BACTERIAL DNA EXTRACTION

Ref. ADNBACT (25 extractions)

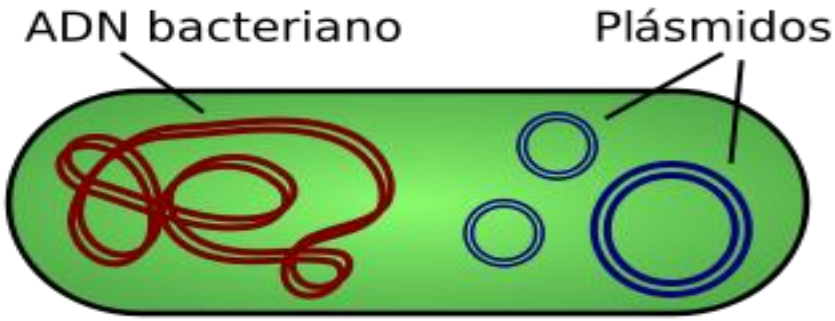
1. EXPERIMENT OBJETIVE

The aim of this experiment is to introduce the principles of chromosomal DNA extraction from bacterial *E. coli* cells.

Students will learn the structure and function of nucleic acids contained in bacteria.

2. BACKGROUND INFORMATION

All genetic information essential to the life of the bacterium is contained in a single molecule of circular, double-stranded **deoxyribonucleic acid (DNA)**, closed by covalent bonding. This molecule is called a **bacterial chromosome**. Many bacteria also possess extrachromosomal DNA, also circular and closed, called **plasmid DNA** because it is contained in the plasmids. These carry gene information for many functions that are not essential for the cell under normal growth conditions.



In biochemical terms the composition and structure of bacterial nucleic acids is the same as for any cell. It should be briefly recalled that nucleic acids are macromolecules composed of nucleotides covalently bound by phosphodiester bonds between the carbons at positions 3 and 5 of two adjacent sugar residues. This structure forms a skeleton of sugars and phosphates constant throughout the macromolecule.

The variation between the nucleotides that constitute the nucleic acid chain, is given by its nitrogen bases; for **DNA** are: **adenine (A), thymine (T), cytokine (C)** and **guanine (G)** and for **ribonucleic acid (RNA)** the **uracil (U)** are instead thymine. A and G are called **purine bases or purines**, while T, U, and C are called **pyrimidine bases or pyrimidines**. Thus, a nucleic acid strand or strand will have a primary structure determined by the sequence of the bases that compose it. DNA, as a macromolecule, is composed of two nucleotide strands or antiparallel strands that bond together to form a double helix. The bonds between the two strands of DNA are determined by hydrogen bridges between the purines of one chain, with the pyrimidines of the other. Then the A forms two bridges of hydrogen with the T, whereas the C forms three bridges of hydrogen with the G. This phenomenon is

known as **complementarity of bases**, that is to say that the A is complementary to the T and the C is it For G. These bonds maintain the stable double-stranded DNA structure.

In this double-stranded DNA structure, **pairs of nucleotides or base pairs (bp)** can be distinguished. These bp can be used as a unit of size or length for the DNA molecules, in this way we can say for example that the chromosomal DNA of *Escherichia coli* has a size of 4.2 million bp or what is the same of 4,200 kilobases Kb).

All cells must face the problem of how to contain molecules as large as DNA in their structure. Returning to the example of *E. coli*, the 4,200 Kb of its genome implies a length of 1.3 mm is a thousand times the length of the cell. Bacteria do not possess histones associated to their genome and consequently do not have the possibility of compacting their DNA in structures like nucleosomes like eukaryotic cells. Therefore, they must pack their DNA in another way. This is achieved because the closed circular DNA is able to adopt a tertiary structure called **supercoiling**, which involves winding the double helix axis on itself. This supercoiling is said to have negative sense because it has the opposite sense to winding one strand of DNA over the other.

This supercoiling structure also assumes for the bacteria a source of energy storage to be used in many physiological processes that require it, for example the separation of the two strands of DNA required for replication and transcription. The bacterial chromosome is sufficiently long to form many circular loops, which as such may overcook into a series of independent topological domains. This organization in domains collaborates to the general compaction of the bacterial genome and prevents that, with the rupture of a strand (in any place of the chromosome) the total supercoiling is lost, keeping the energy stored.

3. EXPERIMENT COMPONENTS for 25 extractions

COMPONENTS	
E. coli bacterial pelets	25 units
Lysis solution	20 ml
Protein precipitation solution	10 ml
Hydration solution	20 ml

Required and NOT supplied components

- Microcentrifuge.
- Microtubes and micropipettes.
- Incubation bath.
- Ethanol 70% and Isopropanol.
- Basic electrophoresis system (apparatus and reagents).
- Nucleic acid detection system.

4. EXPERIMENT PROCEDURES

This practice allows isolating the chromosomal DNA from bacterial cells of *E. coli*, these bacteria are Gram (-) so the treatment with lysozyme is not needed if they need Gram (+) bacteria that are more difficult to lyse and need for them Lytic enzymes.

4.1 Simple Protocol

This protocol allows a fast extraction of chromosomal DNA so that it can be performed quickly in a practical class with students. Bacterial cells were obtained by centrifugation of 1.5 ml of an overnight culture of *E. coli* cells and supplied as a bacterial pellet.

The first step is to incubate the bacterial pellet with a lysis solution that will break the cell membranes and release the nucleic acids. Proteins and cellular debris will then be removed with the addition of a protein precipitation solution. After centrifugation we will remain with supernatant and precipitate the nucleic acids with isopropanol, followed by a 70% ethanol wash and finally the DNA hydration.

Cellular lysis

1. Add 1.5 ml of an overnight culture to a 1.5 ml tube.
2. Centrifuge at 14,000 x g for 30 seconds. Remove the supernatant.
3. Add **600 µl** of **Lysis Solution** to the cell pellet and pipette to resuspend and lyse the cells.
4. Incubate the samples at 80°C for 5-10 minutes. Cool at room temperature.

Protein precipitation

1. Cool sample to room temperature.
2. Add **300 µl** of **Protein precipitation solution**.
3. Vortex vigorously for 20-30 seconds.
4. Centrifuge at **14,000 x g** for **5 minutes**. It will be noted that the protein precipitate forms a pellet.

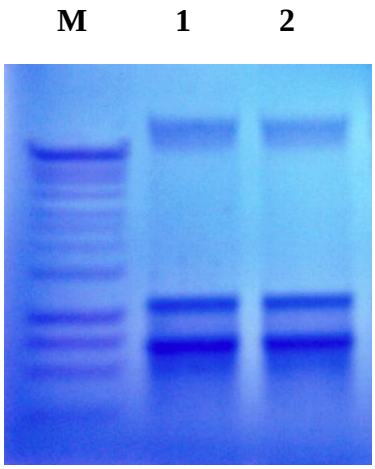
DNA Precipitation

1. Transfer the supernatant containing the DNA to a **1.5 ml** tube containing **600 µl of isopropanol.** Mix by inversion several times.
2. Centrifuge at **14,000 x g** for 3 minutes.
3. Remove supernatant. Add **600 µl** of **70% ethanol** and invert several times to wash the DNA pellet.
4. Centrifuge at **14,000 x g** for 2 minutes. Carefully remove all the ethanol. Watch not lose the DNA pellet
5. Invert the tube and allow to dry on absorbent paper for 15 minutes.

Hydration of DNA

1. Add **500-750 µl** of **DNA hydration Solution**. Resuspend by micropipette the white pellet. Incubation at 55°C with periodic vortex stirring will aid in the dissolution of the DNA.

5. PRACTICE RESULTS



M: Molecular weight marker.

1. *E. coli* Bacterial pellet.
2. *E. coli* Bacterial pellet.

Staining done with our DANABLUE-FLASHBLUE NO-TOXIC system.

Two bands above the last of the molecular weight marker corresponding to the **chromosomal DNA** can be observed. In this fast method we do not use the RNase enzyme that would eliminate **degraded RNA** that we can observe as one or two bands on the bottom of the agarose gel.