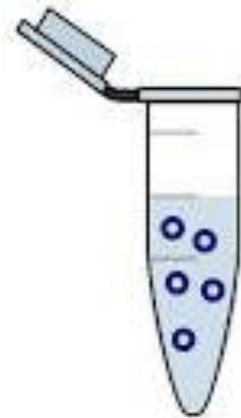
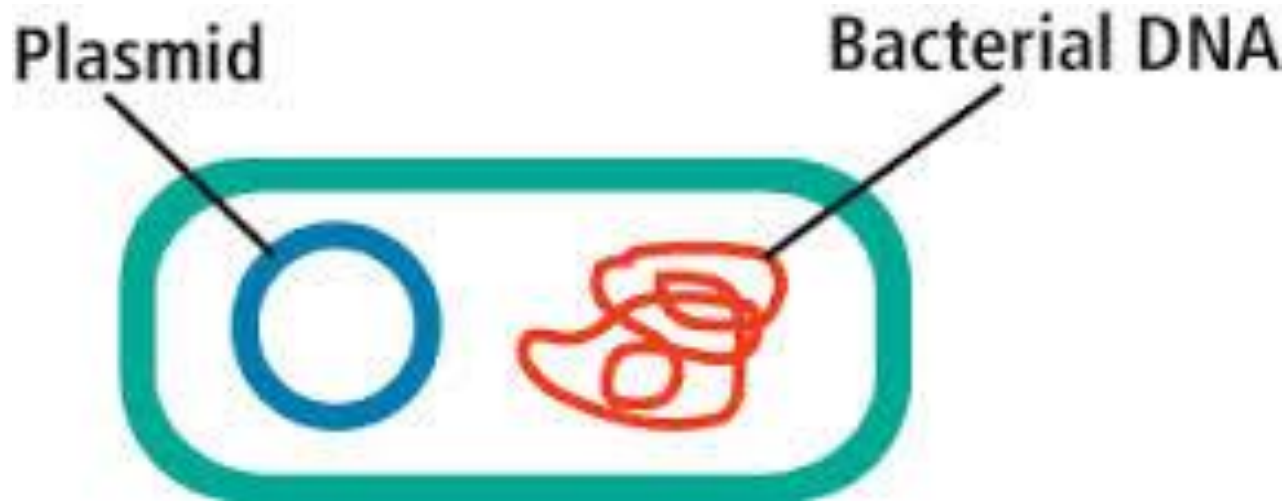


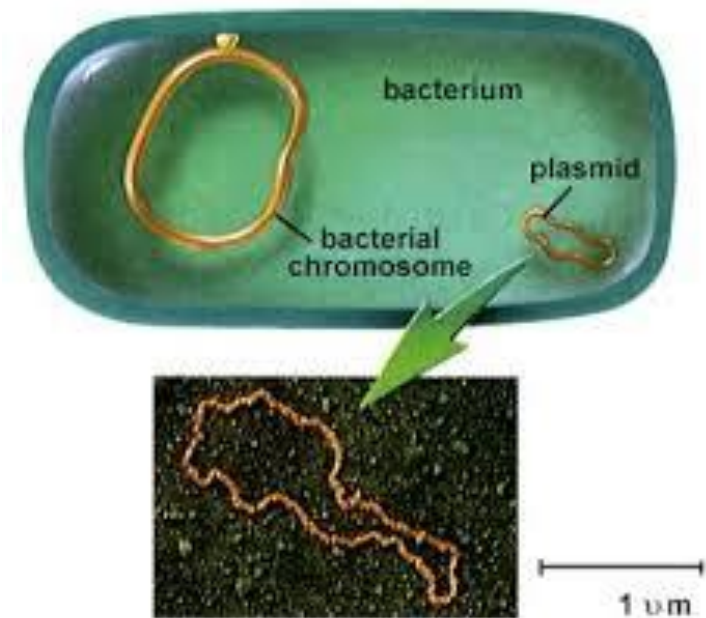
Plasmid Isolation

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Plasmid

- Plasmids are small, double strand , closed circular DNA molecules.
- Isolated from bacterial cells.
- Replicate independently of the bacterial chromosome



Plasmids Features

- Found in a wide variety of bacterial species.
- Extra-chromosomal elements, which replicate independently of the bacterial chromosome.
- Not essential for the bacterium but may confer a selective advantage.
- Using the enzymes and proteins encoded by their host for their replication and transcription.
- Inherited.
- Used in many applications e.g. Drugs production.



Types of Bacterial Plasmids

- There are three general classes for plasmids which can be advantageous for host cell:
- Virulence plasmids encoding toxin genes.**(turn the bacterium into a pathogen).**
- Drug-resistance plasmids that confer resistance to antibiotics.**(containing antibiotic or drug resistant gene).**
- Plasmids encode gene required for bacterial conjugation.

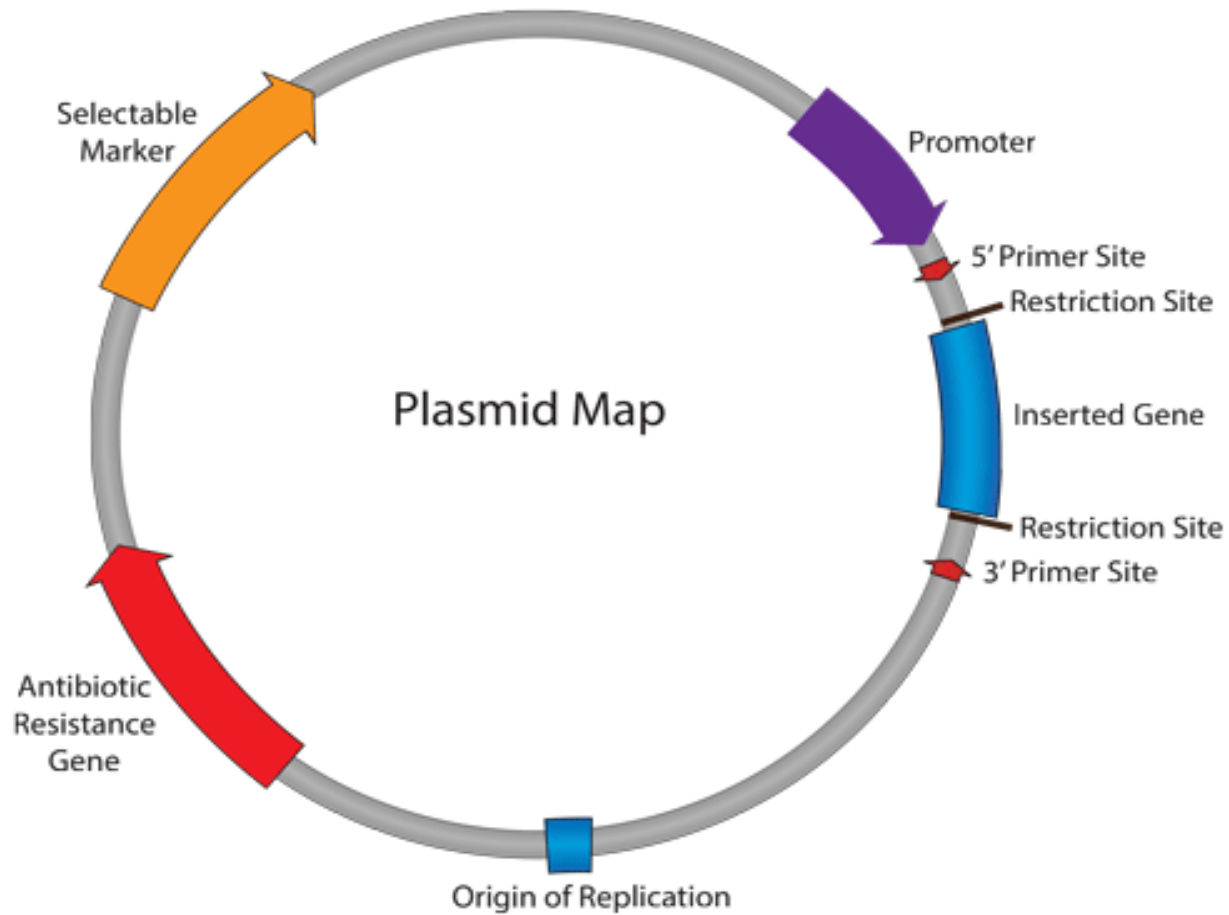
Plasmid Copy Number

- It is one of the most critical factors affecting the yield of plasmid from a given system.
- It is determined primarily by the replicon.
- Replicon is the region of DNA surrounding and including the origin of replication in the plasmid.
- Usually low copy number yield is ~25 copies of the plasmid per bacterial cell.
- High copy number plasmid yield is approximately 200–700 plasmid copies per cell.

Plasmids Applications

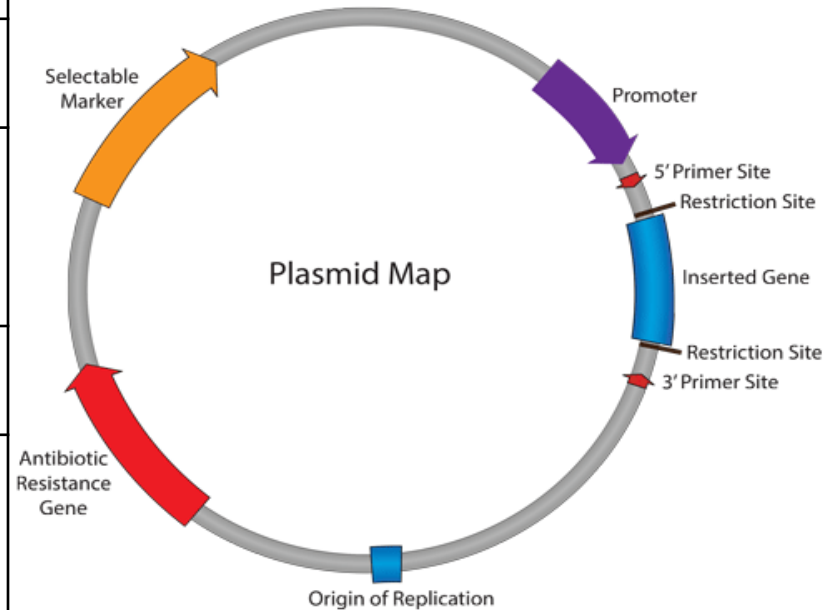
- They are important tools in genetics and biochemistry labs.
- They are commonly used to:
 1. Multiply or express particular genes.
 2. Gene therapy.
 3. Molecular cloning.
- Plasmids which used in genetic engineering are called vectors.

Plasmid Map



Plasmid Map

Element	
Origin of Replication (ORI)	DNA sequence which allows initiation of replication within a plasmid by recruiting transcriptional machinery proteins
Antibiotic Resistance Gene	Allows for selection of plasmid-containing bacteria.
Multiple Cloning Site (MCS)	Short segment of DNA which contains several restriction sites allowing for the easy insertion of DNA. In expression plasmids, the MCS is often downstream from a promoter.
Insert	Gene, promoter or other DNA fragment cloned into the MCS for further study.
Promoter Region	Drives transcription of the target gene. Vital component for expression vectors: determines which cell types the gene is expressed in and amount of recombinant protein obtained.
Selectable Marker	The antibiotic resistance gene allows for selection in bacteria. However, many plasmids also have selectable markers for use in other cell types.
Primer Binding Site	A short single-stranded DNA sequence used as an initiation point for PCR amplification or sequencing. Primers can be exploited for sequence verification of plasmids



Isolation of Plasmid DNA

- It is generally involve three steps :
 - 1-Growth of the bacterial culture .
 - 2-Harvesting and lysis of bacteria.
 - 3-Purification of plasmid DNA.

Isolation of Plasmid DNA

- It is generally involves three steps :

1-Growth of the bacterial culture .

2-Harvesting and lysis of bacteria.

- Using either detergents, alkali, organic solvents, or heat.

- The choice among these methods depends on:

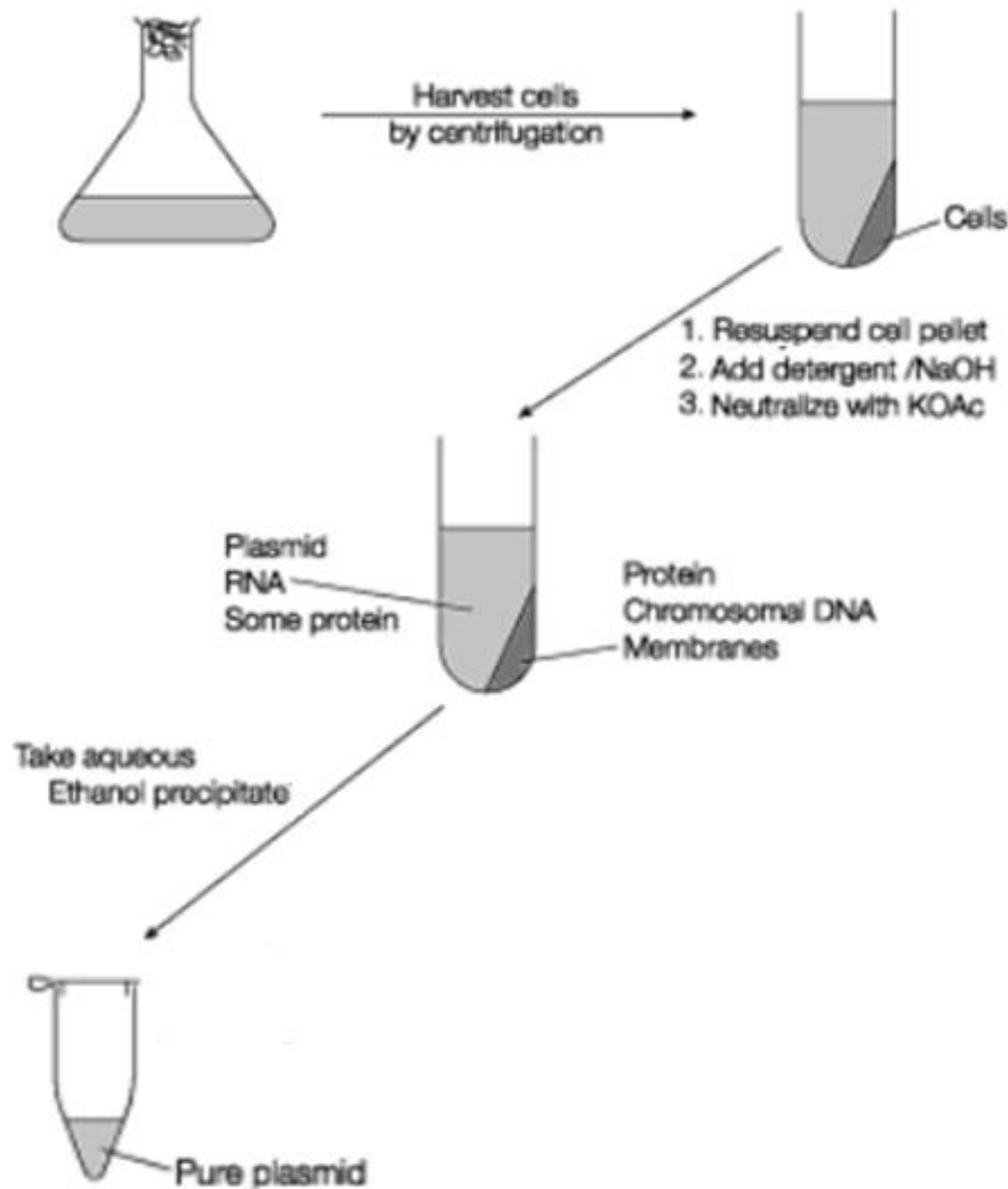
Plasmid size.

Bacterial strain.

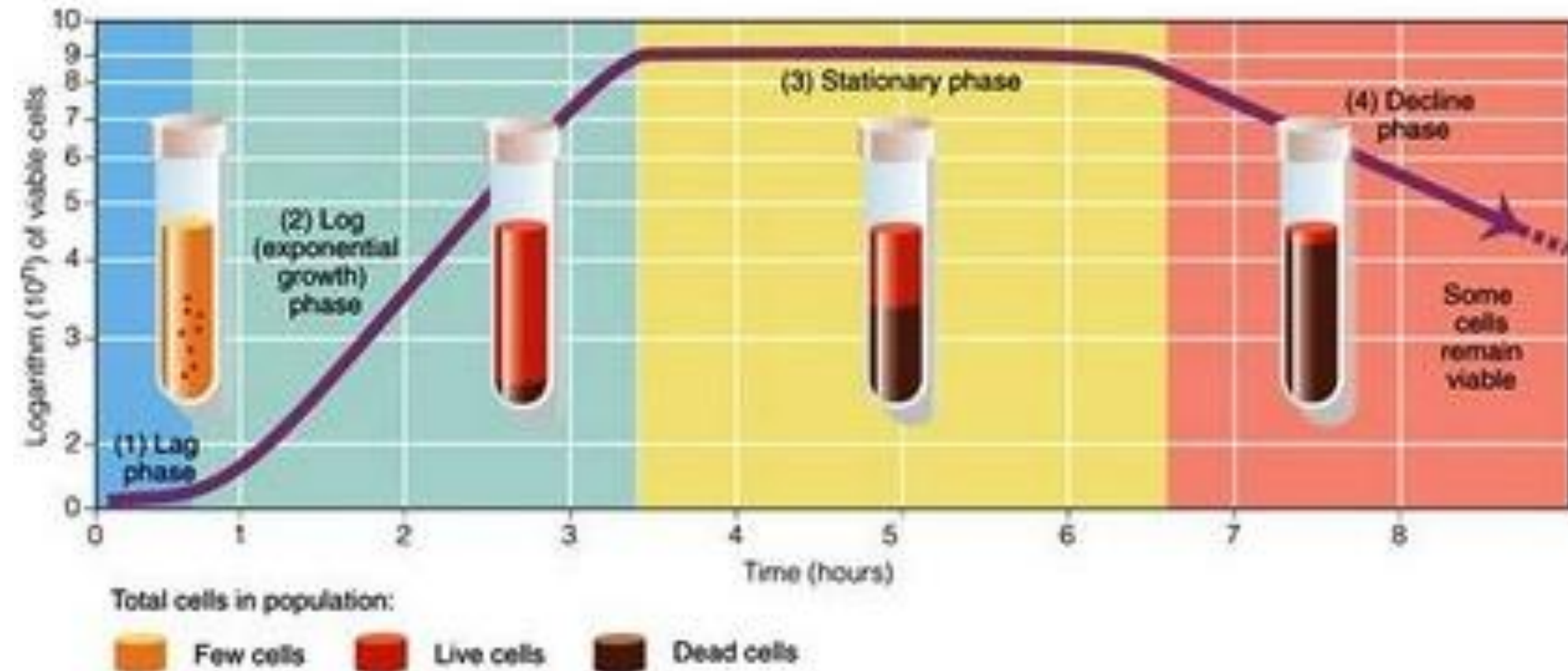
The subsequent technique used to purify the plasmid DNA.

3-Purification of plasmid DNA.

Outline of the Plasmid Isolation



Bacterial Culture Growth Curve



Bacterial Culture Growth Curve

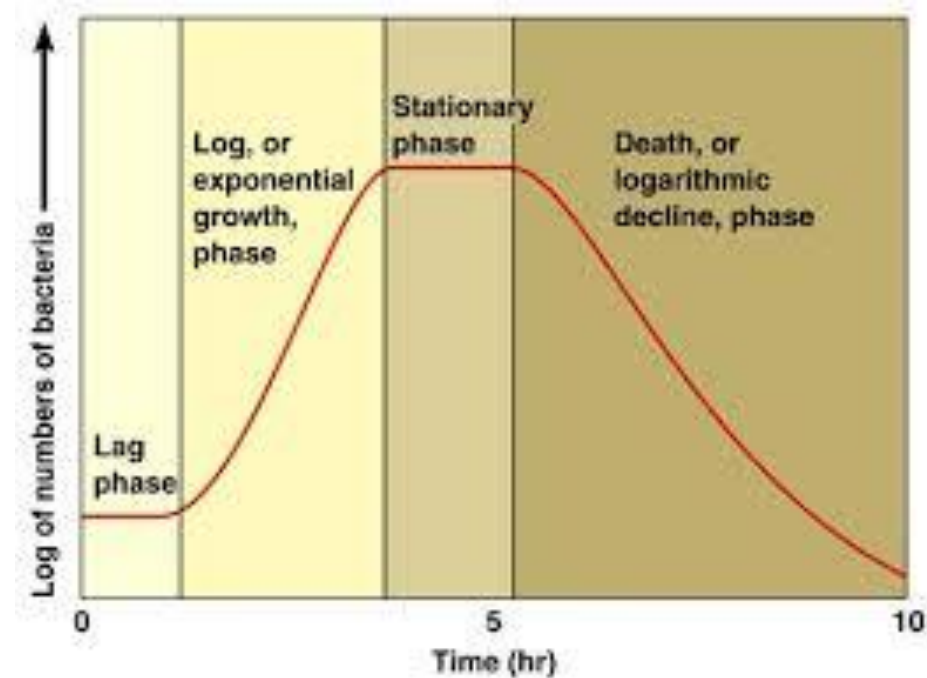
Depending upon nutritional status, bacteria exhibit different growth patterns which include:

Lag phase: in this phase bacteria adapt themselves to growth conditions and synthesis its own DNA, RNA and proteins.

Log phase: it is exponential phase, bacterial cells divide and the production of new cells is proportion to increased time.

Stationary phase: the growth rate slows as nutrients become limited, waste products accumulate and the rate of cell division equals the rate of death.

Death phase: due to continuous accumulation of toxic metabolites and the lack of nutrients, death occurs of the bacteria.

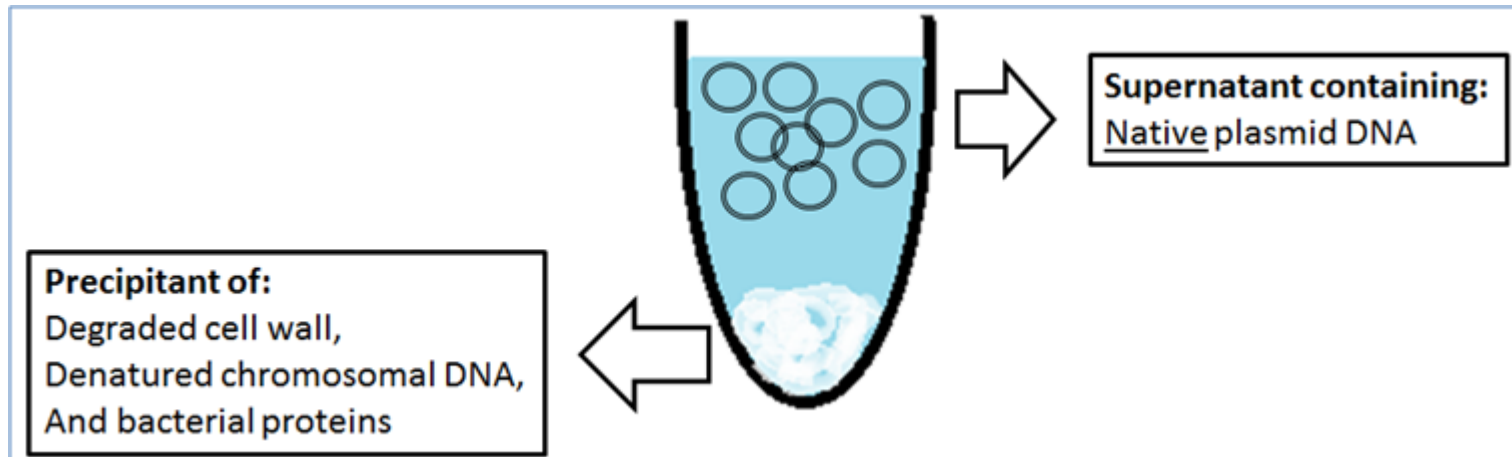


Plasmid isolation and purification

- **Principle of the experiment “Alkaline lysis method”:**

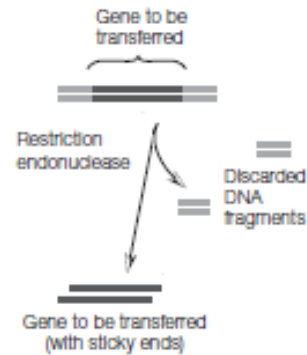
using SDS in an alkaline solution:

- The SDS: will lyse the bacterial cell membrane and denature the proteins too.
- The alkaline pH : will denature the genomic DNA and denature the proteins too.
- The degraded cell wall, denatured chromosomal DNA and bacterial proteins form large aggregated complex which and precipitated and removed by centrifugation.
- Native plasmid DNA can be collected from the supernatant.

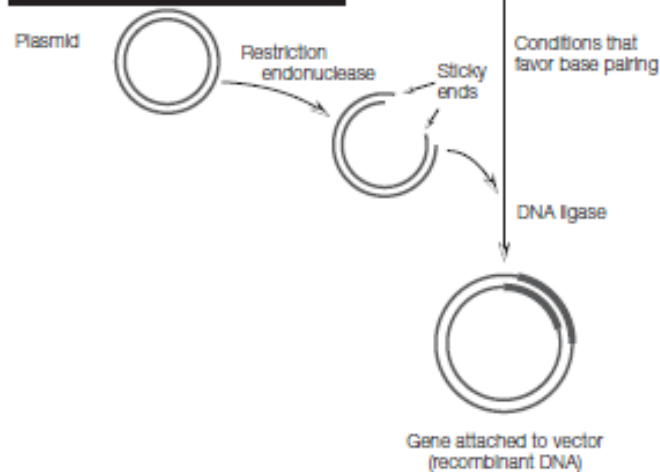


DNA Cloning

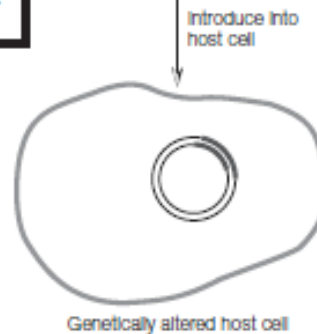
Step 1. Obtain gene to be transferred.



Steps 2 and 3. Attach gene to vector.



Steps 4 and 5. Introduce recombinant DNA into host cell.



DNA Cloning Continue

