BCH 462- Biotechnology & Genetic engineering [Practical]

Lab (1) Plasmid Isolation and Purification

Let’s assume we want to make insulin for the treatment of diabetes.

- Which is better to use cow OR bacteria as a biological factory? Why?

➜ How would the cell produce human insulin ?

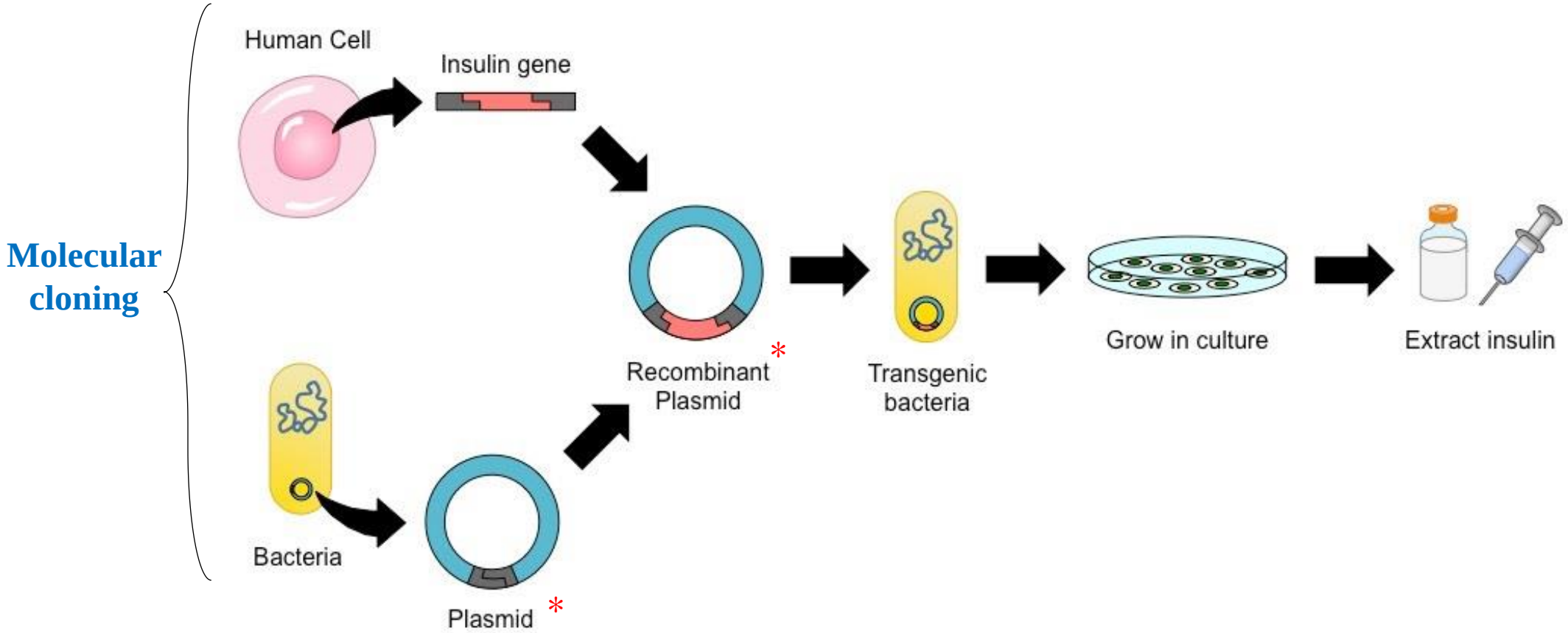


Figure 1. Schematic representation of recombinant insulin production

DNA cloning techniques

Are techniques used to create copies of certain DNA fragments.

1- **PCR** (in vitro)

[polymerase chain reaction].

2- **Cell-based** (in vivo)

[using a vector e.g. **plasmid** carrying the DNA of interest, which eventually inserted to a host cell "usually bacteria" and self replicate].

Remember

Vector ▶ e.g. **Plasmid**

Host ▶ e.g. **Bacteria**

Plasmid

- The DNA of most bacteria is contained in a single circular molecule, called the **bacterial chromosome.**
- Many bacteria contain an extrachromosomal element of DNA, termed a **plasmid.**
- **Plasmid** is a relatively small, covalently closed circular molecule that replicate independently from a bacterial chromosome. (Why ?)
- Every plasmid has its own origin of replication **(replicon)** and use the enzymes and proteins that encoded by its host for its replication and transcription.
- Plasmid found in a wild variety of bacterial species and they are not essential for the bacterium but benefit the survival of the organism (**Symbiotic** relationship with the host ?).

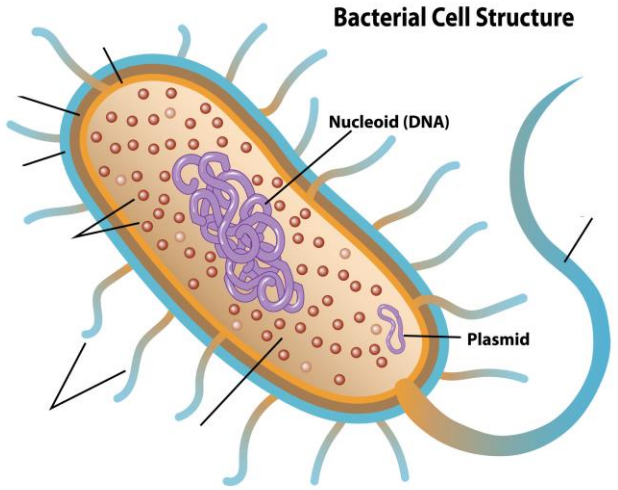


Figure 2. Illustration of a bacterium showing chromosomal DNA and plasmids

Plasmid cont.

- **Plasmids classes:**

I. Virulence plasmids encoding toxin genes.

II. Drug-resistance plasmids that confer resistance to antibiotics.

III. Plasmids encode genes required for bacterial conjugation.

(which can be advantageous for host cell)

- **Plasmids applications:**

i. Molecular cloning

ii. Gene therapy

iii. Drug production

iv. Making a large amount of proteins.

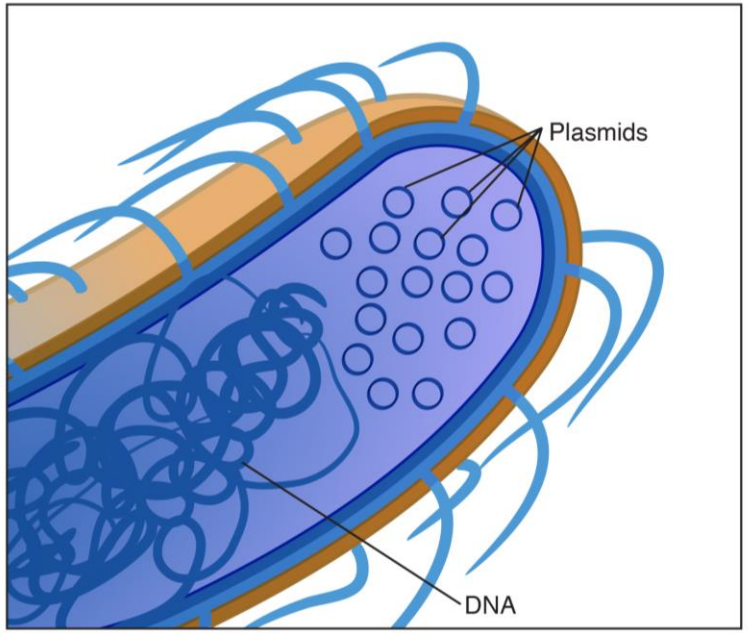


Figure 3. Illustration of *E.coli* showing chromosomal DNA and plasmids

Plasmid cont.

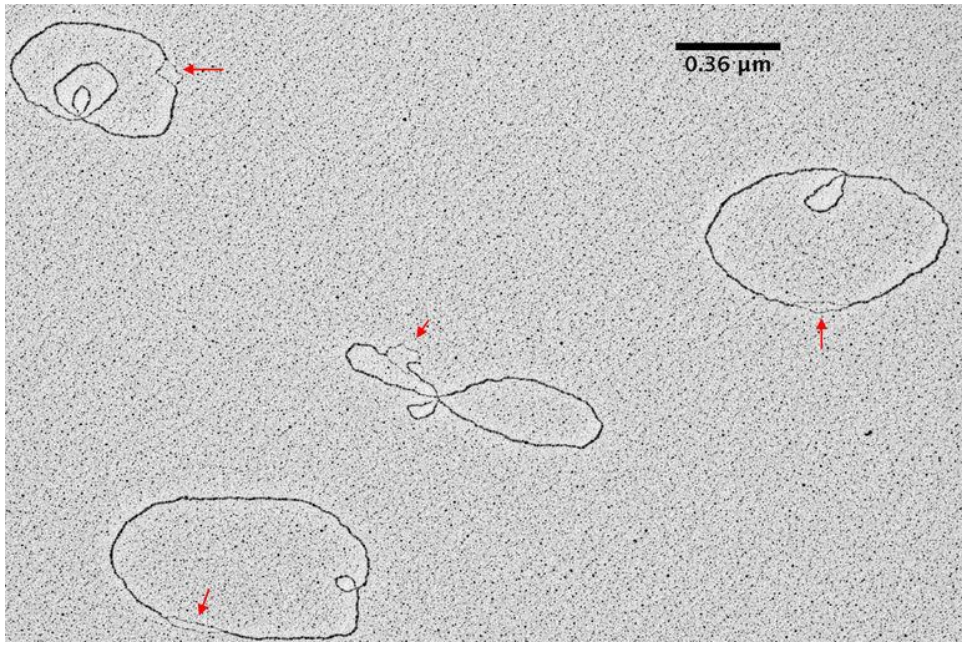


Figure 5. TEM picture of plasmid DNA molecules in solution

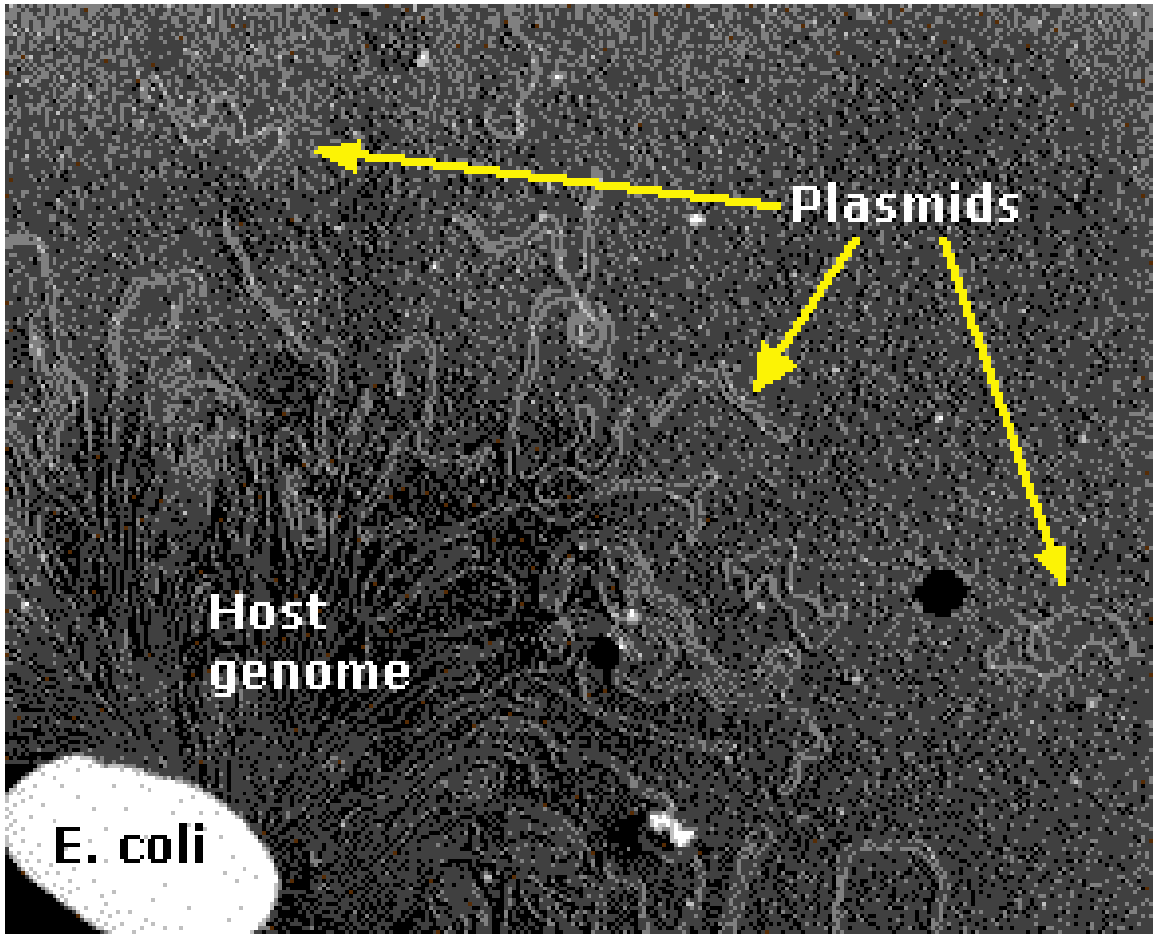


Figure 6. Electron micrograph of an *E. coli* cell ruptured to release its DNA.

Plasmid as a vector

- Plasmids are widely used as **vectors** in **molecular cloning**, serving to drive the replication of **recombinant DNA** sequences within host organisms (It is used to provide a "vehicle" in which to insert a desired DNA fragment).
- **Recombinant DNA,** molecules of DNA from **two different species** (human/bacteria) that are inserted into a **host organism** (bacteria) to produce new genetic combinations (human insulin).
- In the laboratory, the modified plasmids (**recombinant DNA**) are usually reintroduced into a host cell for replication via process called ***transformation.***

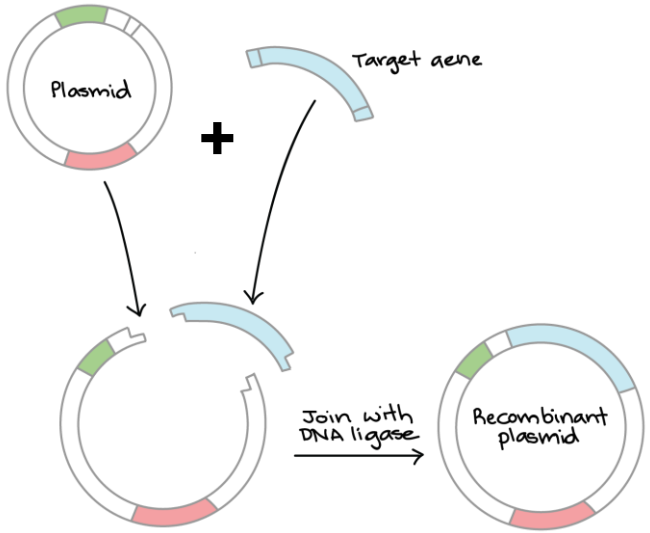


Figure 7. Recombinant DNA

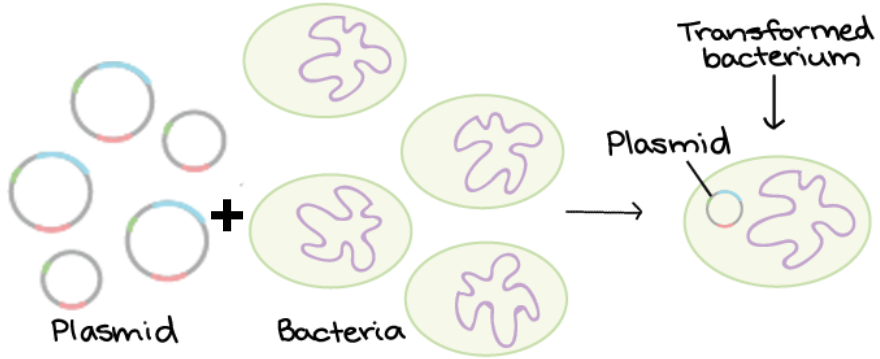
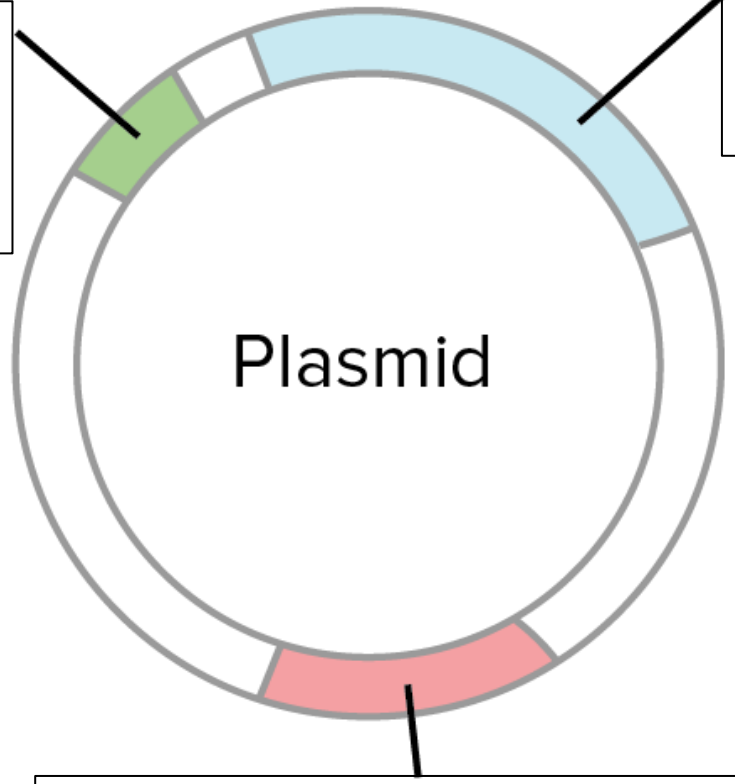


Figure 8. Transformation

Plasmid vectors should contain three important parts:

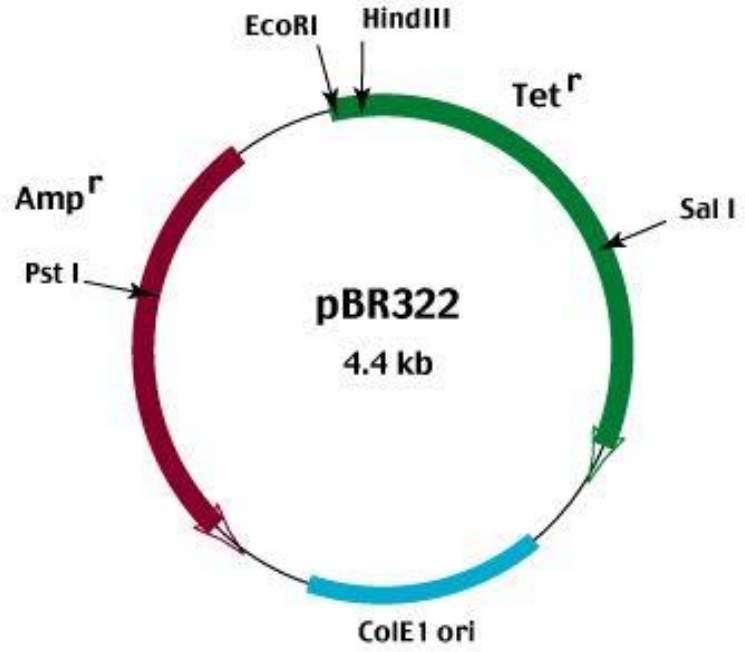
1. **Origin of replication (Ori)**
2. **antibiotic resistance**
3. **gene cloning site**

The **Ori** is a DNA sequence which allows initiation of replication of the plasmid by cellular enzymes.



Cloning site is a short segment of DNA which contains several restriction sites allowing for the easy insertion of DNA (A place to insert foreign DNAs).

Antibiotic resistance gene allows for selection of plasmid-containing bacteria. **(?)**



pBR322 plasmid

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:

I. **Lag phase:** in this phase bacteria adapt themselves to growth conditions and synthesis its own DNA, RNA and proteins.
II. **Log phase:** it is exponential phase, the bacterial cells divide and the production of new cells is proportion to increased time.
III. **Stationary phase:** the growth rate slows as nutrients become limited, waste products accumulate and the rate of cell division equals the rate of death.
IV. **Death phase:** due to continuous accumulation of toxic metabolites and the lack of nutrients, death occurs of the bacteria.

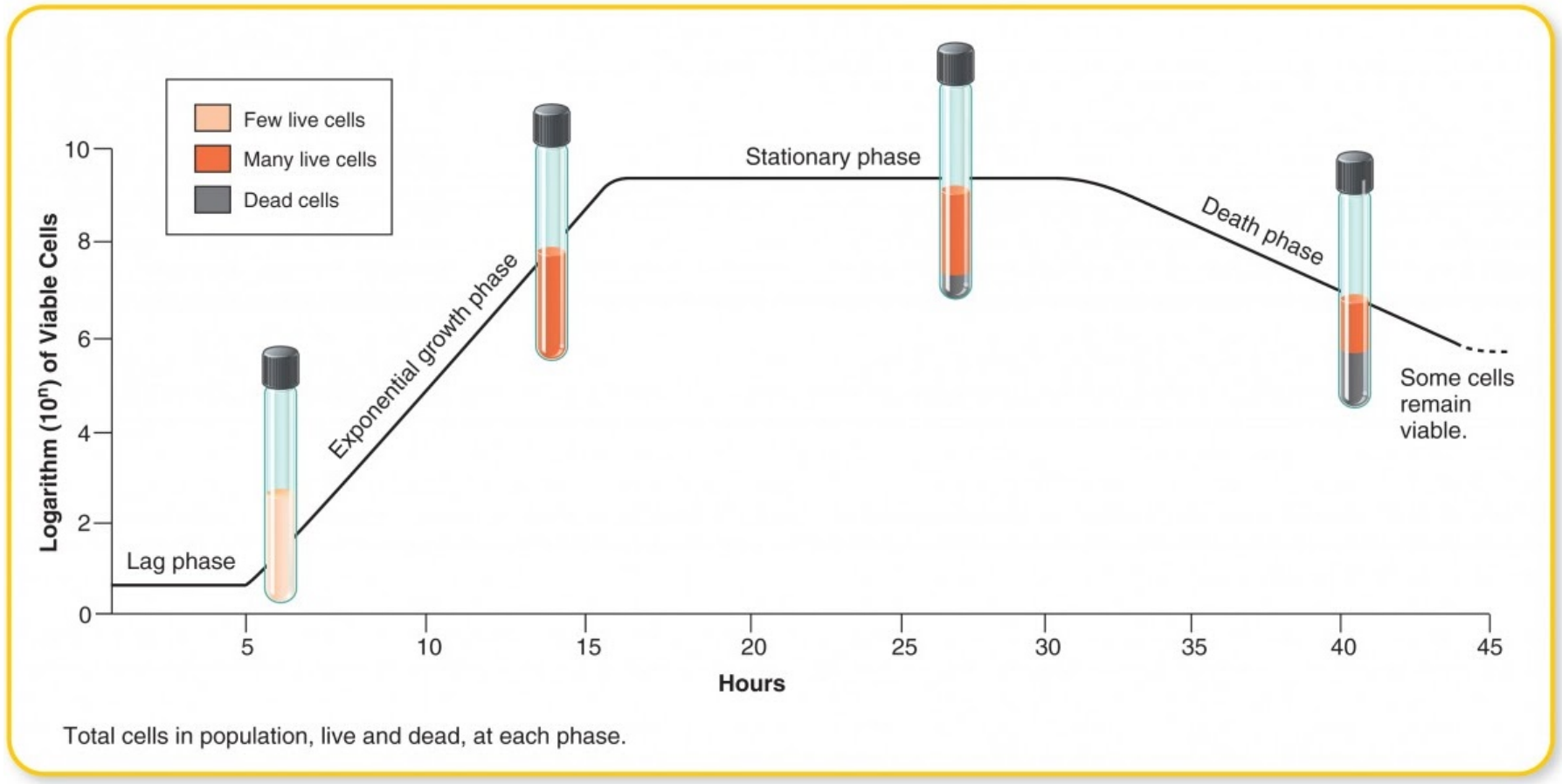


Figure.9. Bacterial culture growth curve.

Pause and Think on which phase should we purify plasmid?

2. Harvesting and lysis of bacteria

1. Bacteria are recovered by **centrifugation.**
2. **Cell lysis** 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

- The plasmid purification procedures, unlike the procedures for purification of genomic DNA, should involve removal of not only protein but also another major impurity **bacterial chromosomal DNA**.

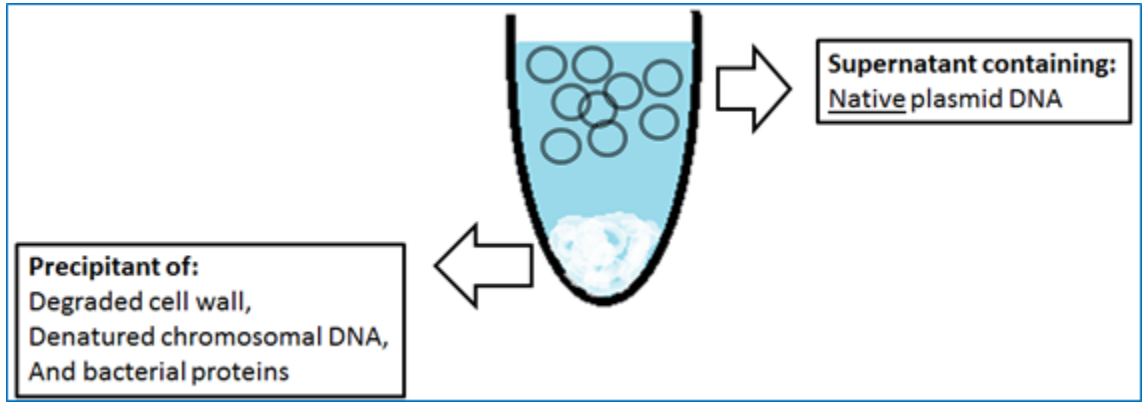
- **There are basic methods of plasmid preparation:**

1. Chemical base lysis methods.
2. Application of affinity matrices for plasmid or proteins.

Practical Part

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.



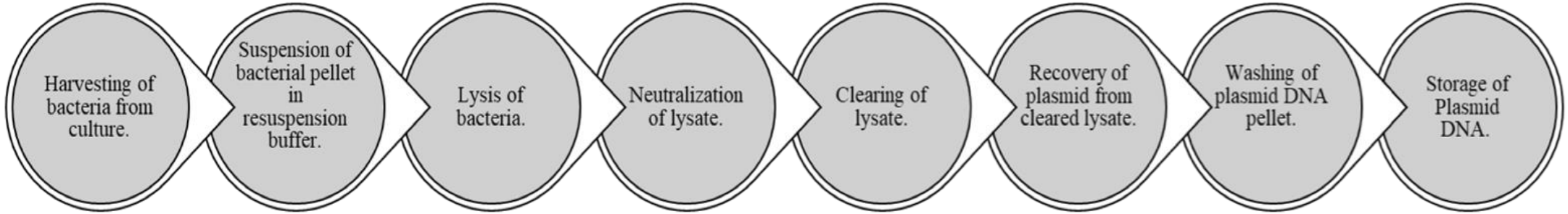


Figure.10. Alkaline lysis purification method performing steps

Practical part

- **Results:**

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

- **Methodology:**

1- Centrifuge the bacterial samples at 4 °C, maximum speed for 5 minutes, using microcentrifuge device.

Bacterial sample was centrifuged at 4°C, maximum speed for 5 minutes using microcentrifuge.

- **References:**

Endnote, Mendeley or Cite This For Me: Web Citer (*extension in Google Chrome*).