جامعة الملك سعود
King Saud University

462 BCH

Biotechnology & Genetic engineering

(Practical)

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All lectures and lab sheets are available on my website:
Fac.ksu.edu.sa\naljebrin

Course Outline

	Title of the Experiments
1	Plasmid Isolation and Purification
2	Competent Cells Formation and Transformation of Competent Cells with plasmid DNA
3	Extraction and Determination of Bacterial Proteins
4	Sodium Dodecyl Sulfate Polyacryl Amide Gel Electrophoresis (SDS-PAGE)
5	Western Blot
6	Enzyme-linked Immunosorbent Assay (ELISA)
7	Single Radial Immunodiffusion
8	Immunoelectrophoresis

Marks Distribution

	Marks
Practical Performances	3 Marks
Report	5 Marks
Quiz	7 Marks
Final	**Theoretical only** -15 Marks
Total	30 Marks

Final exam date:

Wednesday 18/3/1439 – 6/12/2017

How to write a scientific report?

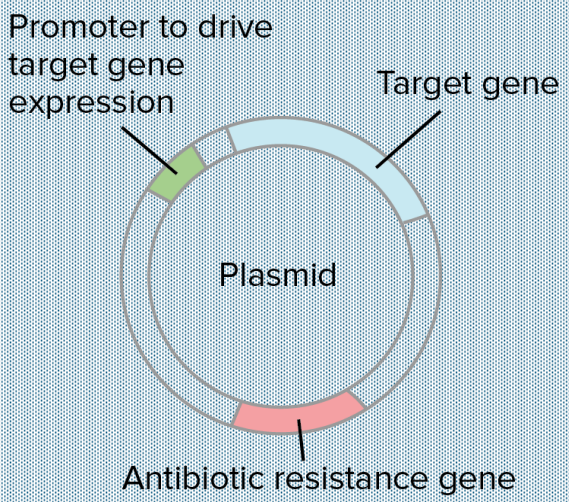
- The laboratory reports should contain the following sections:
  - Title
  - Objectives
  - Brief Introduction **[Theoretical background information]**
  - Materials and Methods **[As seen in the lab sheet]**
  - Results
  - Discussion
    - In this section you are required to describe of what happened in the experiment **[Principle]** , explain your results
    - Even if you obtained unexpected results, the discussion section is the section to justify or explain the reasons why you have obtained such results.
  - Questions

- **In case of acid or base contact with your skin,** wash it with large amount of clean, cold water and inform the instructor immediately.
- **Do not handle broken glassware** with your bare hands.
- **Do not eat, drink**, or chew gum in the laboratory.
- **Do not charge your mobile phones** in the laboratory.
- Do not depart from the lab leaving an experiment unattended. If you need to leave the lab you must inform your instructor before leaving the lab.
- You must **wash your glassware and hands** with soap after finishing the experiment.

Lab# I

BCH 462

Plasmid Isolation and Purification.



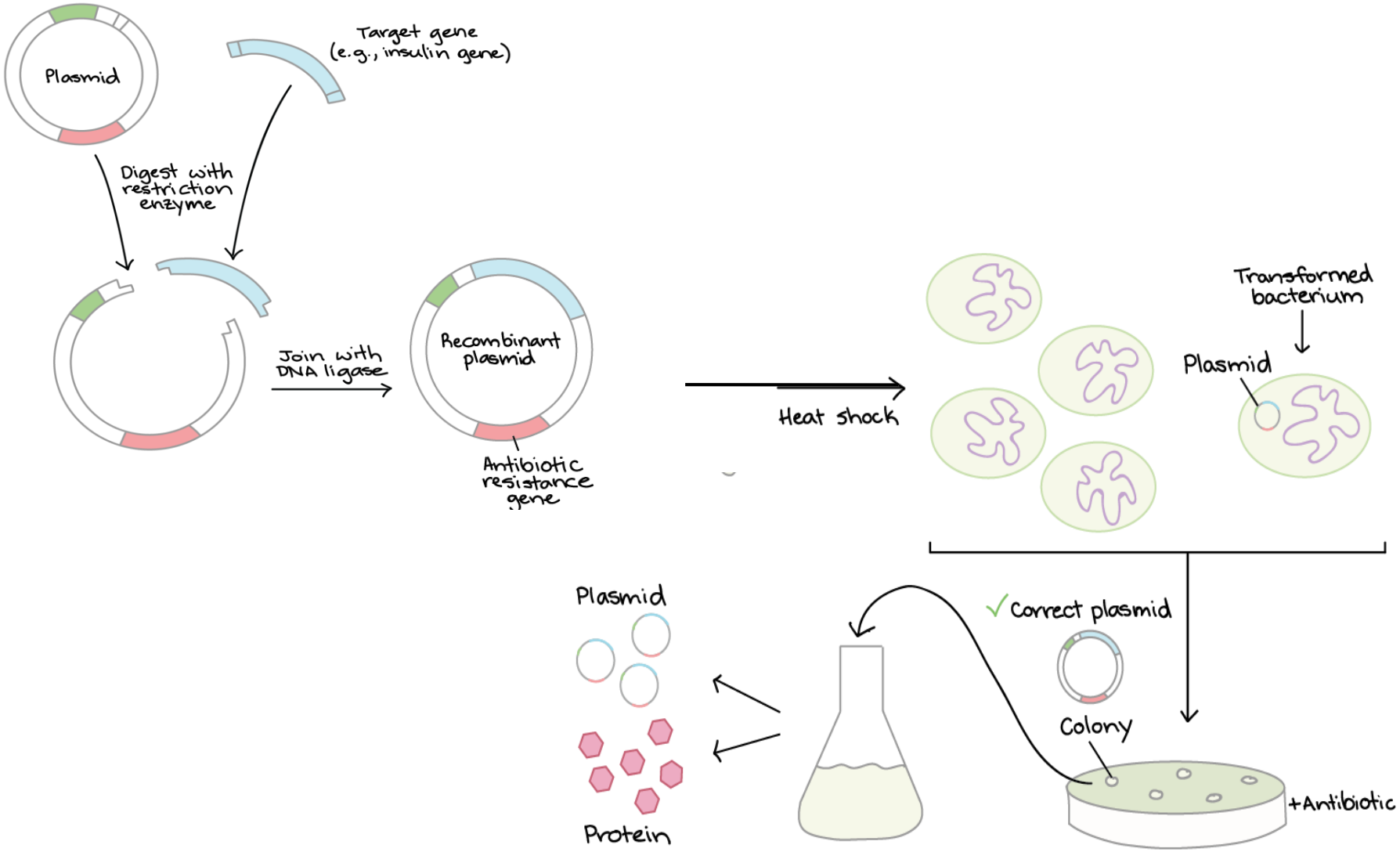
DNA Cloning

•**They are** a molecular biology technique that makes many identical copies of a piece of DNA, such as a gene.

•In a typical cloning experiment, a target gene is inserted into a ***vector e.g. Plasmid***, which eventually is inserted to a host cell "usually bacteria" and self replicate.

•The plasmid is introduced into bacteria via process called **transformation**, and bacteria carrying the plasmid are selected using antibiotics.

•Bacteria with the correct plasmid are used to make more plasmid DNA or, in some cases, induced to express the gene and make protein


Plasmid
Target gene
(e.g., insulin gene)
Digest with restriction enzyme
Join with DNA ligase
Recombinant plasmid
Antibiotic resistance gene
Heat shock
Transformed bacterium
Plasmid
Correct plasmid
Colony
+Antibiotic
Plasmid
Protein

Uses of DNA cloning

•Biopharmaceuticals.

DNA cloning can be used to make human proteins with biomedical applications, such as the insulin, human growth hormone, and tissue plasminogen activator (tPA).

•Gene therapy.

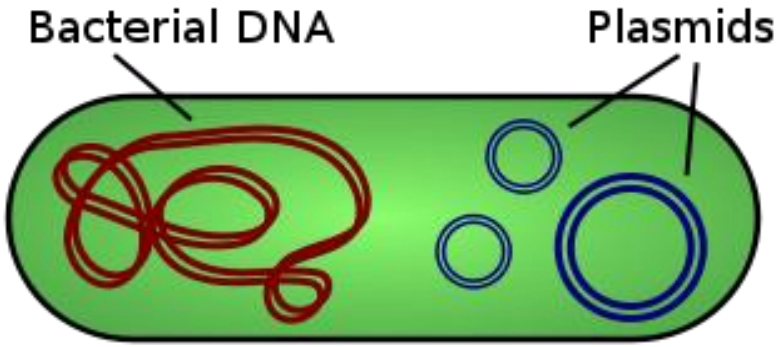
It attempts to provide a normal copy of the gene to the cells of a patient's body with genetic disorders. For example, DNA cloning was used to build plasmids containing a normal version of the gene that's nonfunctional in cystic fibrosis. When the plasmids were delivered to the lungs of cystic fibrosis patients, lung function deteriorated less quickly

•Gene analysis.

In basic research labs, biologists often use DNA cloning to build artificial, recombinant versions of genes that help them understand how normal genes in an organism function.

Plasmid:

Are small, double stranded, closed circular DNA molecules which can replicate independently from a bacterial chromosome.



Features of Plasmid:

1. Found in a wild variety of bacterial species.

2. Inherited extra-chromosomal elements, which replicate independently of the bacterial chromosome.

3. Its replication is maintained by a copy number.

4. Are not essential for the bacterium but may confer a selective advantage.

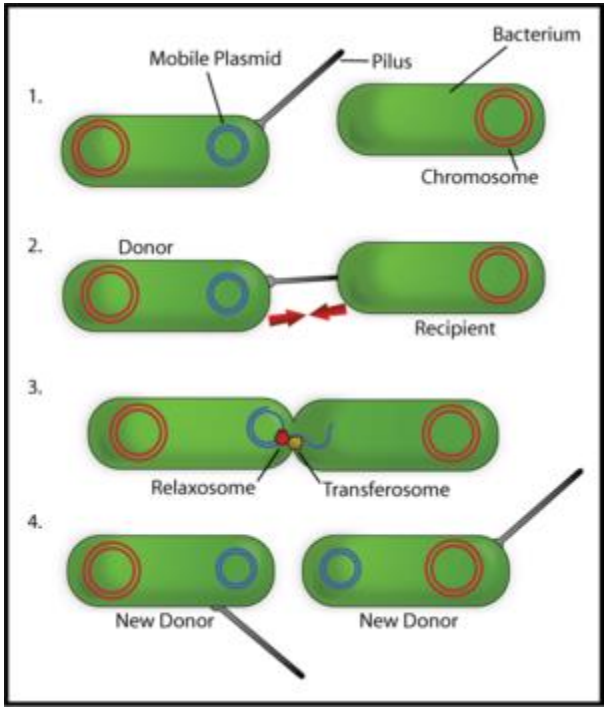
5. Using the enzymes and proteins encoded by their host for their replication and transcription.

There are three general classes for plasmids which can be advantageous for host cell:

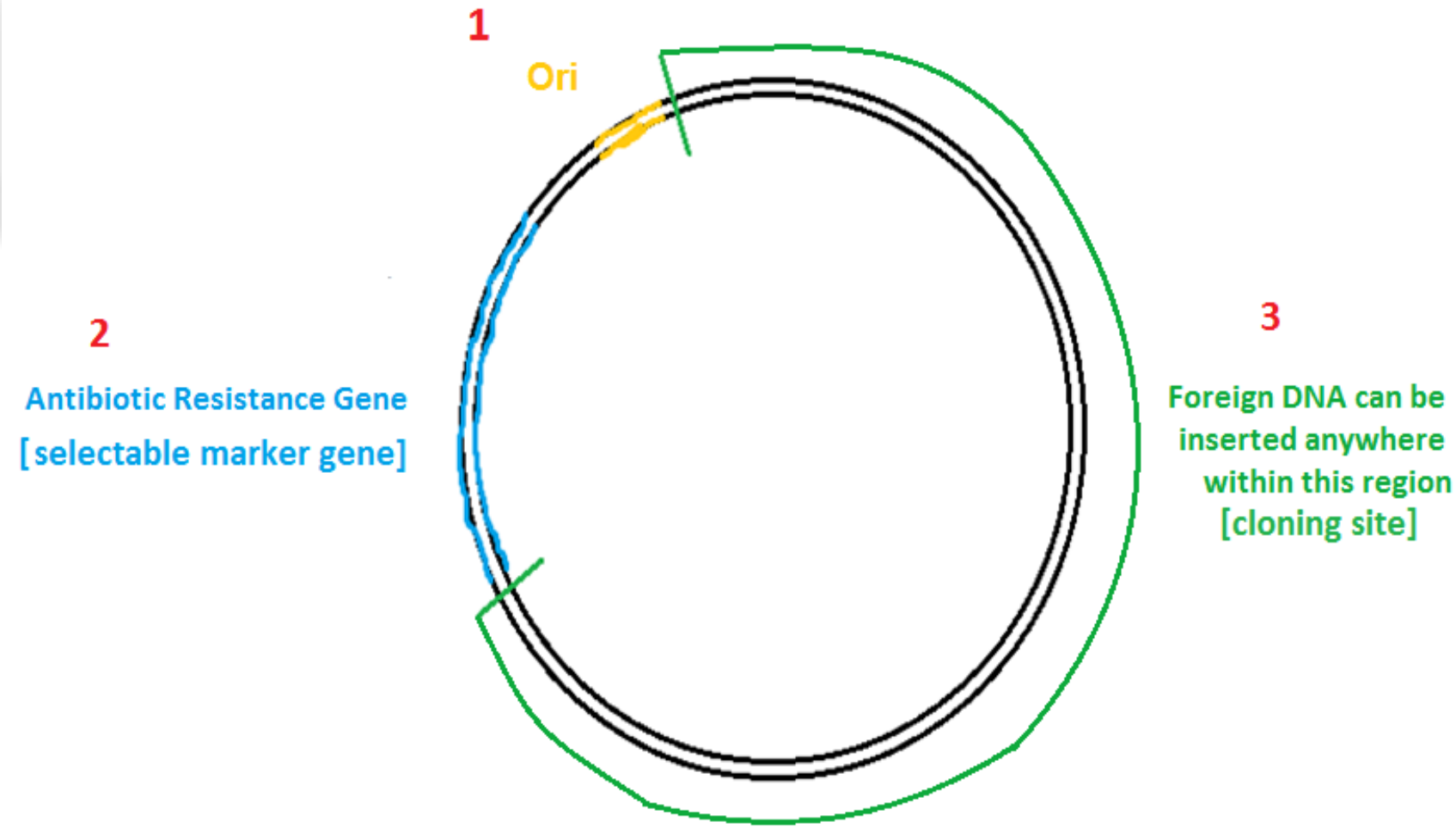
A- Virulence plasmids encoding toxin genes

B- Drug-resistance plasmids

C-Plasmids encode gene required for bacterial conjugation.



Generally plasmid vectors should contain three important parts:



Vector Element	Description
1- Origin of Replication (Ori)	DNA sequence which allows initiation of replication of the plasmid by cellular enzymes (DNA polymerase).
2- Antibiotic Resistance Gene	Allows for selection of" plasmid-containing "bacteria.
3- Cloning site	A place to insert foreign DNAs(the fragment which we are interested in its replication).

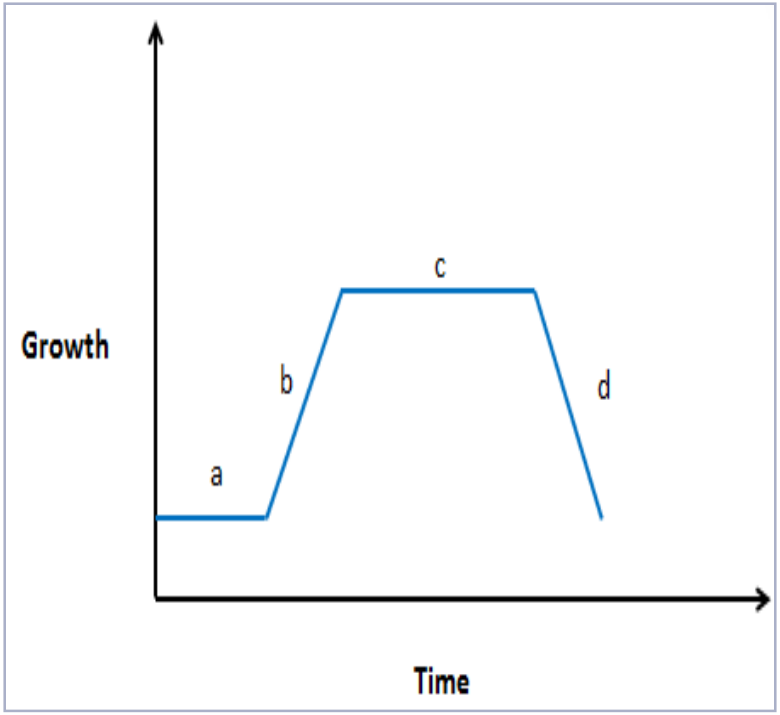
Bacterial growth curve

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

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

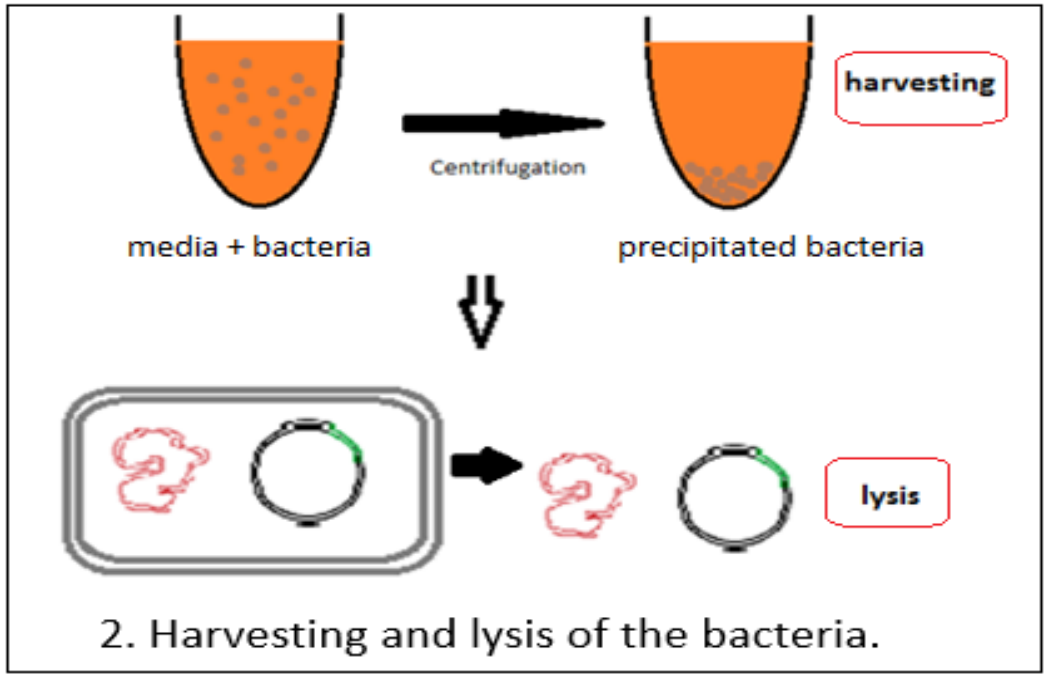
c) **Stationary phase:** the growth rate slows as nutrients become limited, waste products accumulate and the rate of cell division equals the rate of death.

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

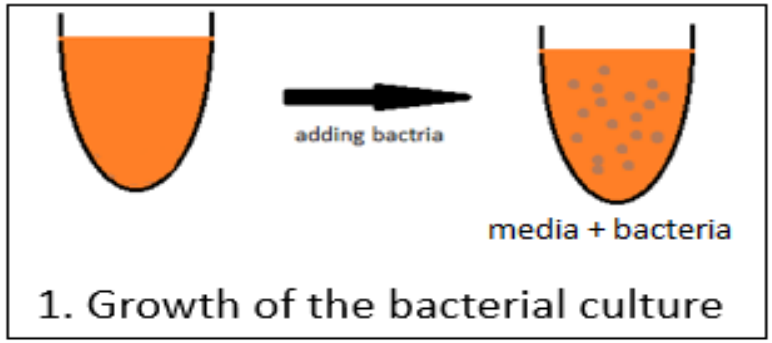


Practical part

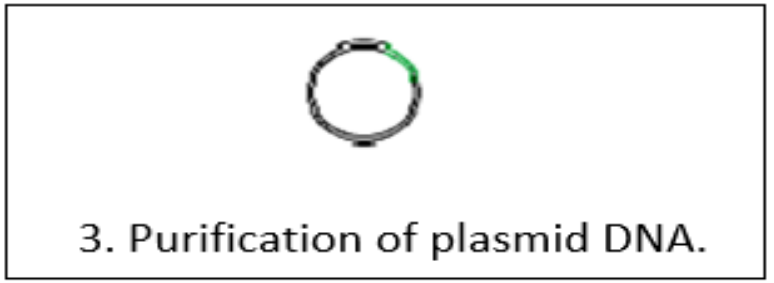
Three general steps involved in plasmid purification:



2. Harvesting and lysis of the bacteria.



1. Growth of the bacterial culture



3. Purification of plasmid DNA.

Bacterial growth curve

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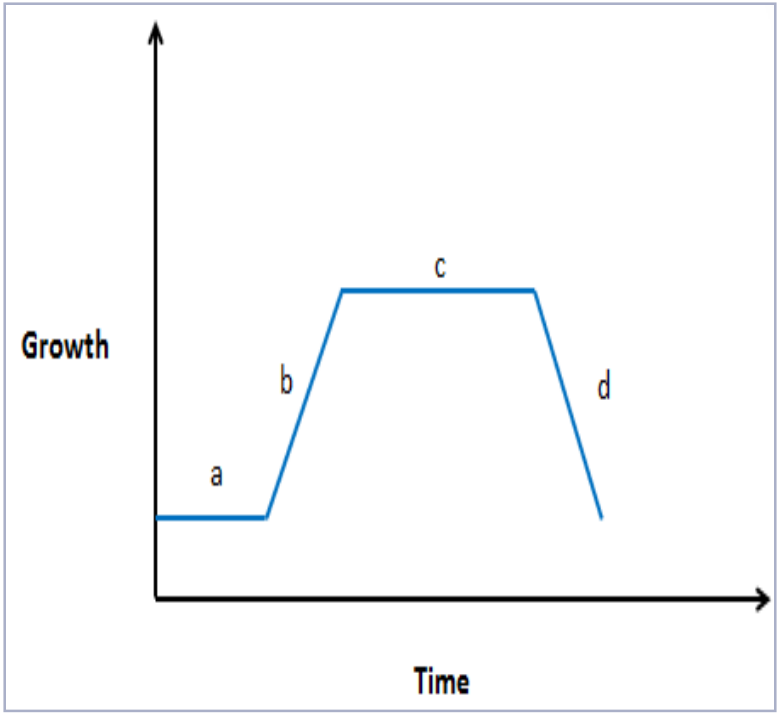


Harvesting and lysis of the culture:

Bacteria are recovered by centrifugation and lysed by any one of a large number of 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 and
- the technique used to subsequently purify the plasmid DNA.

Principle of the experiment "Alkaline lysis method ":

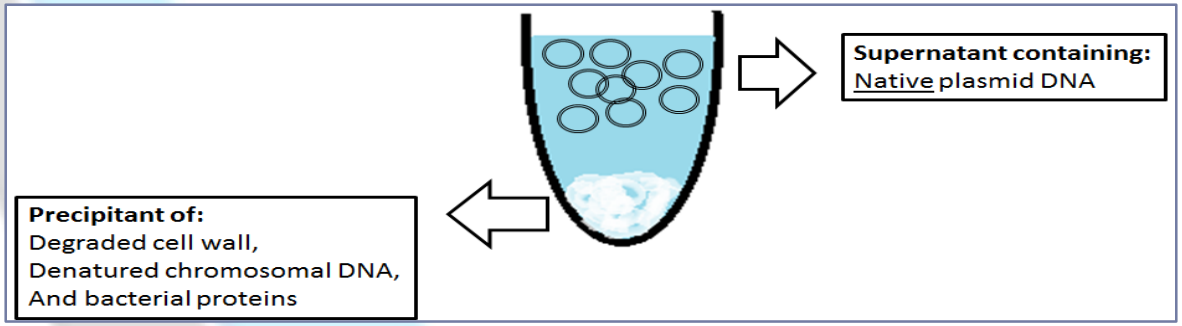
Using SDS in the alkaline solution:

-The SDS: will lyse the bacterial cell membrane and denature the proteins too.

-The alkaline pH: denature the genomic DNA and denature the proteins too.

-The degraded cell wall, denatured chromosomal DNA and bacterial proteins form large aggregated complex which will precipitated during the plasmid isolation and removed by centrifugation.

-Native plasmid DNA can be collected from the supernatant.



A typical isolation procedure by alkaline lysis have the following steps:

1. Harvesting of bacteria from culture by cenrifugation
2. Suspension of bacterial pellet in ***resuspension buffer (Glucose + Tris-Cl +EDTA)***
3. Lysis of bacteria ***(SDS+NaOH)***
4. Neutralization of lysate ***(Acidic potassium acetate)***
5. Clearing of lysate.
6. Recovery of plasmid from cleared lysate.
7. Washing of plasmid DNA pellet.
8. Storage of Plasmid DNA