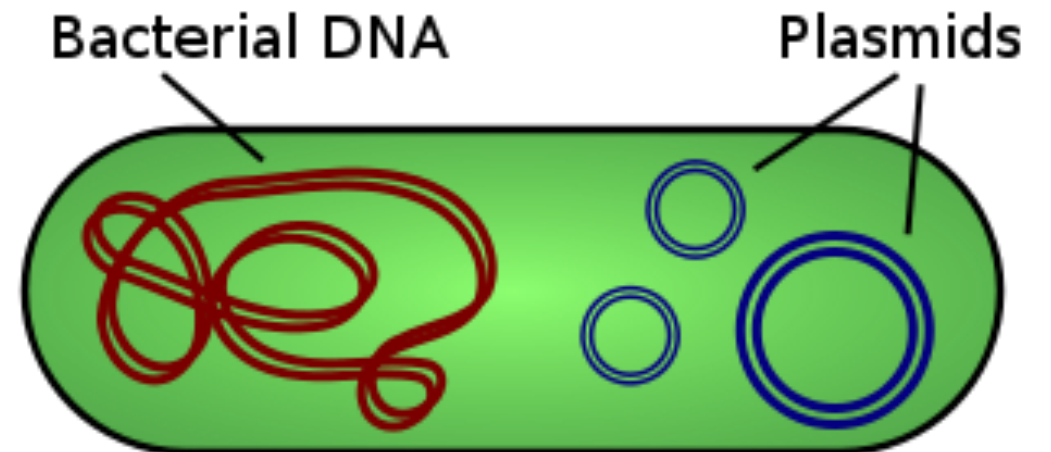


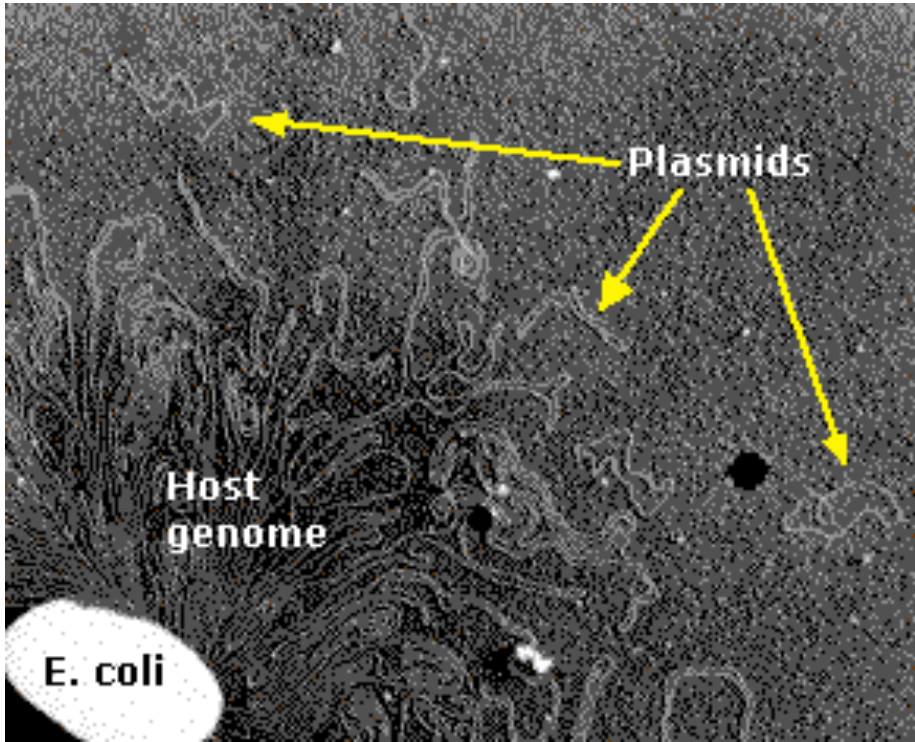
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# Plasmid isolation and purification

# Plasmid:

- Definition.
- Extra hereditary genetic element.
- **Replicon.**
- Symbiotic relationship with the host
- Serve as **vector.**
- Classes of plasmid.
- Applications:
  - ***Molecular cloning,***
  - Gene therapy,.
  - Drug production.
  - Making a large amount of proteins.

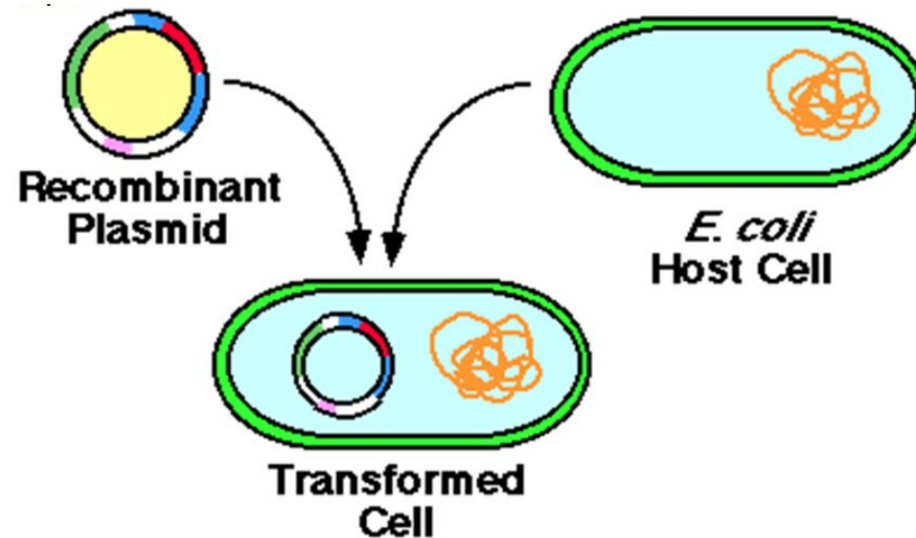




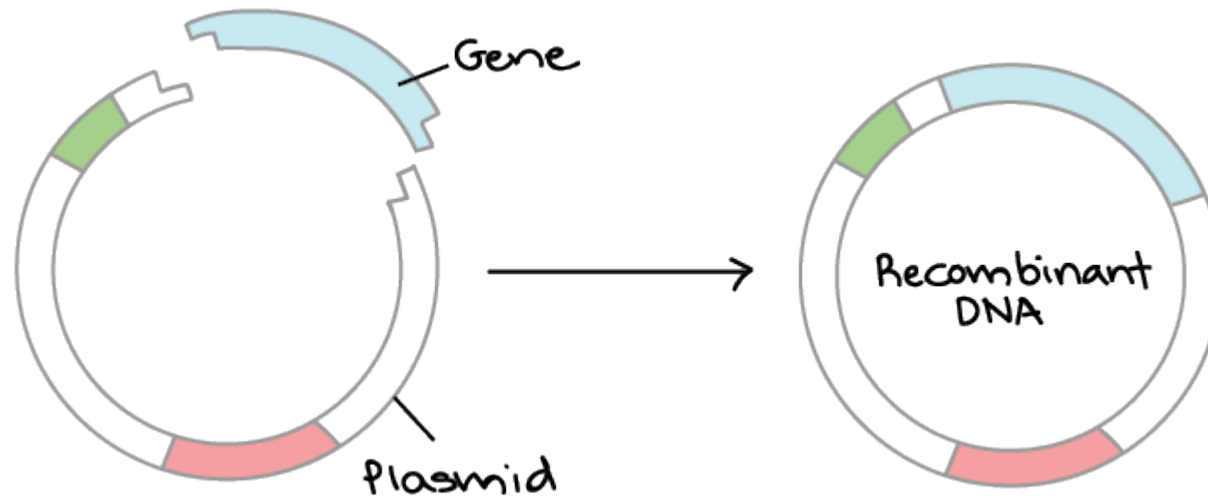
g110355 [RM] © www.visualphotos.com

# Plasmid vectors:

- Plasmids are widely used as vectors in molecular cloning, serving to drive the replication of recombinant DNA sequences within host organisms.
- In the laboratory, plasmids may be introduced into a cell via **transformation**.

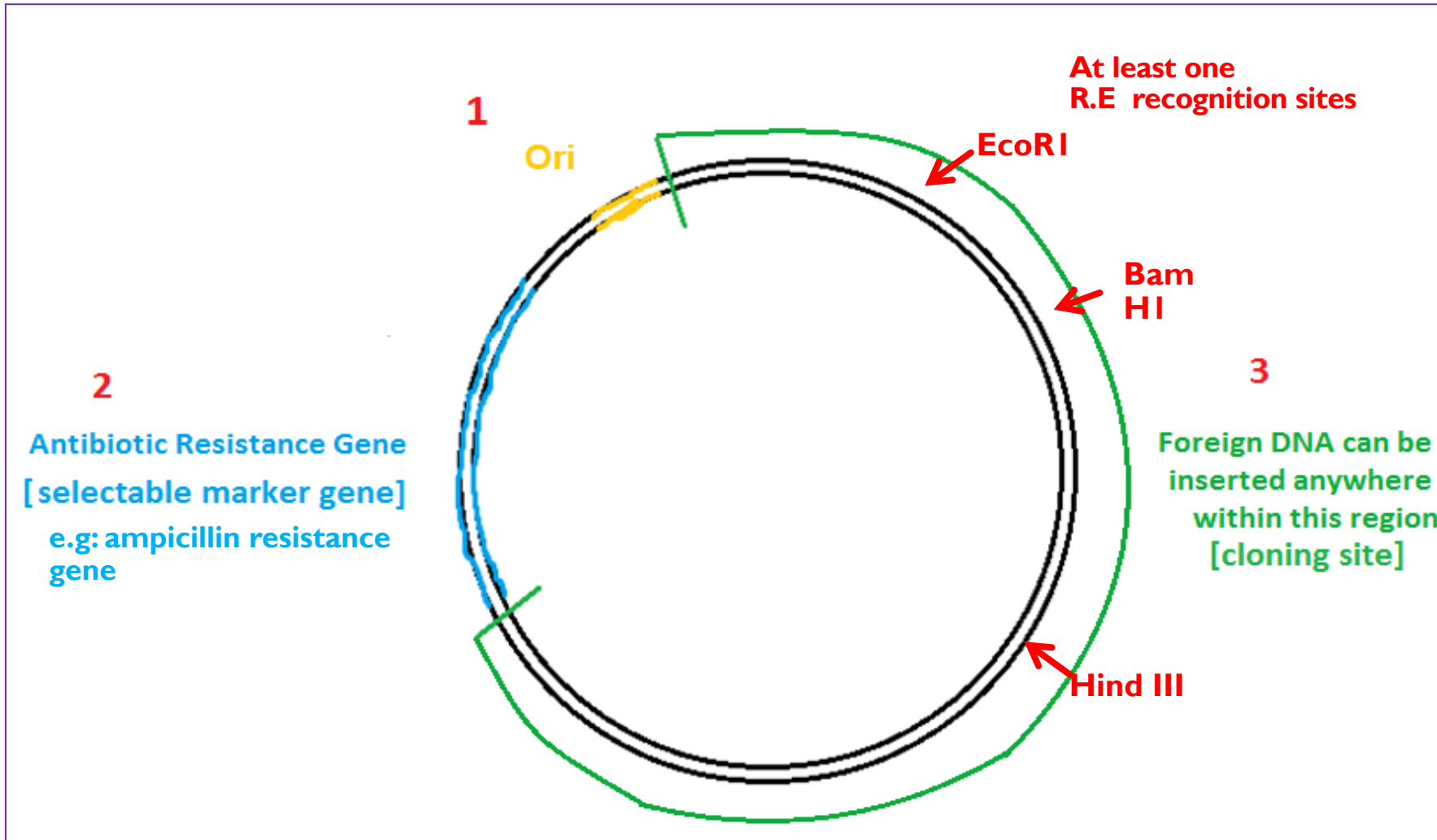


# Recombinant DNA:



↑  
Serve as vector

# Plasmids vectors contain three important parts:

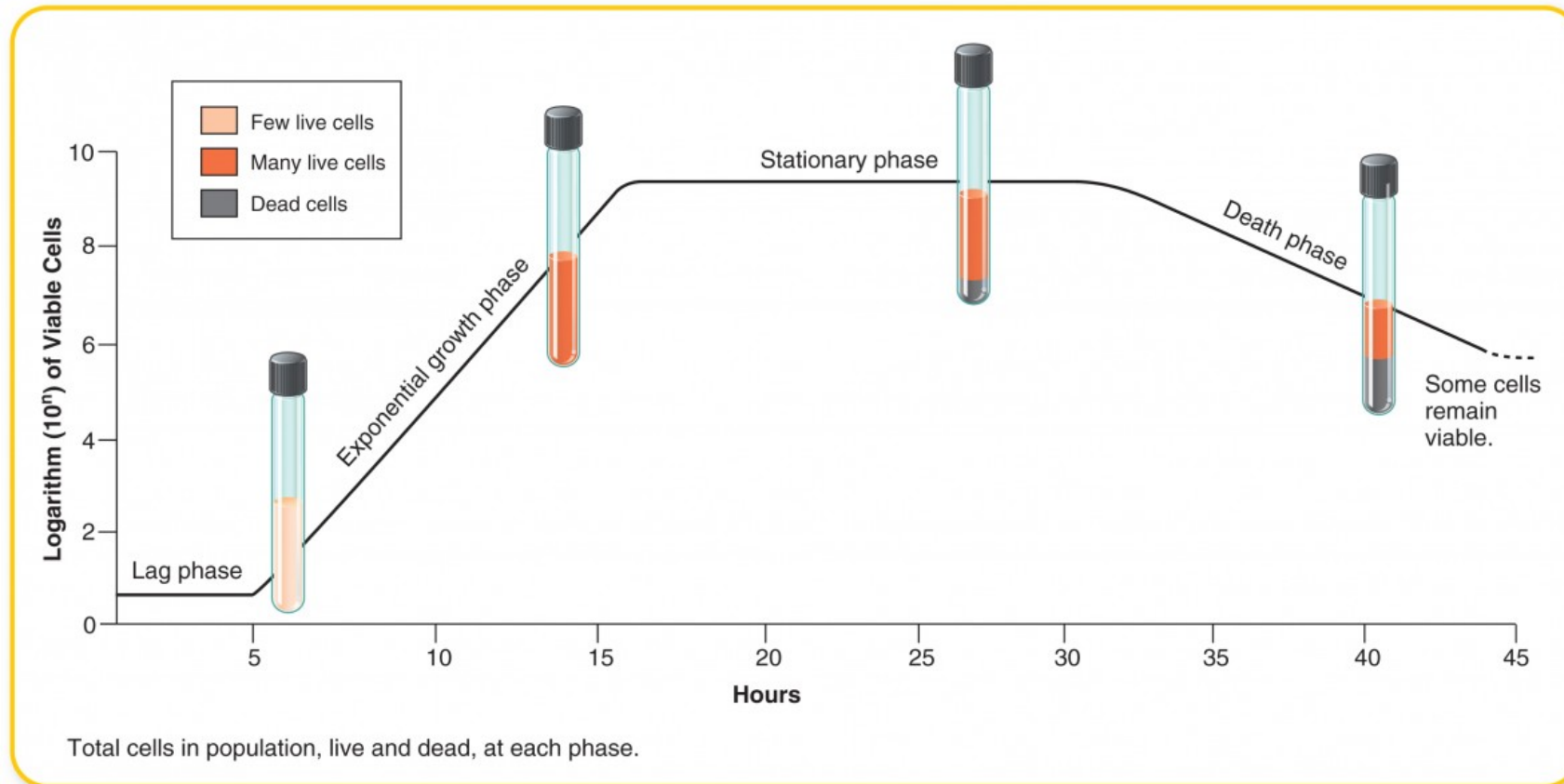


# Plasmid isolation and purification:

- Is an essential step for many molecular biology procedures.
- In general, plasmid purification involved three steps:
  1. Growth of the bacterial culture.
  2. Harvesting and lysis of bacteria.
  3. Purification of plasmid DNA.

# 1. Growth of the bacterial culture:

- Depending upon nutritional status, bacteria exhibit different growth patterns which include:



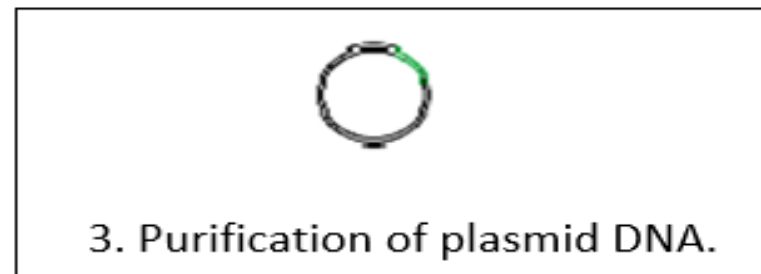
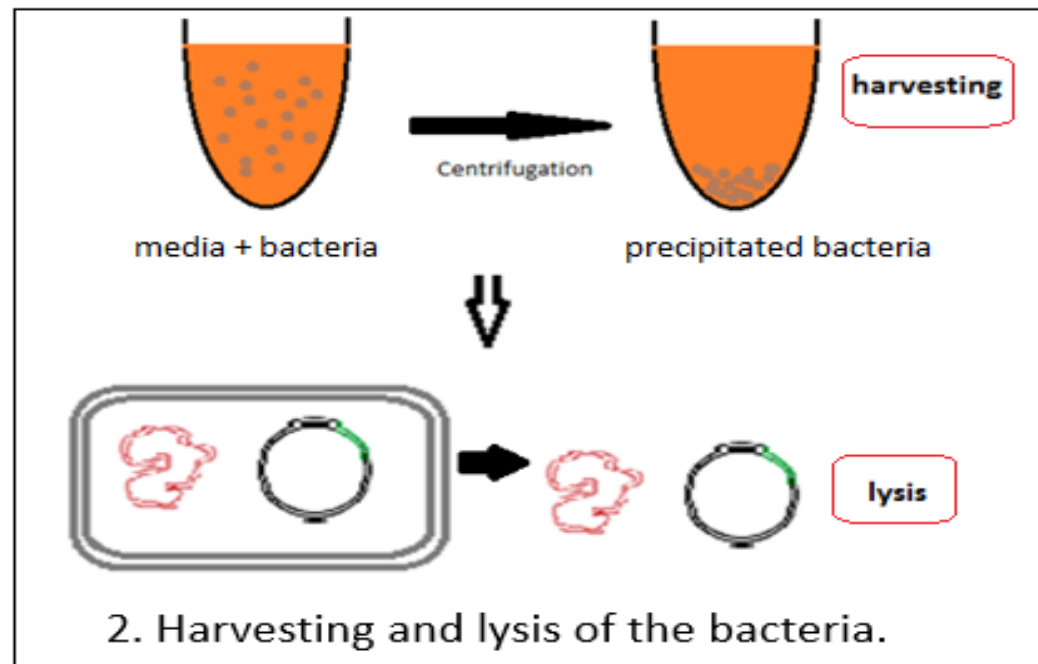
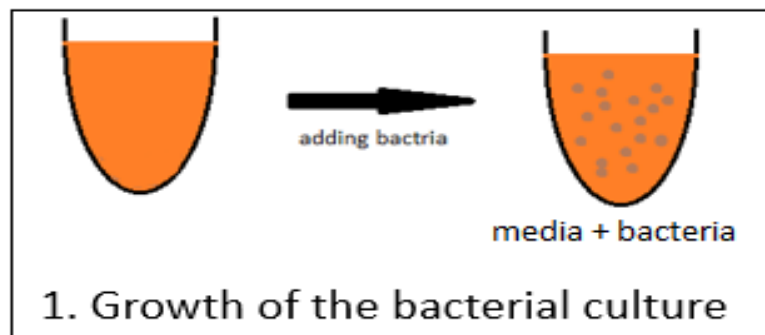


## 2. Harvesting and lysis of bacteria:

- Bacteria are recovered by centrifugation and lysed by any one of many methods, including:
  - ➔ treatment with detergents, alkali, organic solvents, and heat.
- The choice among these methods depends on three factors:
  - The size of plasmid.
  - The bacterial strain.
  - The technique used to subsequently purify the plasmid DNA.

# 3. Purification of plasmid DNA:

- Unlike the procedures for purification of genomic DNA ?
- There are basic methods of plasmid preparation:
  - Chemical base method.
  - Application of affinity matrixes for plasmid or proteins.





# PRACTICAL PART

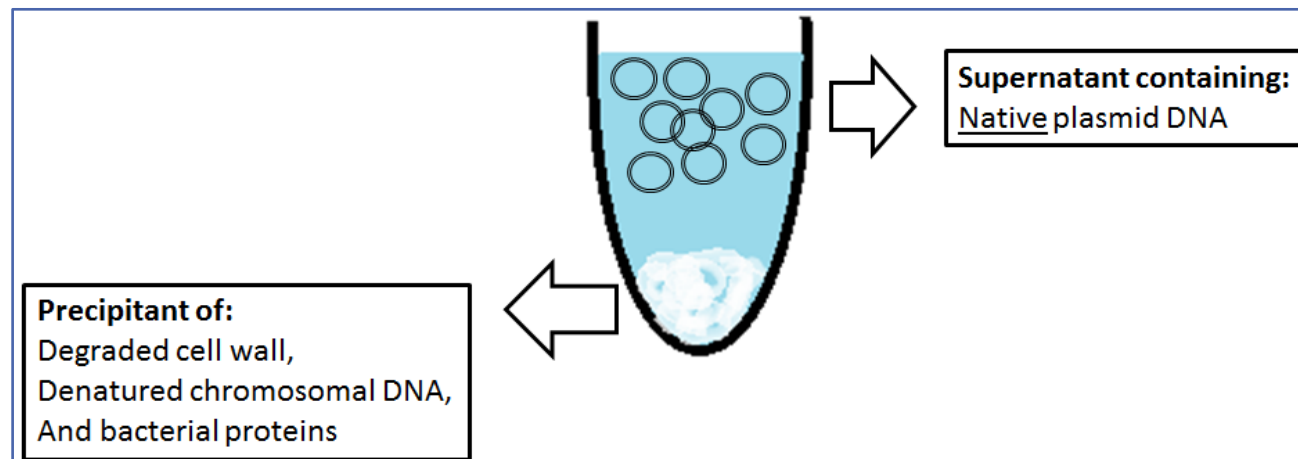


# Aim:

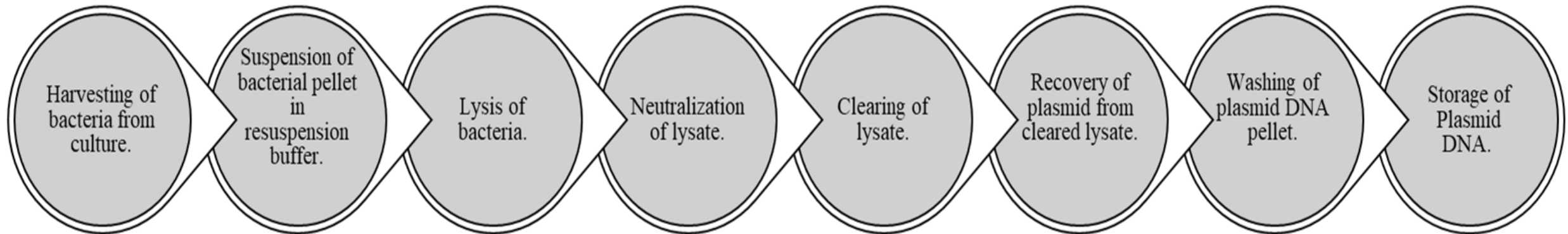
- To isolate pure plasmid DNA from E. coli using alkaline lysis method.

# Principle:

- In the alkaline lysis method, cells are lysed and DNA denatured by **SDS** and **alkaline pH**.
- The **SDS** will lyse the bacterial **cell membrane** and **denature the proteins**.
- **Alkaline pH** will **denature the genomic DNA** and the **proteins too**.
- **Neutralization** of the solution .
- Precipitation of protein-SDS complexes by lowering the temperature.
- Subsequently both complexes, DNA and protein, are removed by **centrifugation** leaving native plasmid molecules in the supernatant.



# Alkaline lysis purification method performing steps:



# Results:

- Concentration of plasmid DNA (ng/μl) = \_\_\_\_\_
- Plasmid purity: A260/A280 = \_\_\_\_\_



# HomeWork

- ❖ What is the importance of antibiotic resistance gene in the plasmid?