Lab# 1

Plasmid Isolation and purification.

BCH 462 [practical]

DNA cloning techniques:

They are techniques, used to create copies of certain DNA fragments.

Using:

1-PCR[polymerase chain reaction].

2-Cell based. [using a vector carrying the DNA of interest ,which eventually inserted to A host cell “usually bacteria” and self replicate].

Isolated and amplified specific DNA fragment

“e.g.: gene A”

Plasmid:

Are small, double stranded, closed circular DNA molecules which can replicate independently from a bacterial chromosome. (can be isolated from bacterial cells).

Features:

1. Found in a wild variety of bacterial species.

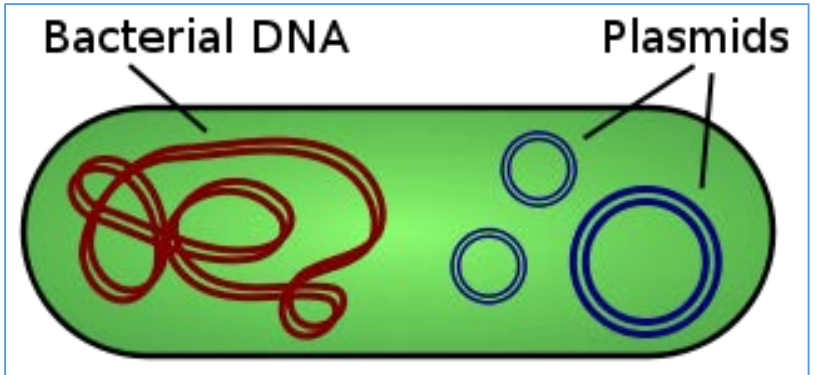
2.Extra-chromosomal elements, which replicate independently of the bacterial chromosome.

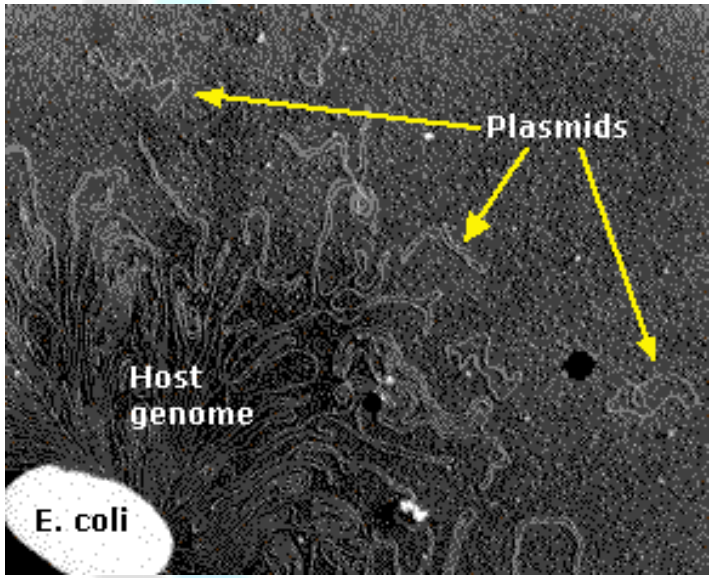
3. Are not essential for the bacterium but may confer a selective advantage.

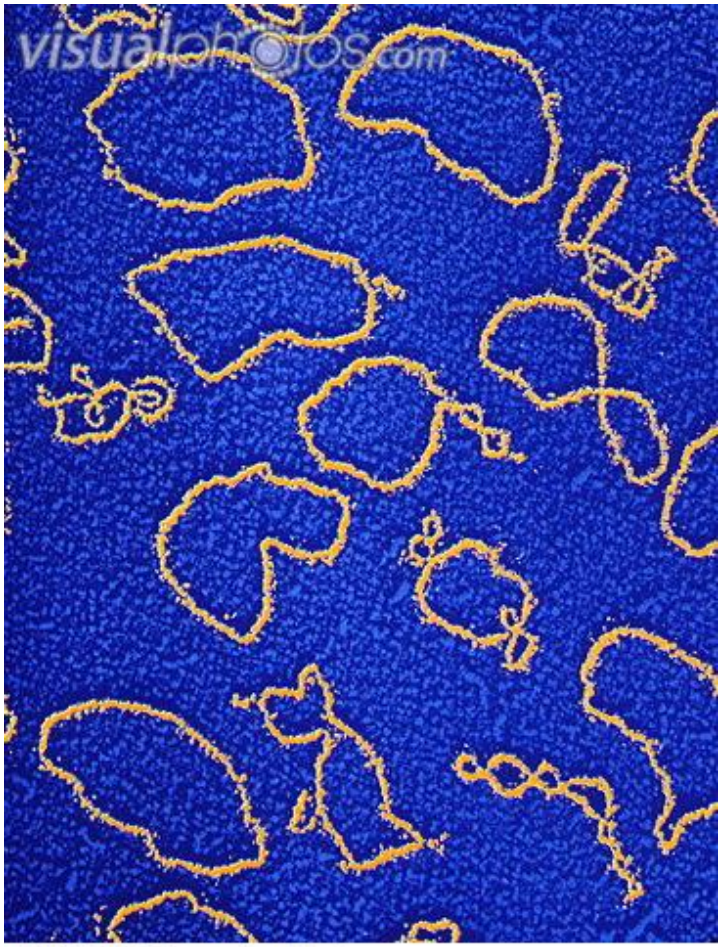
4.Using the enzymes and proteins encoded by their host for their replication and transcription.

5. Are inherited.

6. Used in many applications e.g. Drugs production.







g110355 [RM] © www.visualphotos.com

“Cell based” DNA cloning involve:

1.Insertion of **DNA fragment** in to a cloning vector ”e.g. plasmid”.

2. Introducing a **vector with DNA fragment** in to bacterial cells ” the host”.

3. Amplifying the (vector DNA), using bacterial DNA replication machinery.

1

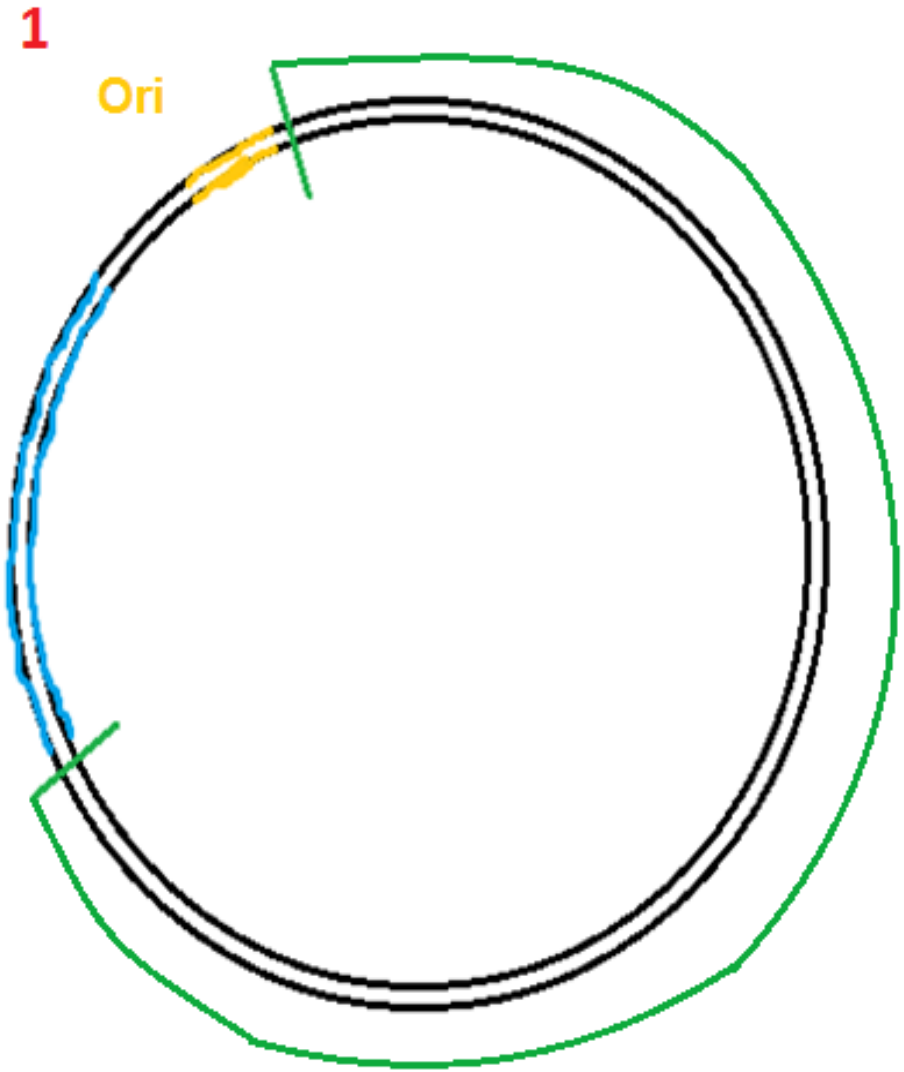
DNA fragment **cloning vector** Recombinant DNA

2

Recombinant DNA the host Transformed bacteria

3

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(?).
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).

Some of plasmid applications :

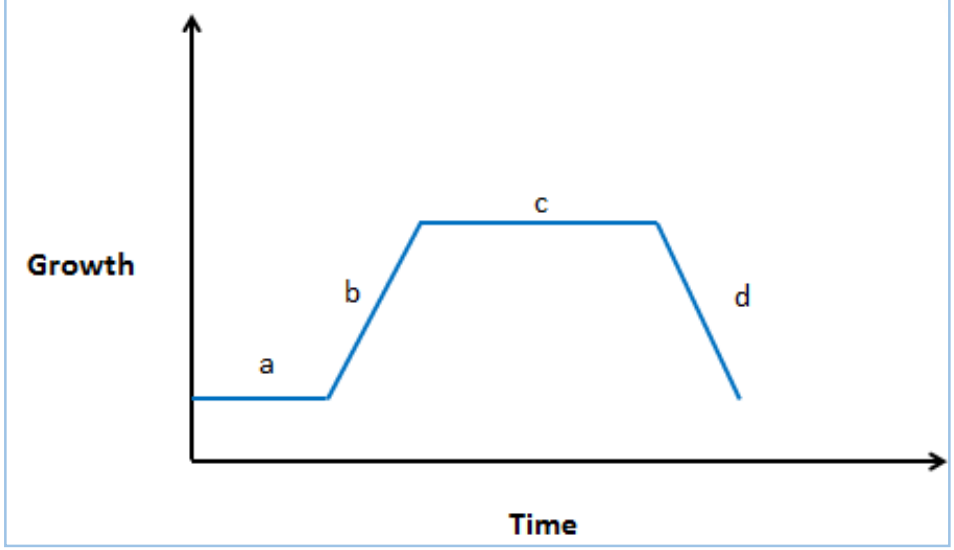
1. Gene therapy.
2. Molecular cloning.
3. Make large amounts of proteins.

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

A- Virulence plasmids encoding toxin genes.

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

C-Plasmids encode gene required for bacterial conjugation.



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

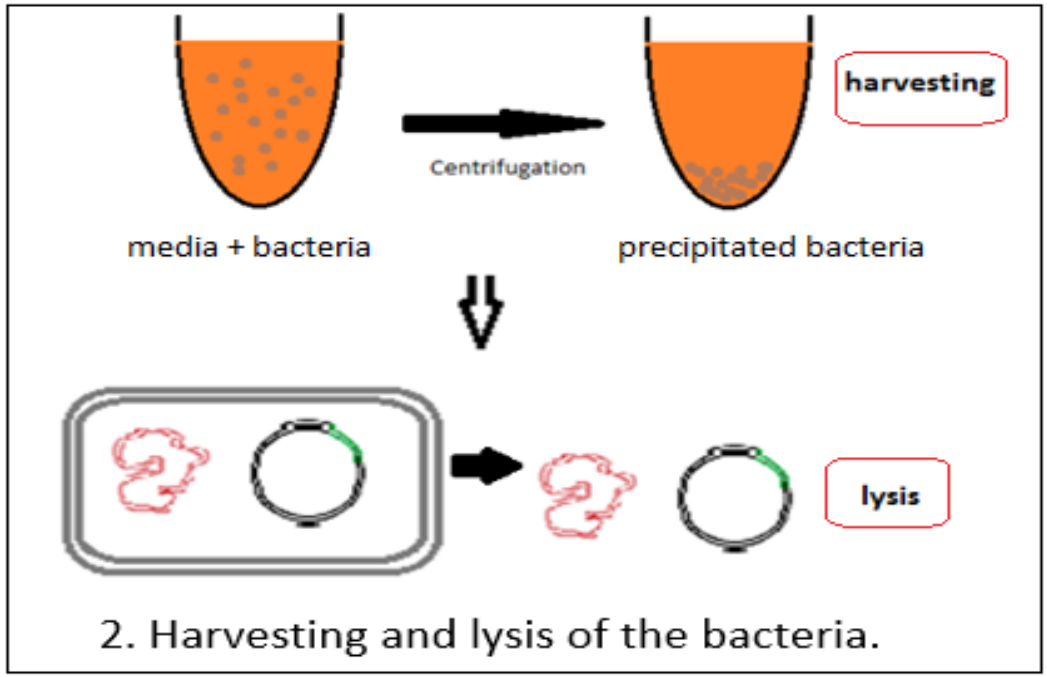
a) **Lag phase**: in this phase bacteria adapt themselves to growth conditions and synthesis its own DNA,RNA and proteins.

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

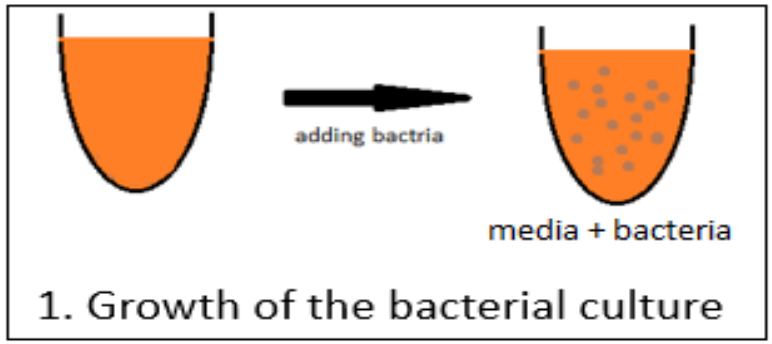
c) **Stationary phase:** the growth rate slows as nutrients become limited, waste products accumulate and the rate of cell division equals the rate of death.

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

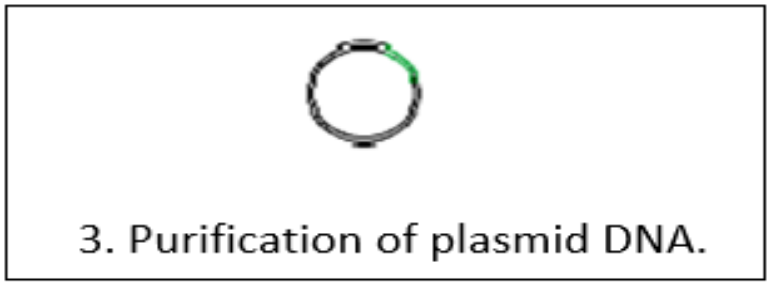
Three general steps involved in plasmid purification:



2. Harvesting and lysis of the bacteria.



1. Growth of the bacterial culture



3. Purification of plasmid DNA.

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.

Practical part

Plasmid isolation and purification

Principle of the experiment "Alkaline lysis method ":

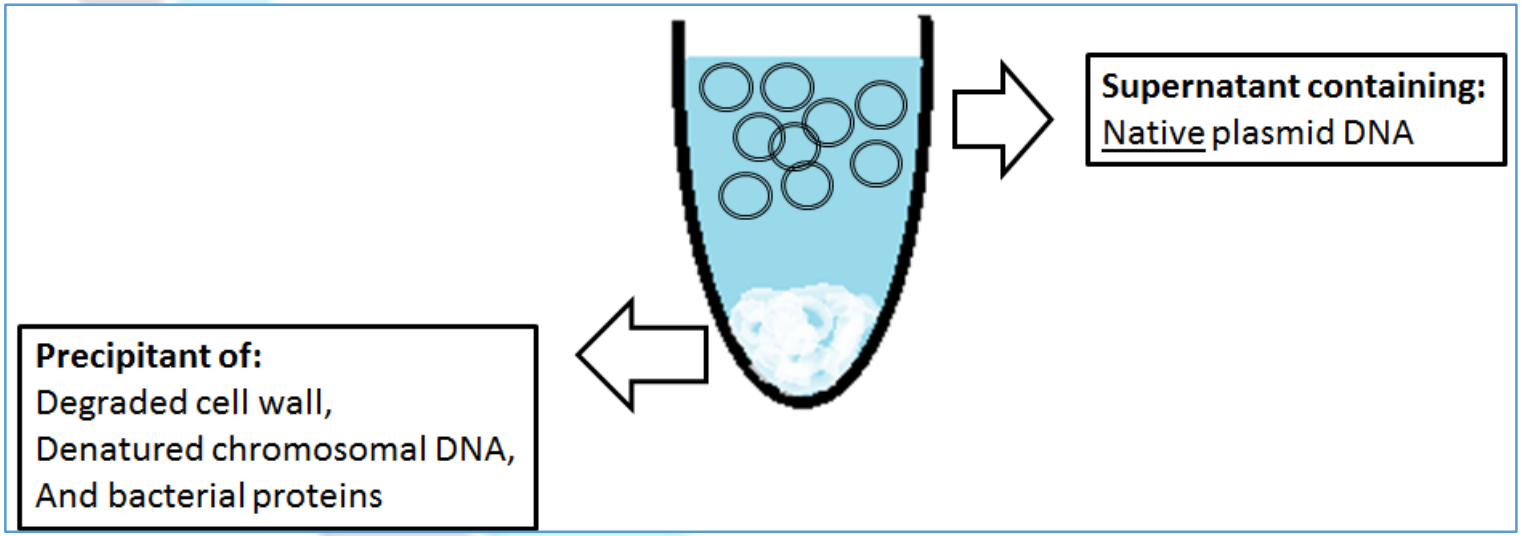
Using SDS in the alkaline solution:

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

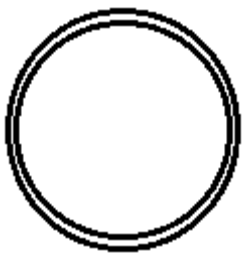
-The alkaline pH :denature the genomic DNA and denature the proteins too.

-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.

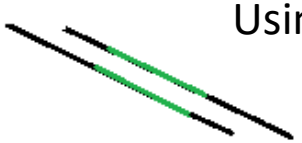


DNA cloning using plasmid



plasmid

Using same R.E used
For cutting gene A



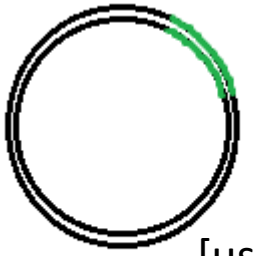
gene of interest

"gene A"

Using R.E

Animal DNA

1



"gene A"

Recombinant Plasmid

Ligation
[using ligase enzymes]

2

Bacterial cell

Introducing

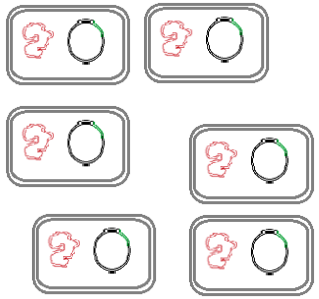
Chromosomal DNA

Transformed bacteria

cloning

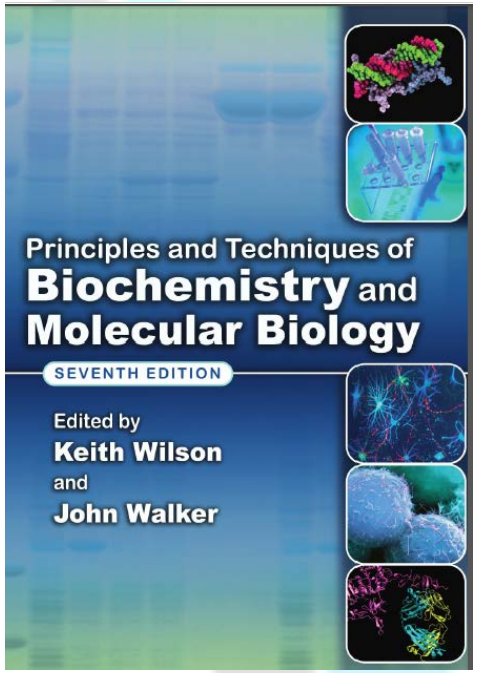
3

Culture plate
[media containing appropriate antibiotic]



Amplified Recombinant plasmid

References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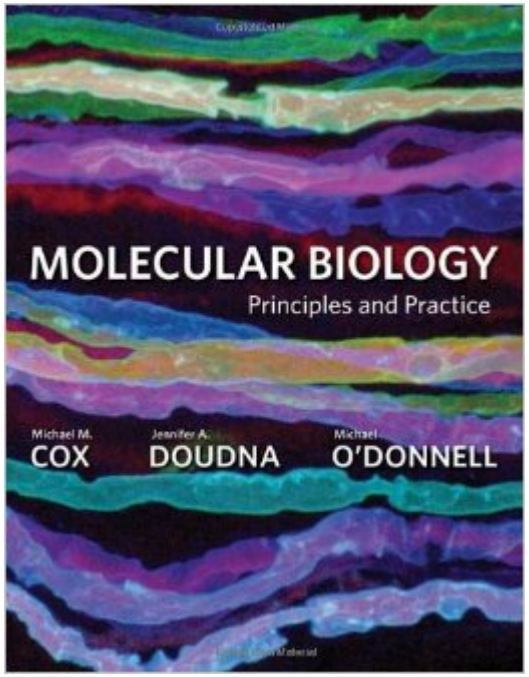
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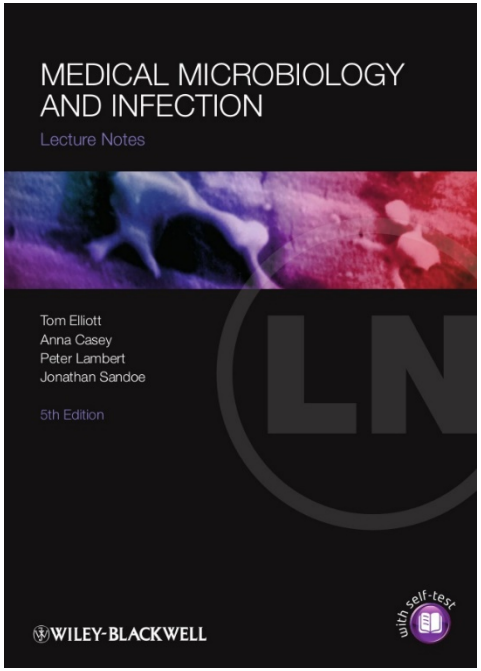
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