete Lysis Solution
Vortex for a full 30 seconds at the highest setting

Incubate at room temperature for 3 minutes

Transfer the lysate to a Spin Column Assembly

Centrifuge for 30 – 60 seconds

Wash DNA with 400 µl of DILUTED Wash Buffer

Centrifuge for 30 – 60 seconds

Decant the filtrate from the waste tube and re-assemble the Spin Column Assembly

Centrifuge for 1 minute

Transfer the Spin Column into a Collection Tube

Add 50 µl of Elution Buffer to the center of the Spin Column

Centrifuge for 30 – 60 seconds

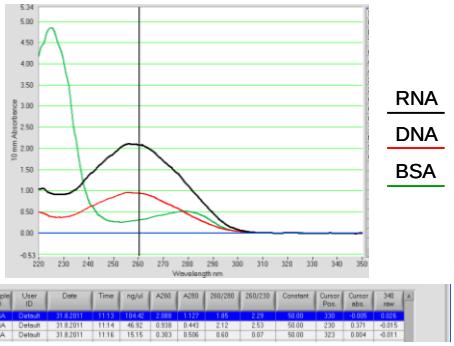
3.2 UV SPECTROPHOTOMETRY OF DNA

OBJECTIVE: **ANALYSIS OF DNA, DETERMINATION OF DNA CONCENTRATION**
Biochemists routinely work with DNA, RNA and proteins and have devised some simple, fast spectrophotometric assays for these molecules. The purpose of this exercise is to use the UV absorbance of biological samples to obtain qualitative and quantitative information about those samples. In this spectrophotometry exercise you will:
1. Produce UV absorbance spectra of DNA
2. Quantify plasmid DNA by measurement of DNA absorbance at 260 nm

1. UV Spectrophotometry of DNA, RNA, and Proteins

Brief Background Review
A. The UV Absorbance Spectra of Nucleic Acids and Proteins .
Most biological molecules do not intrinsically absorb light in the visible range, but they do absorb ultraviolet light. Biologists take advantage of UV absorbance to quickly estimate the concentration and purity of DNA, RNA, and proteins in a sample. In a previous laboratory, you learned how to quantify DNA using the diphenylamine reaction. It is also possible to quantify the amount of DNA in a sample by looking at its absorbance at a wavelength of 260nm or 280nm (in the UV region). The UV method described in this exercise is not highly accurate but it is very widely used since it is easy, quick and little DNA is required.

Proteins have two absorbance peaks in the UV region, one between 215-230 nm, where peptide bonds absorb, and another at about 280 nm due to light absorption by aromatic amino acids (tyrosine, tryptophan and phenylalanine). Certain of the subunits of nucleic acids (purines) have an absorbance maximum slightly below 260 nm while others (pyrimidines) have a maximum slightly above 260 nm. Therefore, although it is common to say that the absorbance peak of nucleic acids is 260 nm, in reality, the absorbance maxima of different fragments of DNA vary somewhat depending on their subunit composition. Figure 1 shows the UV spectra for DNA, RNA, and proteins.



Protocol of spectrophotometry of DNA and proteins

1. Preparation of samples

- Dilute samples first in eppendorf tubes -see table

		sample µl (2-20 µl pipette)	water µl (0,1-1 ml pipette)
1	Plasmid DNA	10	490
2	Blank - water	0	500

2. Measurement

- Prepare samples 1-2 (by pipetting) to Eppendorf tube
- Measure the absorbance of samples against deionized water
- Fill up the table

		A_{230}	A_{260}	A_{280}	A_{330}	A_{260}/A_{280}	A_{260}/A_{230}	
1	Plasmid DNA							

3. Quantification of plasmid DNA concentration from absorbance at 260 nm

For calculation of pure dsDNA, a simple method is used.

If a sample containing pure double-stranded DNA has an absorbance of 1 at 260 nm in 1 cm cuvette, then it contains approximately 50 µg/mL of double-stranded DNA.

CALCULATE CONCENTRATION OF PLASMID DNA

A_{260}=1.....concentration of DNA **50 µg/mL**

A_{260}= .....----------.....X..... concentration of DNA in cuvette

Don't forget on dilution

Dilution = final volume (10+490 µl)/initial volume (10 µl)

Concentration of DNA in sample = dilution * X µg/mL

Your calculation:

3.3 AGAROSE ELECTROPHORESIS OF DNA

Background

Agarose gel electrophoresis is the easiest and most common way of separating and analyzing DNA. The purpose of the gel might be to look at the DNA, to quantify it or to isolate a particular band. The DNA is visualized in the gel by addition of ethidium bromide. This binds strongly to DNA by intercalating between the bases and is fluorescent, meaning that it absorbs invisible UV light and transmits the energy as visible orange light.

1. Preparing and Running Standard Agarose DNA Gels

The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple and include:

- *An electrophoresis chamber and power supply*
- *Gel casting trays, which are available in a variety of sizes and composed of UV-transparent plastic. The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.*
- *Sample combs, around which molten agarose is poured to form sample wells in the gel.*
- *Electrophoresis buffer, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).*
- *Loading buffer, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.*
- *Ethidium bromide, a fluorescent dye used for staining nucleic acids.* ***NOTE: Ethidium bromide is a known mutagen and should be handled as a hazardous chemical - wear gloves while handling****.*
- *Transilluminator (an ultraviolet lightbox), which is used to visualize ethidium bromide-stained DNA in gels. NOTE: always wear protective eyewear when observing DNA on a transilluminator to prevent damage to the eyes from UV light.*

A) To pour a gel, 1 g of agarose powder is mixed with electrophoresis buffer (1X TAE) to the desired concentration, then heated in a microwave oven until completely melted. Most commonly, ethidium bromide is added to the gel (final concentration 0.5 ug/ml) at this point to facilitate visualization of DNA after electrophoresis. After cooling the solution to about 60C, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature or, if you are in a big hurry, in a refrigerator.

B) After the gel has solidified, the comb is removed, using care not to rip the bottom of the wells. The gel, still in its plastic tray, is inserted horizontally into the electrophoresis chamber and just covered with buffer. Samples containing DNA mixed with loading buffer are then pipette into the sample wells, the lid and power leads are placed on the apparatus, and a current is applied. You can confirm that current is flowing by observing bubbles coming off the electrodes. **DNA will migrate towards the positive electrode, which is usually colored red.**

C) Calculate the volumes of DNA and RNA samples needed for preparing appropriate amounts of nucleic acids to load on gel. Complete the tables. Prepare 13 samples for electrophoresis according to the table and load them on gel.

sample	Volume of DNA μl	6xLB
Plasmid DNA	5	
Plasmid DNA	10	

corresponding to supercoiled forms and nicked circles. The image to the right shows an EtBr-stained gel with uncut plasmid in the left lane and the same plasmid linearized at a single site in the right lane.

After placing the gel on UV transilluminator, we can photograph it.