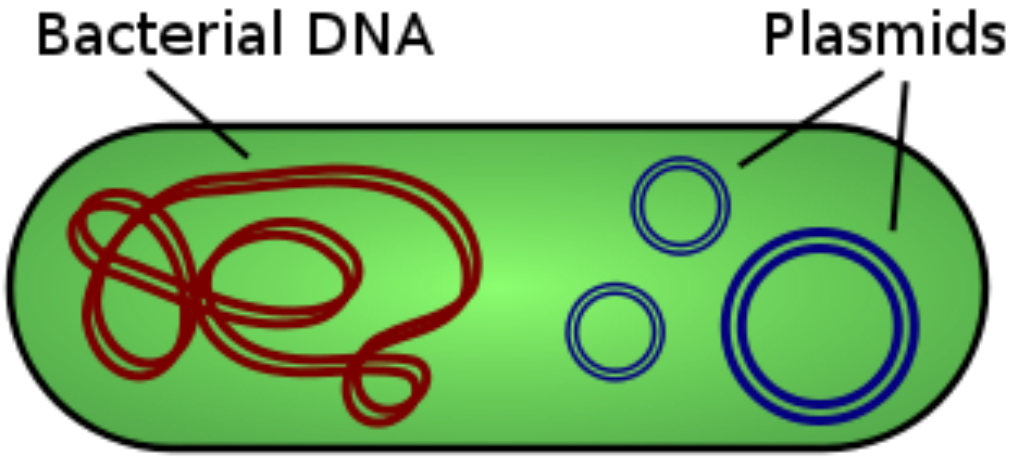
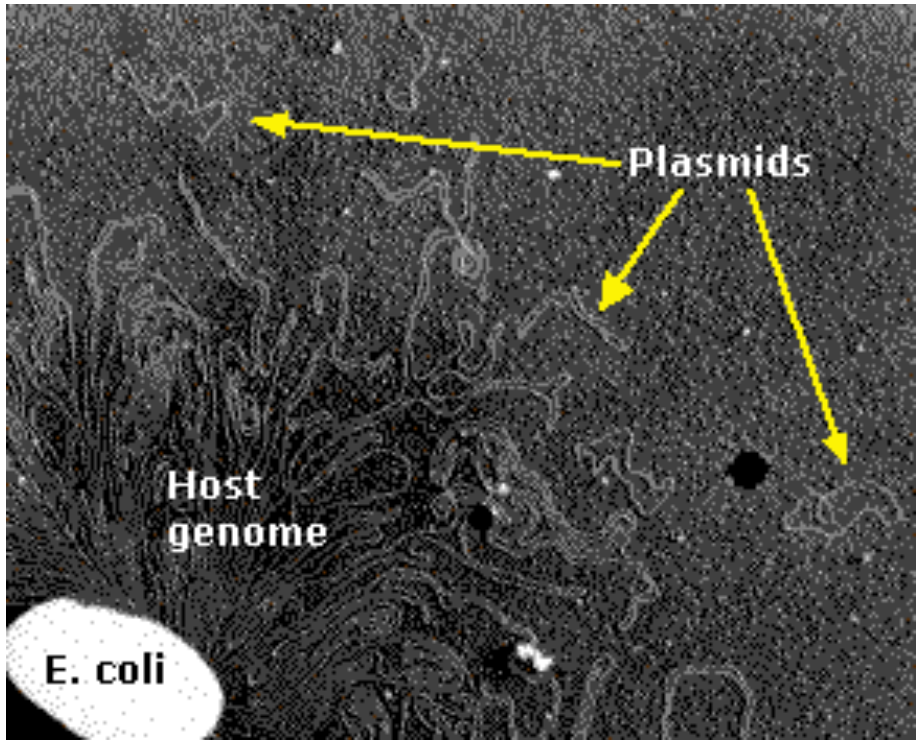
Plasmid isolation and purification

BCH462- Practical

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.




Plasmids
Host genome
E. coli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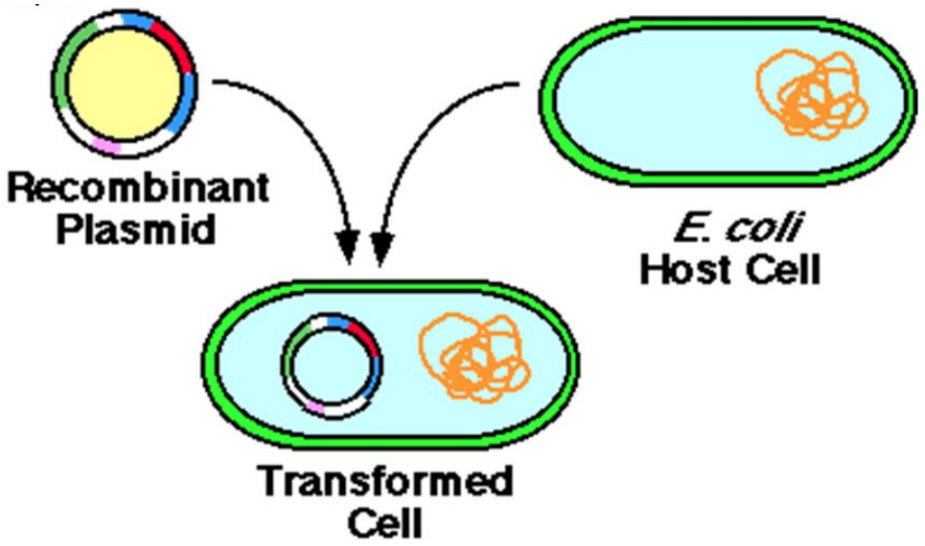
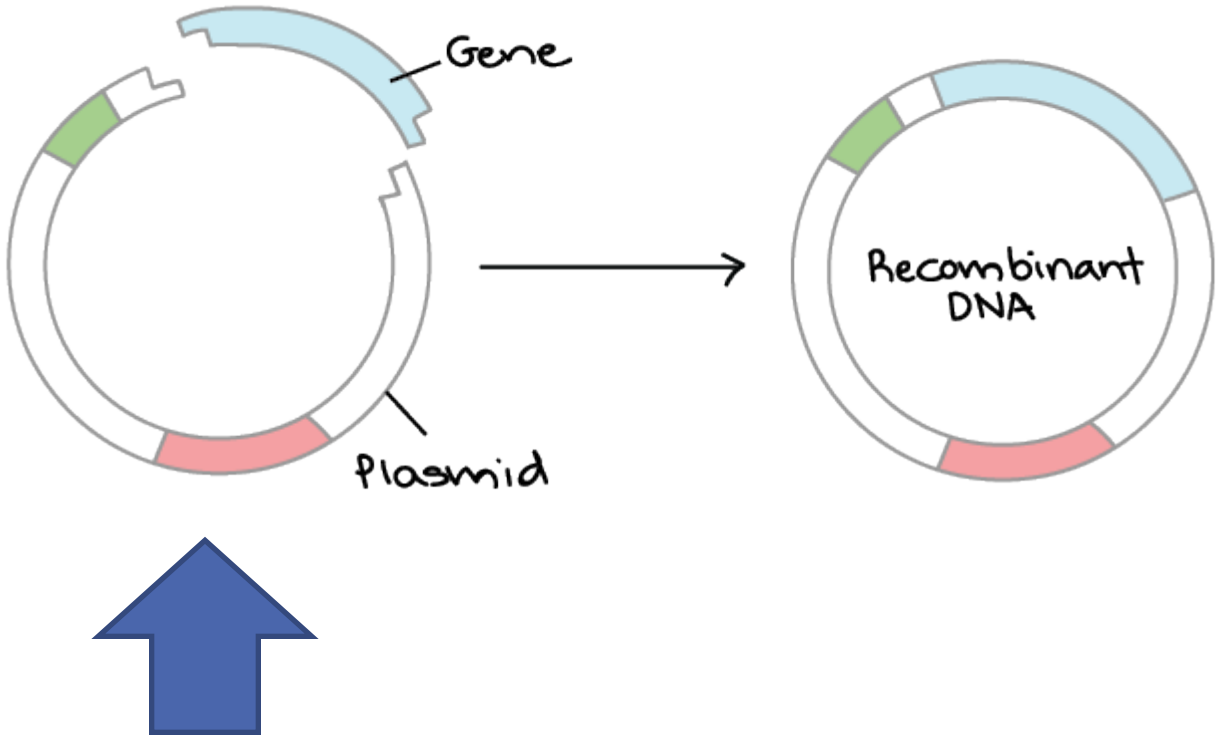
Plasmid vectors:

- 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.**

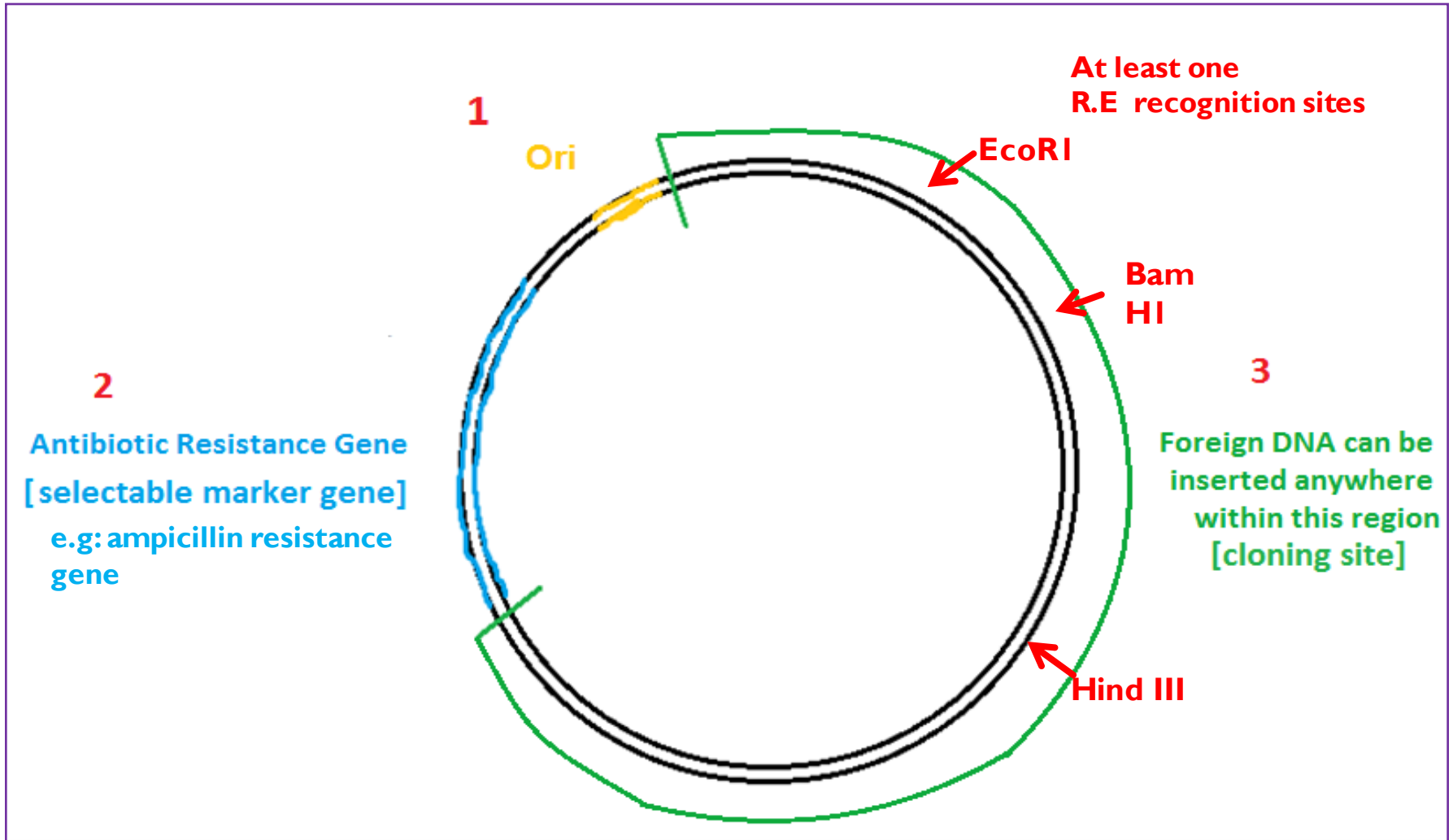


Recombinant DNA:



Serve as vector

Plasmids vectors contain three important parts:

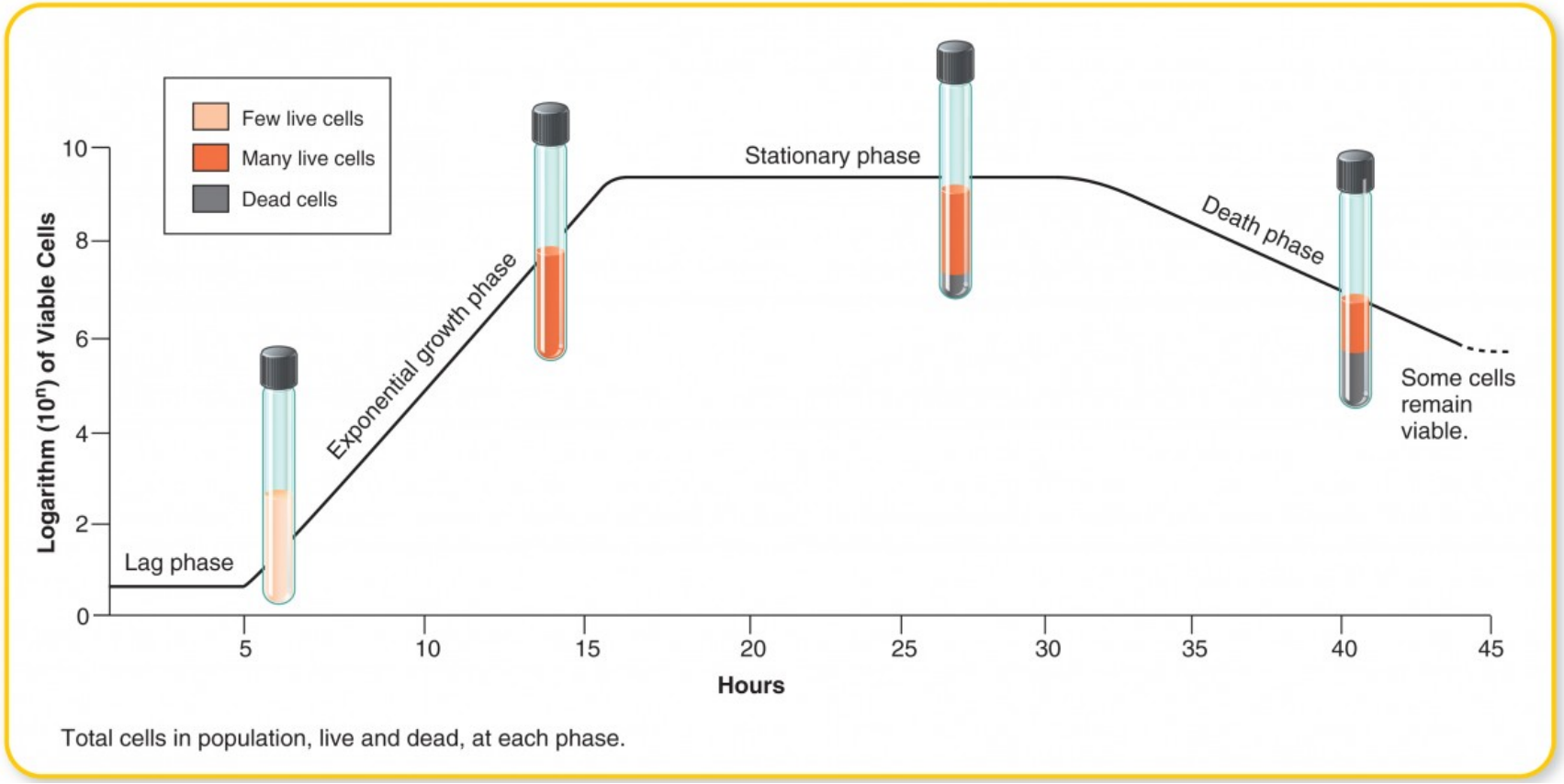


Plasmid isolation and purification:

- 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:



Total cells in population, live and dead, at each phase.

2. Harvesting and lysis of bacteria:

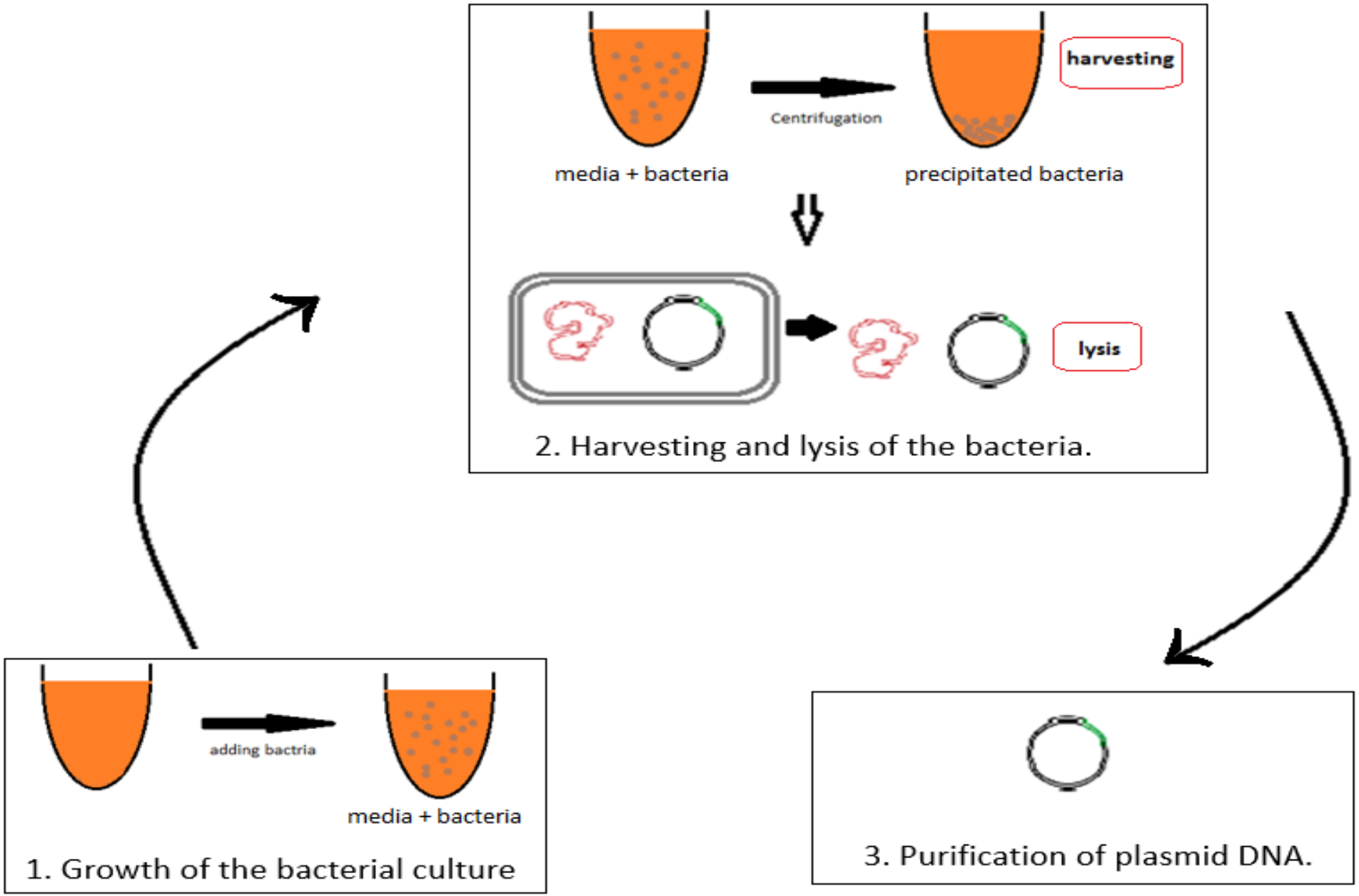
- Bacteria are recovered by centrifugation and lysed by any one of many methods, including:

➔ 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 lysis method.
  - Application of affinity matrixes for plasmid or proteins.


harvesting
Centrifugation
media + bacteria
precipitated bacteria
lysis
2. Harvesting and lysis of the bacteria.
adding bactria
media + bacteria
1. Growth of the bacterial culture
3. Purification of plasmid DNA.

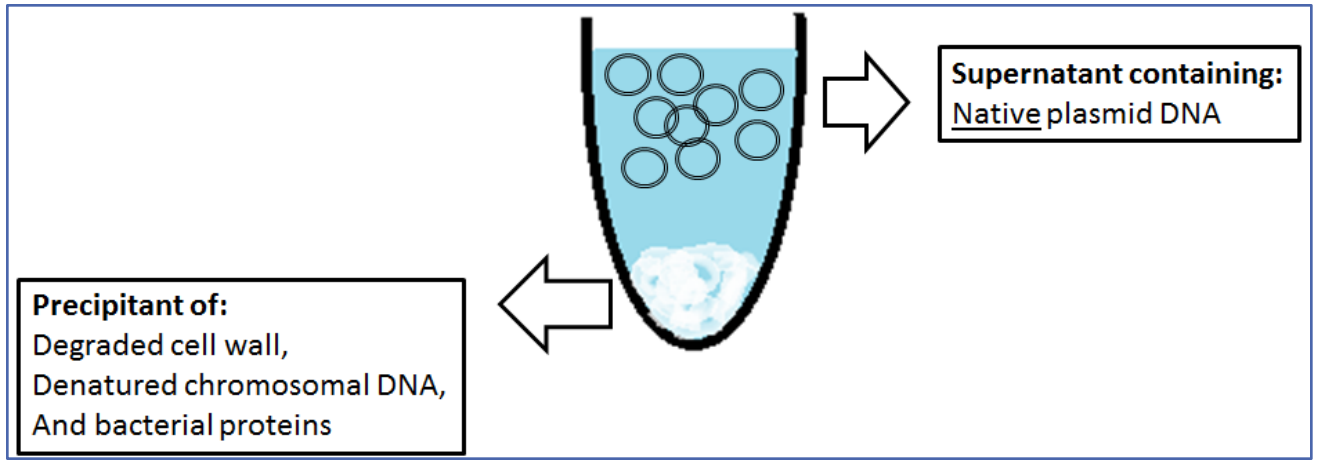
PRACTICAL PART

Aim:

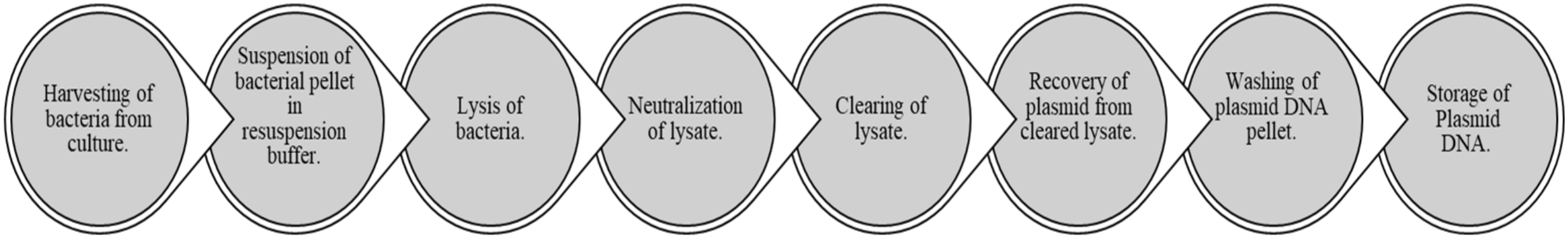
- To isolate pure plasmid DNA from E. coli using **alkaline lysis method.**

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.
- 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 = _____

Questions..

- What is plasmid copy number ? How does it affect the isolation protocol?
- What is the importance of antibiotic resistance gene in the plasmid?