

Lab seven :

Plasmids isolation and extraction:

What is the Plasmid DNA ????

Plasmid is autonomously replicating, extra-chromosomal circular DNA molecules, distinct from the normal chromosomal DNAs and nonessential for cell survival under nonselective conditions. Bacterial plasmids are closed circular molecules of double-stranded DNA that range in size from 1 to >200 kb. They are found in a variety of bacterial species, where they behave as additional genetic units inherited and replicated independently of the bacterial chromosome. Plasmids often contain genes that code for enzymes that can be advantageous to the host cell in some circumstances. The encoded enzymes may be involved in resistance to antibiotics, resistance to toxins found in the environment (e.g., complex organic compounds), or the production of toxins by the bacteria itself. The term plasmid was first introduced by the American molecular biologist Joshua Lederberg in 1952. In the same year, J. Lederberg reviews the literature on cell heredity and suggests the term "Plasmid" for all extra-chromosomal hereditary determinants.

That the size of the plasmids is very small compared with the size of the bacterial chromosome and older plasmids is only about 0.8% of the size of the bacteria *E. coli* chromosome despite the presence of other plasmids smaller than this size much despite the similarity of the Pl. DNA & Ch. DNA in a circular being a single binary string but there inside the cell differently from the chromosome where the plasmids strongly wrapped around itself, creating what is known Super coiled plasmid or Covalently closed circular (C.C.C.). If phenotypic markers of a plasmid (e.g. antibiotic resistances) are known, it is recommended to grow the cells under selective pressure to avoid plasmid loss.

Types of Bacterial Plasmids: Based on their function ,there are five main classes:

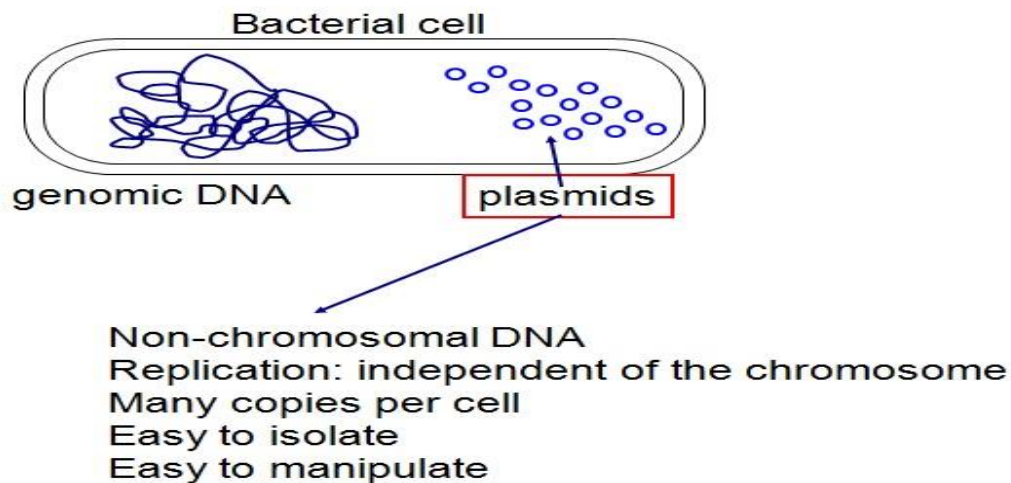
1-Fertility-(F) plasmids: they are capable of conjugation or mating .

2-Resistance-(R) plasmids :containing antibiotic or drug resistant genes. Also known as R-factors ,before the nature of plasmids was understood.

3- Col-plasmids :contain genes that code for colicines, proteins that can kill other bacteria .

4-Degrative plasmids :enable digestion of unusual substances, e.g ,. toluene or salicylic acid .

5-Virulence plasmids: convert the bacterium into a pathogen



Plasmids preparation:

Boiling method :

The boiling lysis method of plasmid isolation is quick and is recommended for isolation of small plasmids (up to 10 kb). Plasmid with size larger than 10 kb should be isolated by other methods (e.g., alkaline lysis method). The quality of plasmid, isolated by this method, is not as good as the plasmid isolated by alkaline lysis method. However, the quality is good enough for restriction digestion analysis. In this method, the bacterial cells are given brief heat treatment in boiling water bath in presence of lysozyme and triton X-100. Plasmid DNA, due to its small size, comes out from the bacterial cell, whereas, genomic DNA remains trapped inside the cell. Subsequent high speed centrifugation separates the plasmid DNA from rest of the cell debris, which form pellet. Pellet is removed and plasmid DNA is recovered by ethanol or isopropanol precipitation method.

modified alkaline lysis method

NOTE: In this experiment use E. coli carries Ampicillin resistance marker on plasmid RY121, which carries the gene, encodes for the β -lactamase.

- 1- Inoculate bacteria into selective Brain heart infusion agar plates (Ampicillin) and incubate plates overnight at 37°C in the shaker and then harvesting.
- 2- Put 2ml of TE buffer in Eppendrofe tube and transfer loop full of bacterial growth into this Eppendrofe and centrifuge at 11000 rpm for 10 minutes. resuspende the pellet in 40 μ l of the same TE buffer.
- 3- Prepare freshly lysis buffer, add (600 μ l) to the cell suspension and mixed gently.
- 4- Incubate the lysis at 37°C for 20-30 minutes.
- 5- Add 2M Tris HCL (pH 7), 30 μ l for neutralization, and mix the mixture.
- 6- Add 5M NaCl, about 240 μ l for precipitation of chromosomal DNA and protein, then incubate in ice for 4 hrs and then centrifuge at 11000 rpm for 10 minutes.
- 7- Transfer the supernatant into another tube and add a double volume of absolute ethanol, then incubate in ice for overnight, after incubation centrifuge at 11000 rpm for 10minutes.
- 8- Remove the supernatant and dry the pellet, then add 1 ml of 70% ethanol, centrifuge at 11000 rpm for 10 minutes. Remove the supernatant was again then dry the pellet and melt with 20 μ l of TE2 buffer before been stored at -20°C.

Benefits of additives :

- 1- **EDTA** : Lysis cell wall of bacteria, It has the ability to pull and remove ions Ca^{++} & Mg^{++} That contribute to maintaining the stability of protein complex and the stability of nuclear and cellular membranes and it inhibits nuclease activity .
- 2- **SDS**: Denaturation proteins which acting on breaking peptide bonds and thus disengagement amino acids and Remove the fat which found in cell walls.
- 3- **NaCl**: Ionized to Na^+ or Cl^- and associated with proteins and the sedimentation .

