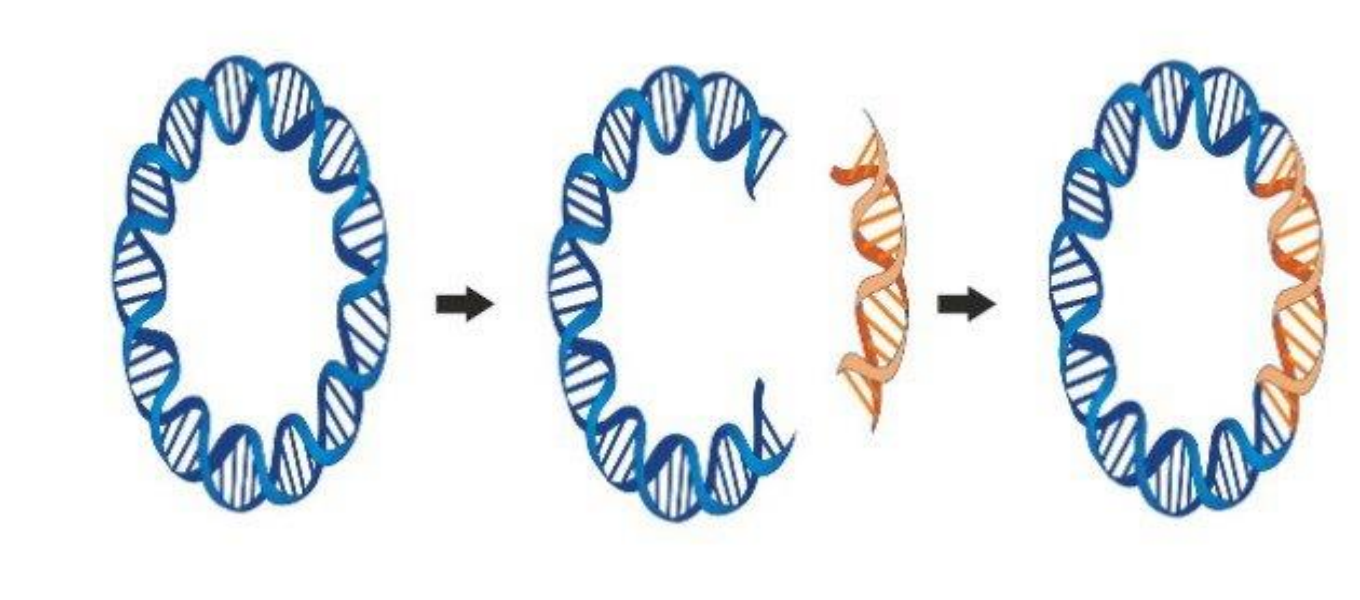
Lec 5 \ **Cloning**



Cloning

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The process of isolating a specific piece of DNA and then transferring this piece to a plasmid vector, which allows us to replicate, know the sequences, and store this piece to conduct functional studies on it or use it in genetic engineering..

Steps of this technique *

1- Amplification of the gene to be cloned using PCR technology.

2- Preparing the gene to be transferred (after amplifying it) using a specific cutting enzyme.

3- Prepare the cloning vector by cutting it with the same cutting enzyme that used the gene.

4- Transferring the prepared gene to the prepared cloning vector and completing the ligation process.

5- Test to ensure the success of the cloning process.

Common elements in this process

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1- Gene amplification using Polymerase Chain Reaction (PCR) technology
2- Cutting enzymes.
3- Clone carriers.
4- Mutant bacteria and how to detect transformation.

Cloning vectors

DNA molecules are used to transfer genes to host cells (microbes, animals, plants) and to provide elements for controlling replication and gene expression. In general, cloning vectors are divided into *

- Vector for bacterial cells
- Vector for plant cells
- Vector for Mammalian cells

Vector for Bacterial cell *

A- Plasmid Vectors

B- Bacteriophage Vectors

C- Cosmids

D- Phagemids or Phasmid

A- Plasmid Vectors *

It is one of the most common cloning vectors. These plasmids are capable of carrying a DNA segment of up to 15 kb. The efficiency of the cloning and transformation process increases as the molecular size of the plasmid decreases, and vice versa. Structurally, plasmid vectors consist of the following: تزداد كفاءة عملية الاستنساخ والتحول مع انخفاض الحجم الجزيئي للبلازميد

1 • The replication initiation region is known as OriV: It is worth noting that the plasmid is capable of self-replication and is called a Replicon, and is also able to integrate with the host bacterial chromosome and is called an Episome.

Plasmid Vectors *

2 • Cloning site: It consists of a region with a distinct sequence that is made and inserted into the plasmid. This region can be distinguished by several types of cutting enzymes. These sites are called MCS. The benefit of them is that they contain cutting sites for many cutting enzymes.

3 • Selectable Marker :

These represent genes that are resistant to some antibiotics, such as the ampicillin resistance gene, symbolized by AmpR, and the tetracycline resistance gene, TetR. The benefit of them is to select transformed bacterial cells (which took the plasmid vector), as the transformed cells grow on a medium containing these antibiotics, while non-transformed cells (which do not Did not take the plasmid vector) do not grow on a medium containing antibiotics.

Plasmid Vectors *

4• Reporter gene

It is a distinctive gene through which the success or failure of cloning can be determined, such as the lacZ gene .

5• Polyhistidine sequence

An optional, laboratory-created but very common sequence region. The resulting protein is characterized by containing a short polyhistidine region. The benefit of it is to extract the resulting protein easily, as nickel separation columns are used, as the resulting protein combines with it through this region, and thus we have obtained the required protein in one step.

There are two types of plasmids: *

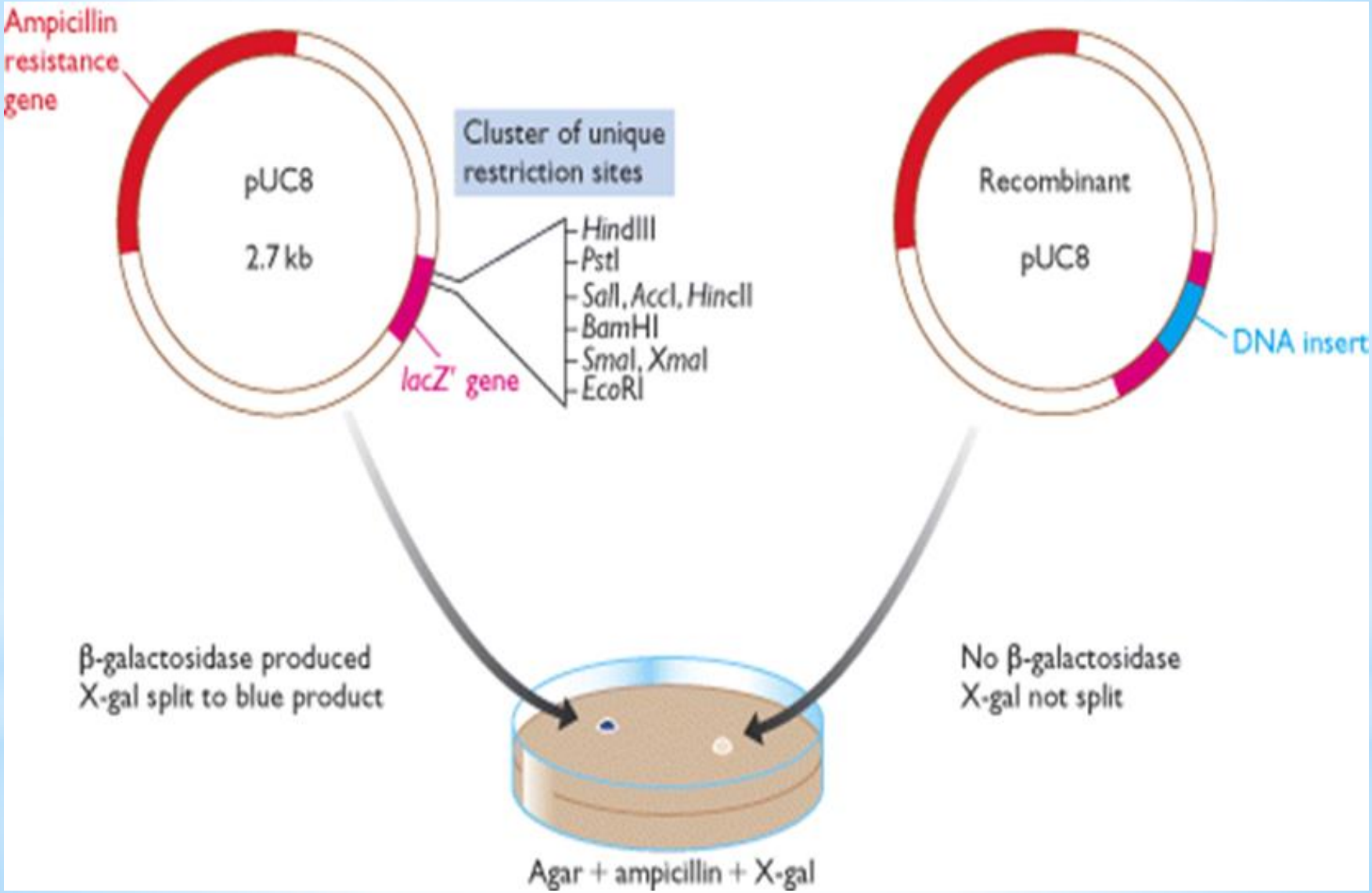
Low Copy Number

The plasmids that are found inside the bacterial cell represent 1-25 copies of the plasmid, an example of which is pBR322 plasmid. It is preferable to use this type of vector if there is a possibility of causing harm to the bacterial cell to which the plasmid was transferred.

There are two types of plasmids: *

High Copy Number

The plasmids that exist inside the bacterial cell represent 100 or more copies of the plasmid, such as pUC plasmid. It is preferable to use this type if I want to obtain a large number of DNA transferred to the host (i.e. a larger product).


Ampicillin resistance gene
pUC8
2.7 kb
Cluster of unique restriction sites
HindIII
PstI
SalI, AccI, HincII
BamHI
SmaI, XmaI
EcoRI
lacZ' gene
Recombinant
pUC8
DNA insert
β-galactosidase produced
X-gal split to blue product
No β-galactosidase
X-gal not split
Agar + ampicillin + X-gal

Vector for Bacterial cell *

B- Bacteriophage Vectors

It is characterized by its ability to carry a piece of DNA up to 53 kb, such as phage λ and M13 phage.

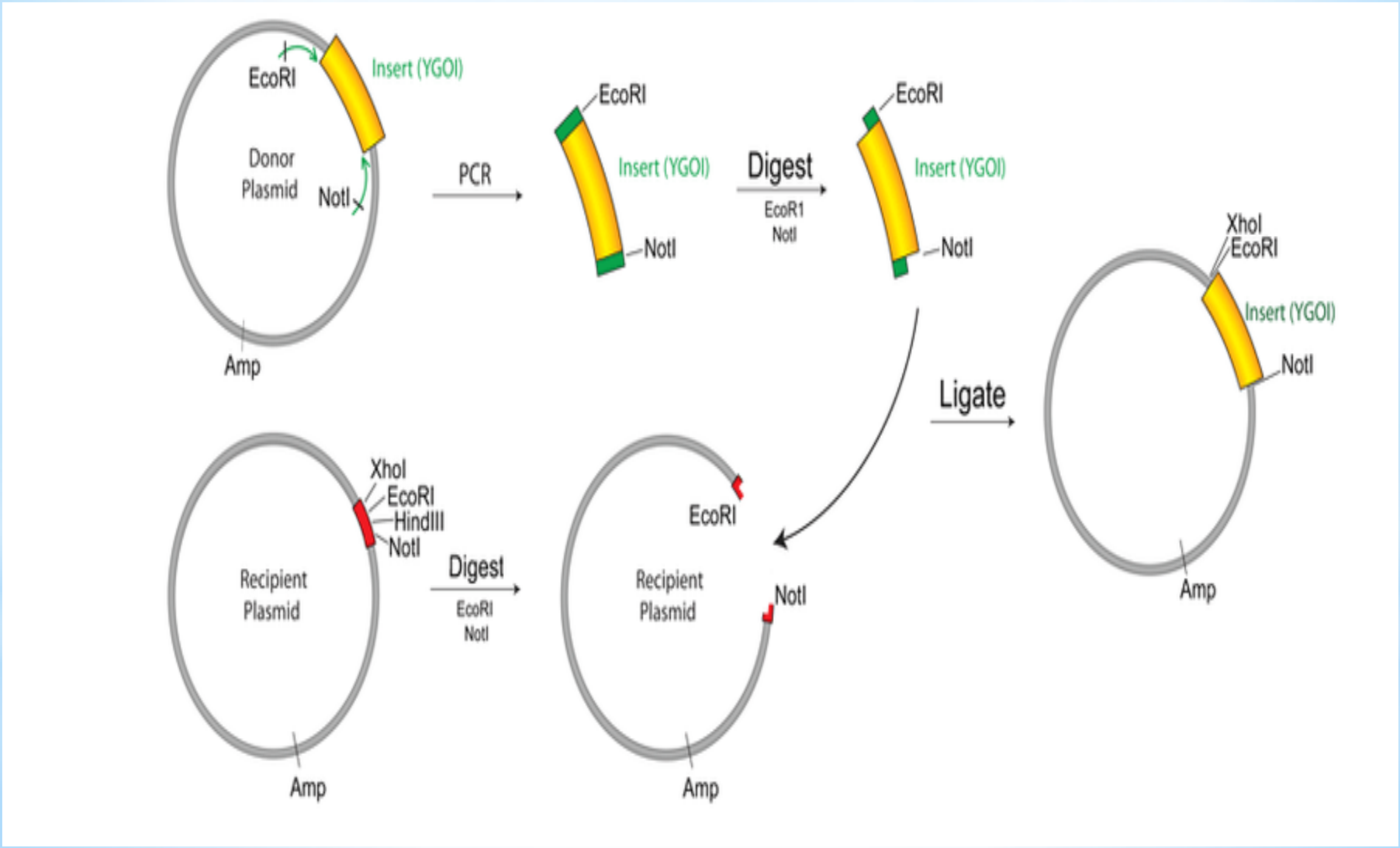
C- Cosmids

They are plasmid vectors that contain the cohesive site of phage λ. They are characterized by their ability to carry a piece of DNA of up to 45 kb.

Vector for Bacterial cell *

D- Phagemids or Phasmid

It is characterized by being composed of a filamentous bacteriophage and a plasmid. It is characterized by containing two replication sites, one for the phage and the other for the plasmid, as in the pBluescript vector, which contains the f1 ori of the filamentous phage f1 phage and the pUC ori of the pUC plasmid.


EcoRI
Insert (YGOI)
Donor Plasmid
NotI
Amp
PCR
EcoRI
Insert (YGOI)
NotI
Digest
EcoR1
NotI
EcoRI
Insert (YGOI)
NotI
Ligate
XhoI
EcoRI
Insert (YGOI)
NotI
Amp
XhoI
EcoRI
HindIII
NotI
Recipient Plasmid
Amp
Digest
EcoRI
NotI
EcoRI
Recipient Plasmid
NotI
Amp