



Biotechnology

SCBC 317

Cloning

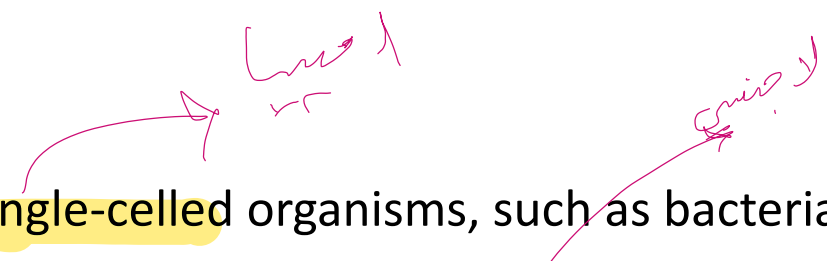
Definition:

- Produce genetically identical copies of a biological entity. The copied material, which has the same genetic makeup as the original, is referred to as a clone.
- Biological entity may be a gene , cell ,whole organism.

-Cloning can be : Natural cloning or artificial cloning

• Natural cloning :

A. some plants and single-celled organisms, such as bacteria, produce genetically identical offspring through a process called asexual reproduction. In asexual reproduction, a new individual is generated from a copy of a single cell from the parent organism



B. organisms that reproduce sexually, cloning can occur. i.e identical twins.

These twins are produced when a fertilized egg splits, creating two or more embryos that carry almost identical DNA . Identical twins have nearly the same genetic makeup as each other, but they are genetically different from their parent.

- Artificial cloning:

- Three different types of artificial cloning:

1. Gene cloning: copies of genes or segments of DNA
2. Reproductive cloning: copies of whole animals.
3. Therapeutic cloning: produces embryonic stem cells for experiments aimed at creating tissues to replace injured or diseased tissues.

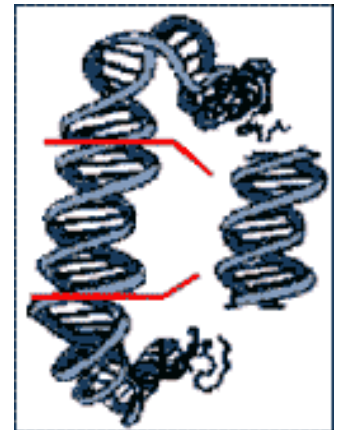
Gene cloning

Definition:

procedures to produce multiple copies of a single gene or segment of DNA

Two types of gene cloning:

- 1) In vivo gene cloning : The copies of gene are produced inside a host cell using restriction enzyme and ligase enzyme
- 2) In vitro gene cloning: The copies of gene are produced in a reaction tube using polymerase chain reaction



In vivo gene cloning : Terms used

- Vector

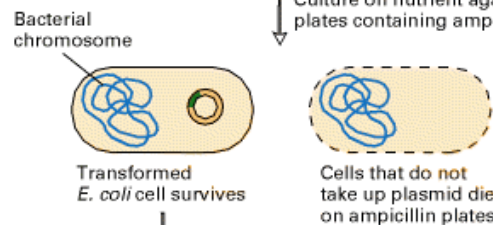
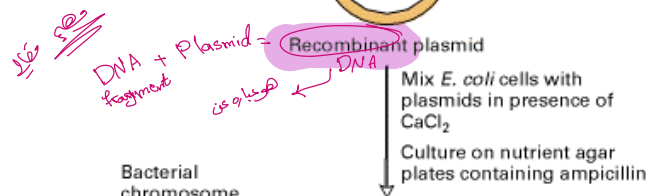
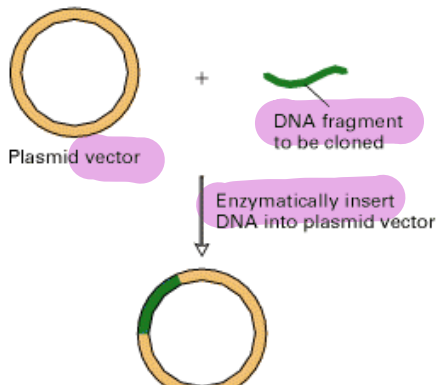
- Restriction enzymes

- Recombinant DNA

Vectors:

- the vector is a DNA molecule that serves as the carrier for the transfer or insertion of foreign gene(s) into another cell, where it can be replicated and/or expressed.
- vector is used to amplify a single molecule of DNA into many copies.
- The insertion of the fragment into the vector is carried out by treating the vector and the foreign DNA with the same restriction enzyme, then ligating the fragments together.

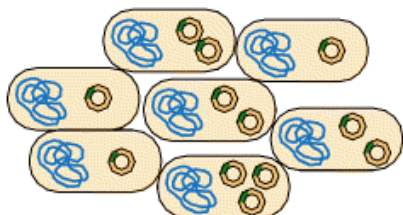
Deconstruction Side



Independent plasmid replication

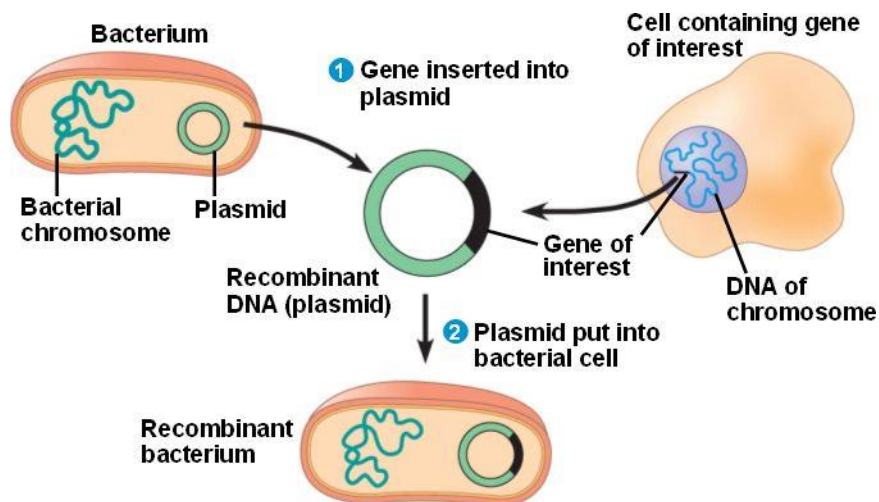


Cell multiplication



Colony of cells each containing copies of the same recombinant plasmid

فونسیون



بجمله 1
2. اورده ختاه

ساده و vector
بجمله 2

am

مميزات

- **vectors common properties:**

1. Ability to replicate. *تضاعف*

2. Contain a genetic marker for selection.

3. Unique restriction sites to facilitate cloning of insert DNA.

مكان القس

Types of vectors

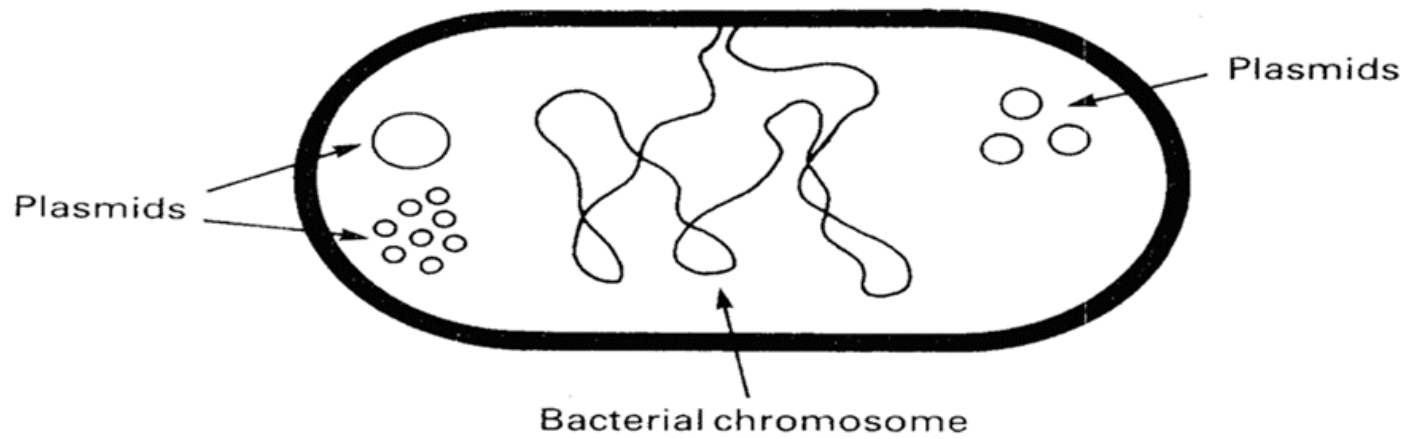
- The vector is chosen according to the size and type of DNA to be cloned

	Maximum insert size (kilobases or kb [1000bp])
Bacterial plasmid	6-12
bacteriophage	25
Cosmids	35
yeast artificial chromosome	200-1000

ازواج
نوعی
میکروارگانیسم

1-Plasmids : ↙ X

- Plasmids are circular, double-stranded DNA molecules that exist in bacteria
- They can replicate independently of the host cell.
- Occur naturally in bacteria and usually carry genes that are useful to survival
- There can be as many as several hundred copies of a single plasmid in each bacteria.



Plasmids: independent genetic elements found in bacterial cells.

Plasmids have:

1-an origin of replication:

is a DNA segment recognized by the cellular DNA-replication enzymes.

- Without replication origin, DNA cannot be replicated in the cell.

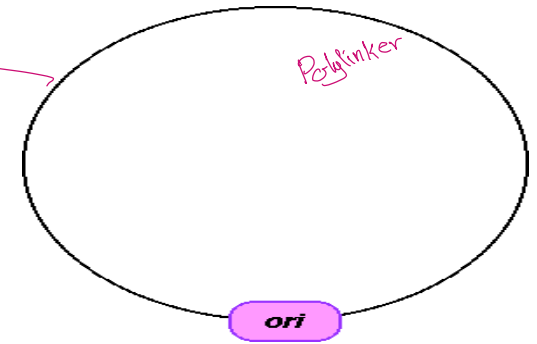
2- Antibiotic resistance genes as markers,

To ensure the retention of plasmid DNA in bacterial populations, an antibiotic resistance gene (i.e., a gene whose product confers resistance to ampicillin) is included in the plasmid.

These bacteria are then grown in the presence of ampicillin. bacteria without the plasmid DNA would not survive antibiotic treatment.

مواصفات

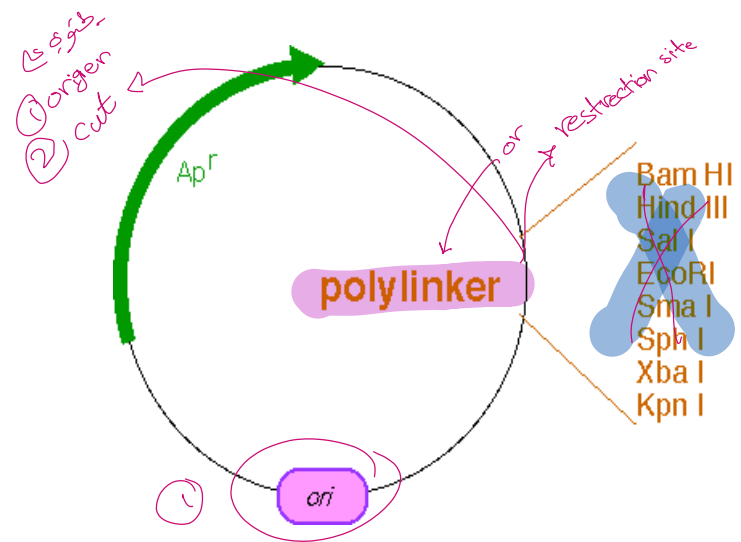
النسب داخلها؟



have 3 name

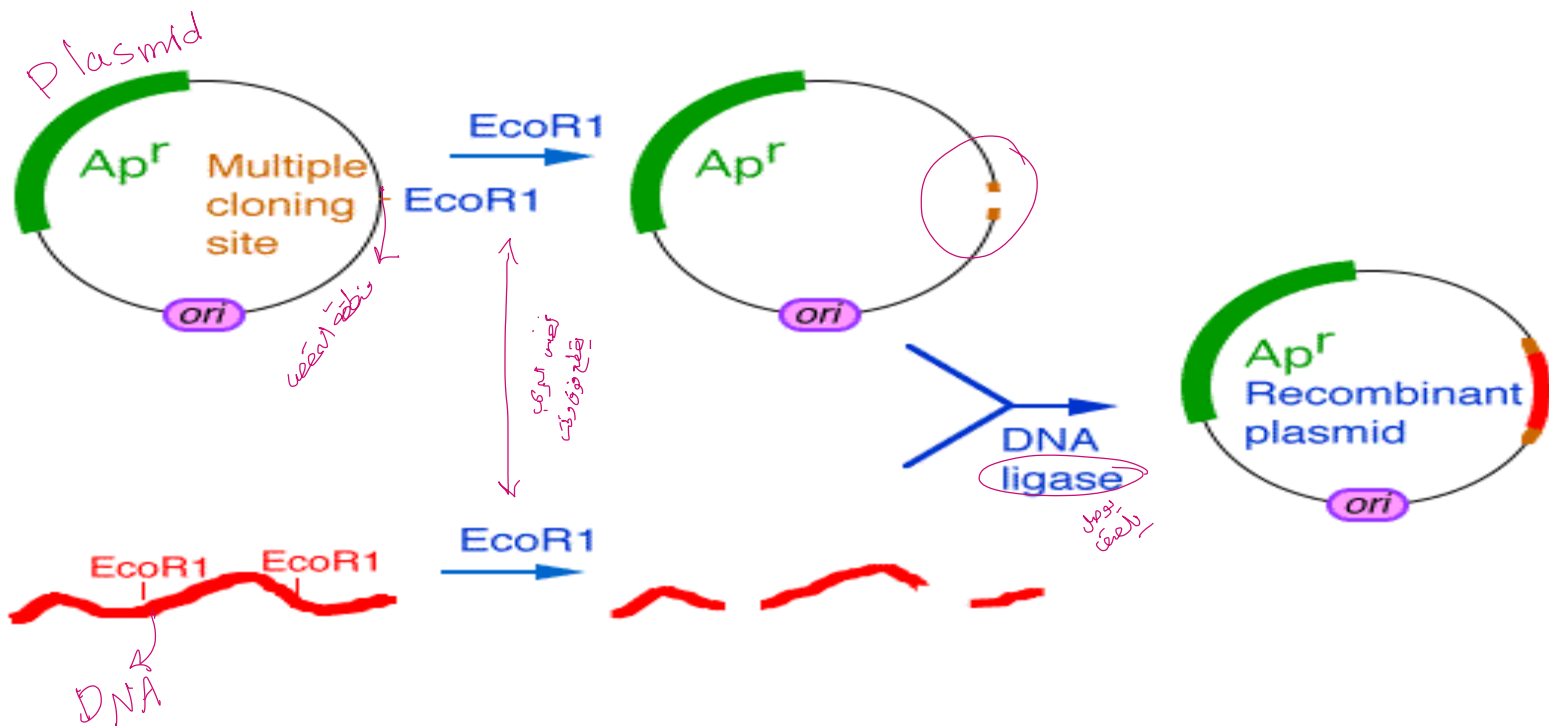
3-Several unique restriction sites:

Many vectors contain a multiple cloning site or polylinker:



- A DNA segment with several unique sites for restriction enzymes located next to each other
- Restriction sites of the polylinker are not present anywhere else in the plasmid.
- Cutting plasmids with one of the restriction enzymes that recognize a site in the polylinker does not disrupt any of the essential features of the vector

Gene can be introduced into the cloning vector at one of the restriction sites present in the polylinker



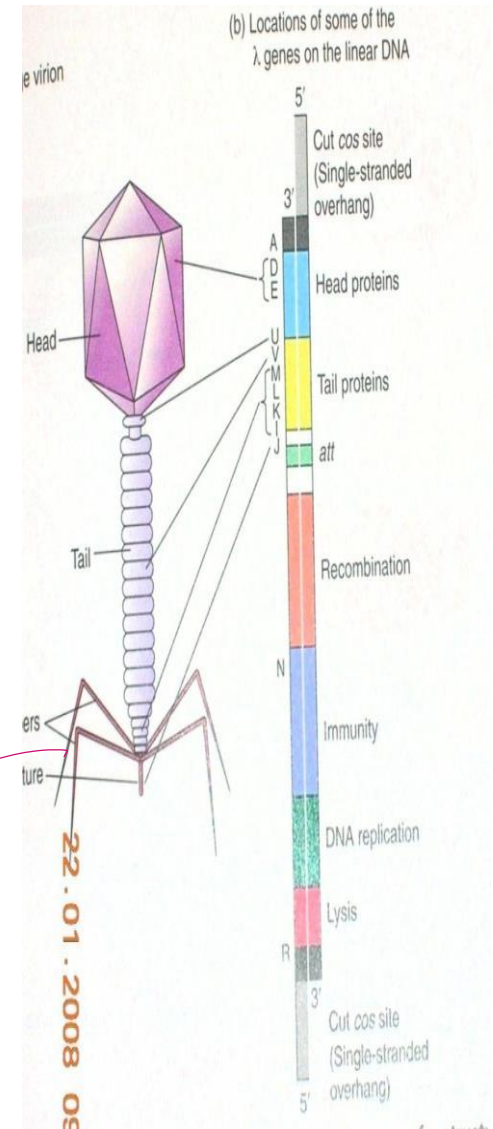
2-BACTERIOPHAGE LAMBDA

Phage lambda is a **bacteriophage** or **phage**, i.e. bacterial virus, that uses *E. coli* as host.

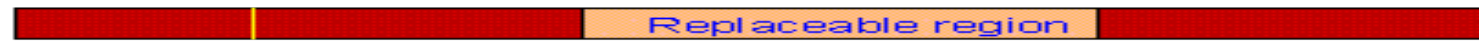
- Its structure is that of a typical phage: **head, tail, tail fibres**.

- **Lambda viral genome:** 48.5 kb linear DNA with a 12 base ssDNA "sticky end" at both ends; these ends are complementary in sequence and can hybridize to each other.

- **Infection:** lambda tail fibres adsorb to a cell surface receptor, the tail contracts, and the DNA is injected. and lambda begins its life cycle in the *E. coli* host.



λ -Phage genome



0

49 kb

DNA recombination

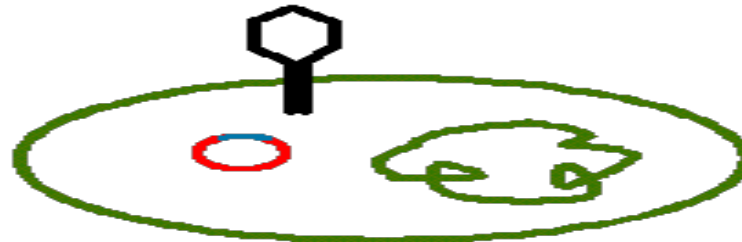


Dna
فایروس

Packaging,
assembly



Infect *E. coli*



3-Cosmid vectors:

are developed by combining the features of the **plasmid** vector and the **bacteriophage** vector. Origin of replication, *multiple cloning* site and selectable marker are obtained from the plasmid and only the cohesive site or **cos site** region is taken from lambda phage. These are fused together to obtain the cosmid vector.

4-The yeast artificial chromosome (YAC) vector

- YACs is a human-engineered DNA molecule used to clone DNA sequences in **yeast** cells.
- YAC vector is capable of carrying a large DNA fragment (up to 200 Kb), but its transformation efficiency is very low.