PLASMIDIC DNA EXTRACTION

Ref. ADNPLASMIDO (25 extractions)

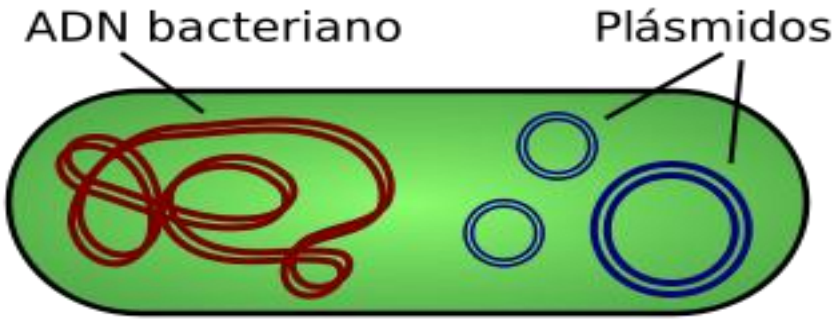
1. EXPERIMENT OBJETIVE

The aim of this experiment is to introduce the principles of extracting plasmid DNA from bacterial cells.

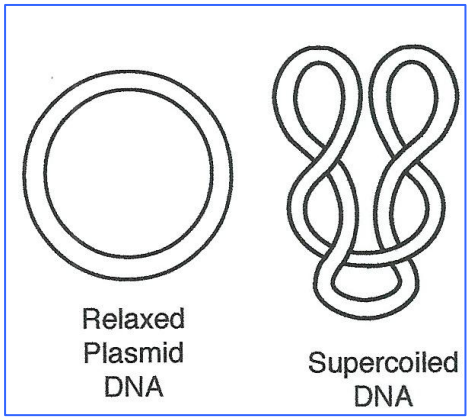
Students will learn the structure and function of DNA plasmids.

2. BACKGROUND INFORMATION

Plasmids are extracromosomal fragments of nucleic acids (**DNA or RNA**) that appear in the cytoplasm of some prokaryotes. They are of variable size, although smaller than the main chromosome. Each bacterium may have one or more bacteria at a time.



The plasmids have a variable conformation that can be linear, circular or supercoiled DNA.



Naturally, plasmid DNA exists as a **supercoiled molecule** (Form I), this supercoiled DNA is itself bent and has a more condensed and entangled structure than the same DNA when it is relaxed.

The purified DNA must be a closed circle covalently and be in the form of supercoiled structure, this structure in the cell is caused by the action of some enzymes called **gyrases** and this has important biological consequences. If one or more phosphates attached to the skeleton of the supercoiled DNA are removed or broken (nicks), the molecule passes into a relaxed form called **circular DNA** (Form II). The 2 strands of the circular DNA are not covalently closed and held together by hydrogen bonds between the bases.

The supercoiled plasmid DNA purified over time and slowly develops nicks and becomes Form II, this is because the DNA in Form I is not as stable as the circular or relaxed forms.

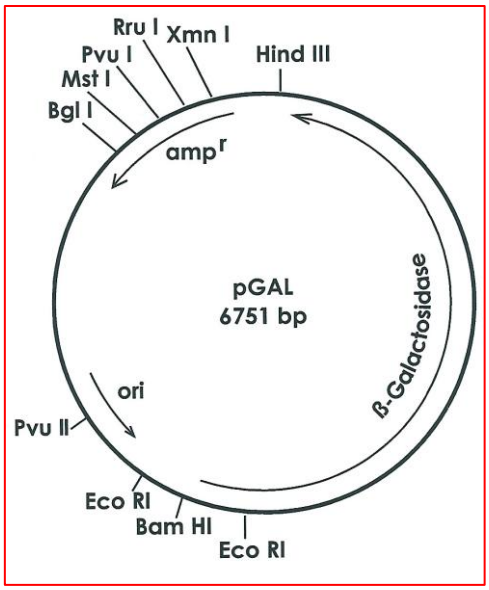
Each type of plasmid presents a control of its different replication, existing plasmids whose replication is coupled with the replication of the bacterial chromosome and others whose replication is not related to the one of the chromosome. The type of genes that carry the plasmids is varied, generally being genes that provide adaptive advantages to the bacteria that carry them: antibiotic resistance genes, genes producing substances toxic to other bacteria or genes that encode enzymes useful for degrading chemical substances.

Plasmids can be classified according to different criteria. One of these criteria is the type of genes they carry. This defines the group of plasmids with substance degradation genes, another group with fertility genes, the one that carries virulence genes or the group that carries resistance genes.

Plasmids are very useful tools in genetic engineering for gene transformation and the genetic manipulation of prokaryotes and eukaryotes cells. Plasmids used in genetic engineering are called **vectors**. They are very useful for synthesizing in large quantities proteins of interest, such as insulin or antibiotics, by a process known as **transformation**. The transformation process begins with the selection of a suitable plasmid, which introduces the genes to be expressed with specific protocols using restriction enzymes and DNA ligase. Subsequently a type of bacteria is transformed with the modified plasmid and the transformed bacteria are selected that produce the desired substances. These bacteria are grown in bioreactor type systems for growth in large quantities. In this process, plasmids with characteristics that allow the selection of transformed bacteria in a culture medium such as plasmids with antibiotic resistance genes or with enzymes genes that synthesize colored compounds.

Although plasmids cannot synthesize a protein envelope and are difficult to transfer from one cell to another it has been hypothesized that they could be the precursors of the first viruses.

In this experiment the 6751 base pair **pGAL plasmid** that has been engineered will be isolated from bacterial cells that are supplied with the kit. The pGAL plasmid contains the *E. coli* gene coding for **β-galactosidase** which in the presence of the artificial **galactoside X-GAL** will produce blue colonies, this is because when β-galactosidase breaks the X-GAL produces a blue product, this property will make it possible to distinguish in a culture dish that bacterial cells have the pGAL plasmid. In addition, this plasmid confers resistance to the antibiotic **ampicillin** since it contains the gene coding for **β-lactamasa** which inactivates ampicillin.



3. EXPERIMENT COMPONENTS for 25 extractions

COMPONENTS	
Bacterial pellets containing the plasmid pGAL	25 units
DANAGENE Plasmid Mini Kit	25 extractions

Required and NOT supplied components

- Microcentrifuge.
- Microtubes and micropipettes.
- 100% Ethanol.
- Basic electrophoresis system (apparatus and reagents).
- Nucleic acid detection system.

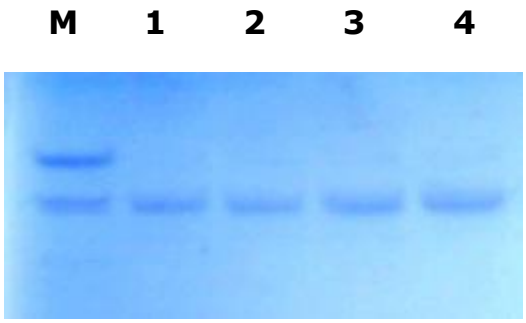
4. EXPERIMENT PROCEDURES

This practice, which allows isolating the **pGAL plasmid** from bacterial cells, is carried out with the commercial kit **DANAGENE Plasmid Mini Kit**. This kit allows the extraction of the plasmid DNA using a method called alkaline lysis and treatment with RNAse that allow the production of a clear cell lysate with minimal amount of DNA and RNA contaminating. In the presence of chaotropic salts, the plasmid DNA binds to the silica membranes of columns present in the kit. The contaminants are removed with wash buffer and the plasmid DNA is eluted with an elution buffer.

Follow the operating instructions included in the kit.

1. If a basic system of electrophoresis is not available, contact the technical department of DanaGen-BioTed info@bioted.es who can supply all the materials necessary to carry out the electrophoresis of the results of plasmid DNA extraction.
2. If you do not have your own DNA visualization system (ethidium bromide, SBRY Green, etc.), we can supply you our **DANABLUE** or GELSAFE (you need a UV transilluminator).

5. PRACTICE RESULTS



M: Molecular weight marker.

1. Plasmid pGAL.
2. Plasmid pGAL.
3. Plasmid pGAL.
4. Plasmid pGAL.

Staining done with our NO TOXIC DANABLUE system