Dr. Maha Mikhlef Kadum

Plant Genetic Engineering

Lecture no .3

Fourth grade/ sub-dep. Fungi and Plant Sciences

2022-2023/ course no. 1

What is RFLP

RFLP is a difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples.

Method of DNA analysis by RFLP

The method of analysis of DNA by RFLP involves the following steps:

1- In the first step fragmentation of a sample of DNA is done by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction digest.

2- The resulting DNA fragments are then separated by length through a process known as agarose gel electrophoresis.

3- Then transferred to a membrane via the Southern blot procedure.

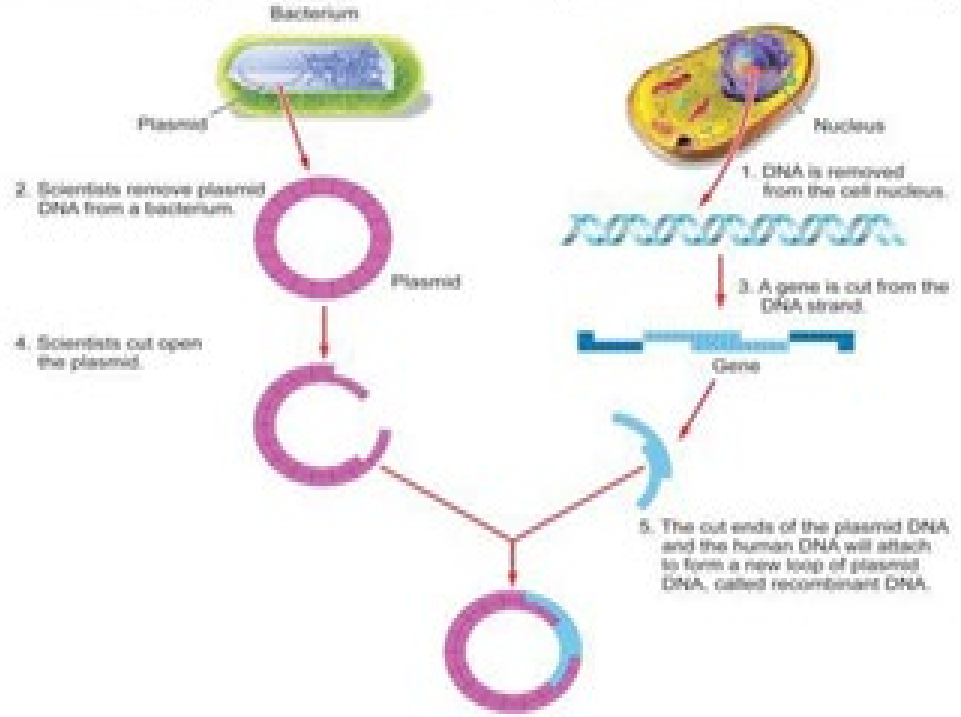
4- Hybridization of the membrane to a labeled DNA probe will done and then determines the length of the fragments which are complementary to the probe.

5- Then we will observe the fragments of different length.

An RFLP occurs when the length of a detected fragment varies between individuals. Each fragment length is considered an allele, and can be used in genetic analysis.

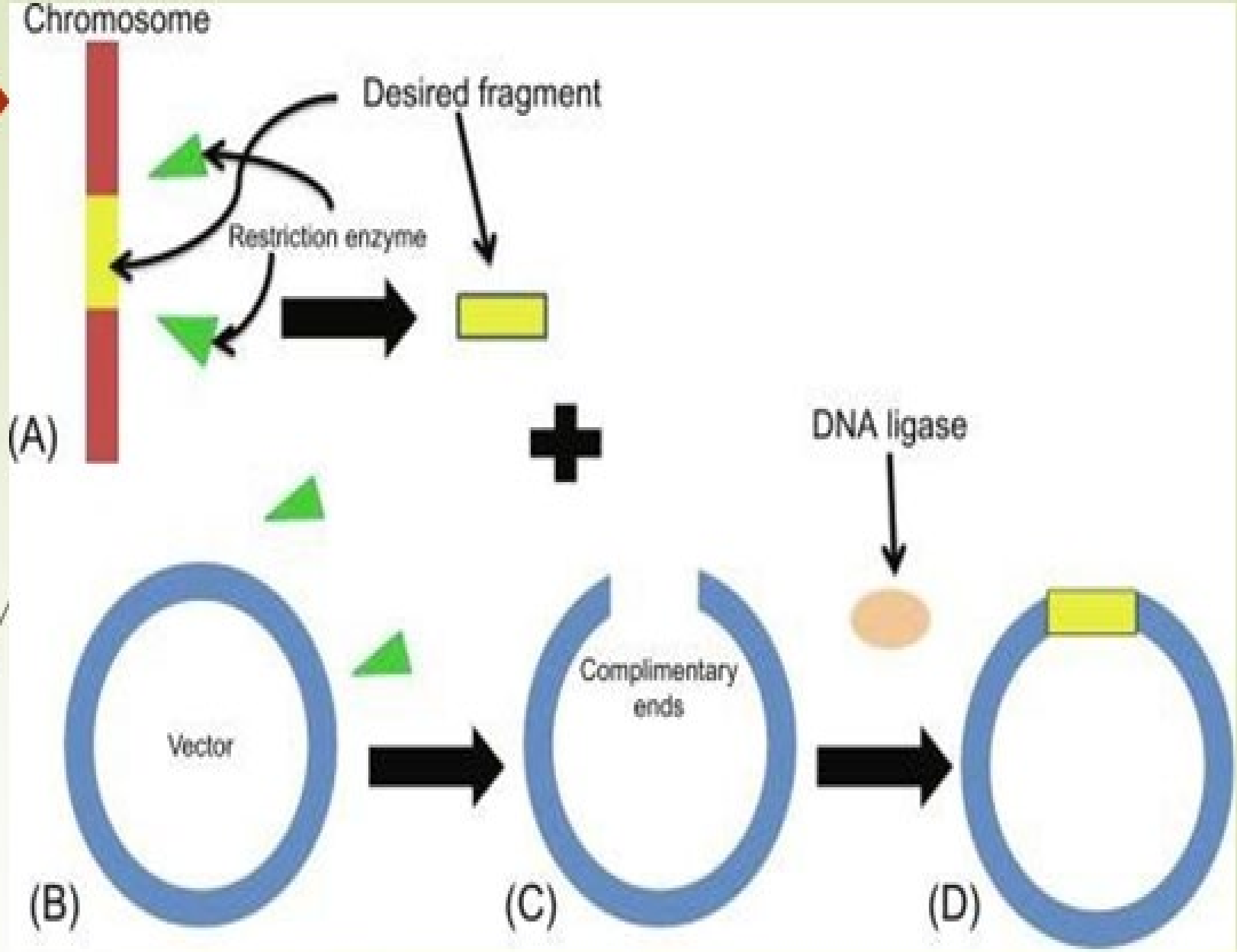
What is recombinant DNA?

- Recombinant DNA refers to the creation of new combinations of DNA segments that are not found together in nature.
- Artificial DNA sequences constructed as per your requirement
- **DNA molecules are constructed outside the living cells that is, *in vitro* by joining natural or synthetic DNA segments that can replicate in a living cell**



TOOLS OF RECOMBINANT DNA TECHNOLOGY

- 1.Restriction Enzymes-
- restriction endonuclease- cut DNA at a particular point.
- First restriction endonuclease- *Hind II.*
- Exonucleases- remove nucleotides from the ends of DNA.
- Each restriction endonuclease recognises a specific *palindromic nucleotide sequences* in the DNA.
- Restriction enzymes cut the strand of DNA a little away from the centre of the palindromic site leaving single stranded streches called *STICKY ENDS.*
- Sticky ends are joined together by DNA ligase.


Chromosome
Desired fragment
Restriction enzyme
(A)
DNA ligase
Vector
Complimentary ends
(B)
(C)
(D)

Steps of Recombinant DNA formation

Isolation of desire DNA and Plasmid

↓

Cut both the DNA by same restriction endonuclease

↓

Then Ligate both DNA with the help of DNA ligase

↓

Introduce recombinant DNA into host

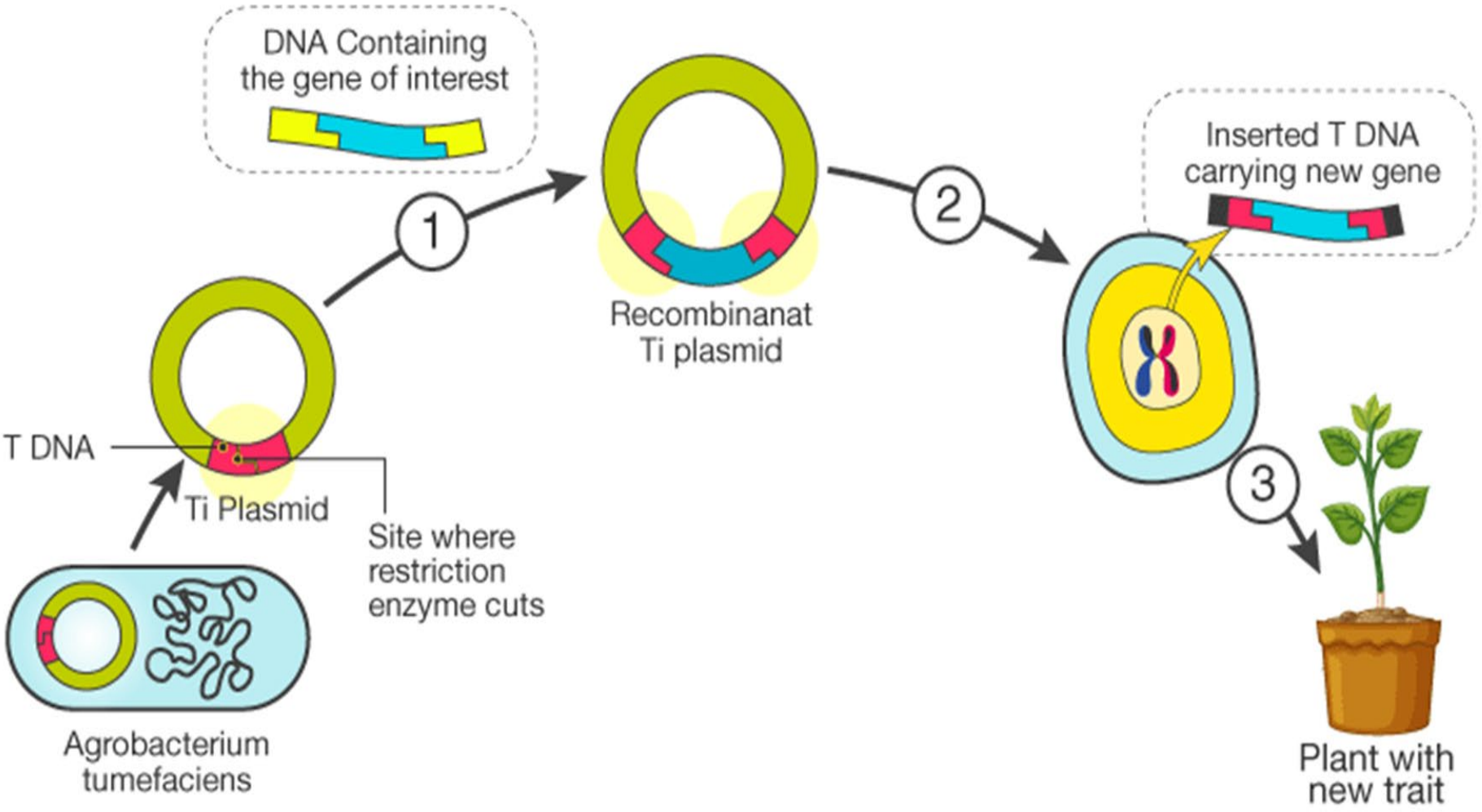
↓

Culture on the Nutrient agar medium containing antibiotics

↓

Isolation of living bacteria grow in culture midium

RECOMBINANT DNA TECHNOLOGY



1. Treat foreign DNA and plasmid with restriction enzyme and DNA ligase.
2. Introduce the recombinant plasmid into cultured plant cells.
3. Regenerate new plant from cultured cells.

Vectors

TYPES OF VECTORS

- If a vector is used for reproducing the DNA fragment, it is called a "**cloning vector**".
- If a vector is used for expressing certain gene in the DNA fragment, it is called an "**expression vector**".

Cloning vector

A vector used for transfer and multiplication of desired DNA in suitable host is called as cloning vector.

Plasmid: The extra chromosomal, self replicating, double stranded & circular DNA molecules of bacteria that carries the characters like sex factor, drug resistance, colicin factor etc. is called as Plasmid.

Cosmid are essentially plasmids that contain minimum of 250 bp of lambda DNA.

Phagemid: A plasmid vector that contains **origin of replication** from a phage, in addition to that of plasmid.

Phasmid: a gene-cloning vector consisting of an artificial combination of a plasmid with a phage such that its genome contains functional origins of replication of both; it may thus be propagated either as a plasmid or as a phage in appropriate host strains.

What is Bacteriophage?

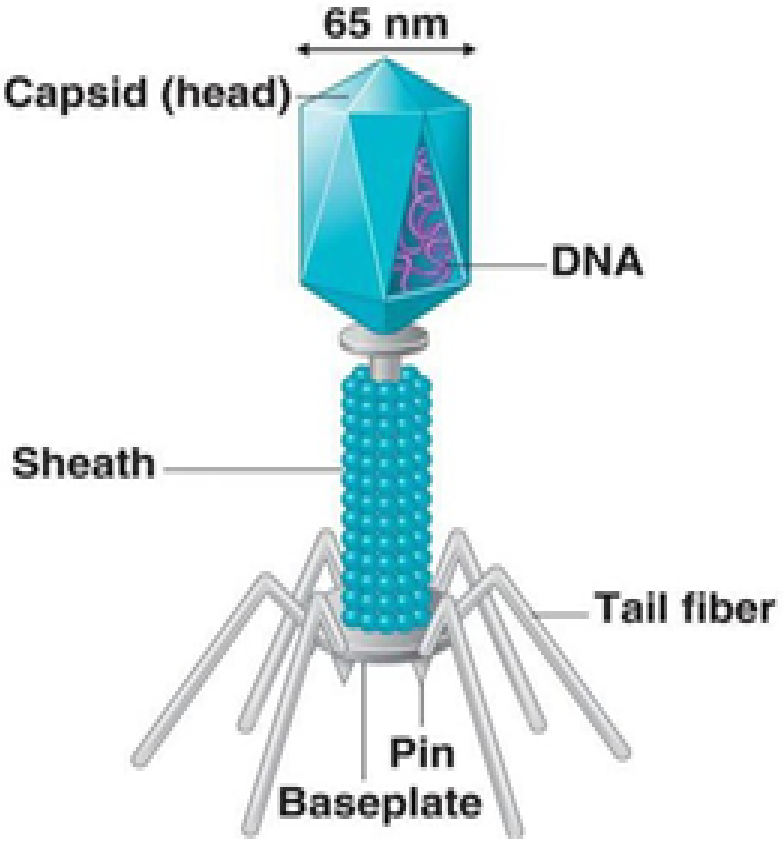
A virus that infects and replicates within bacteria.

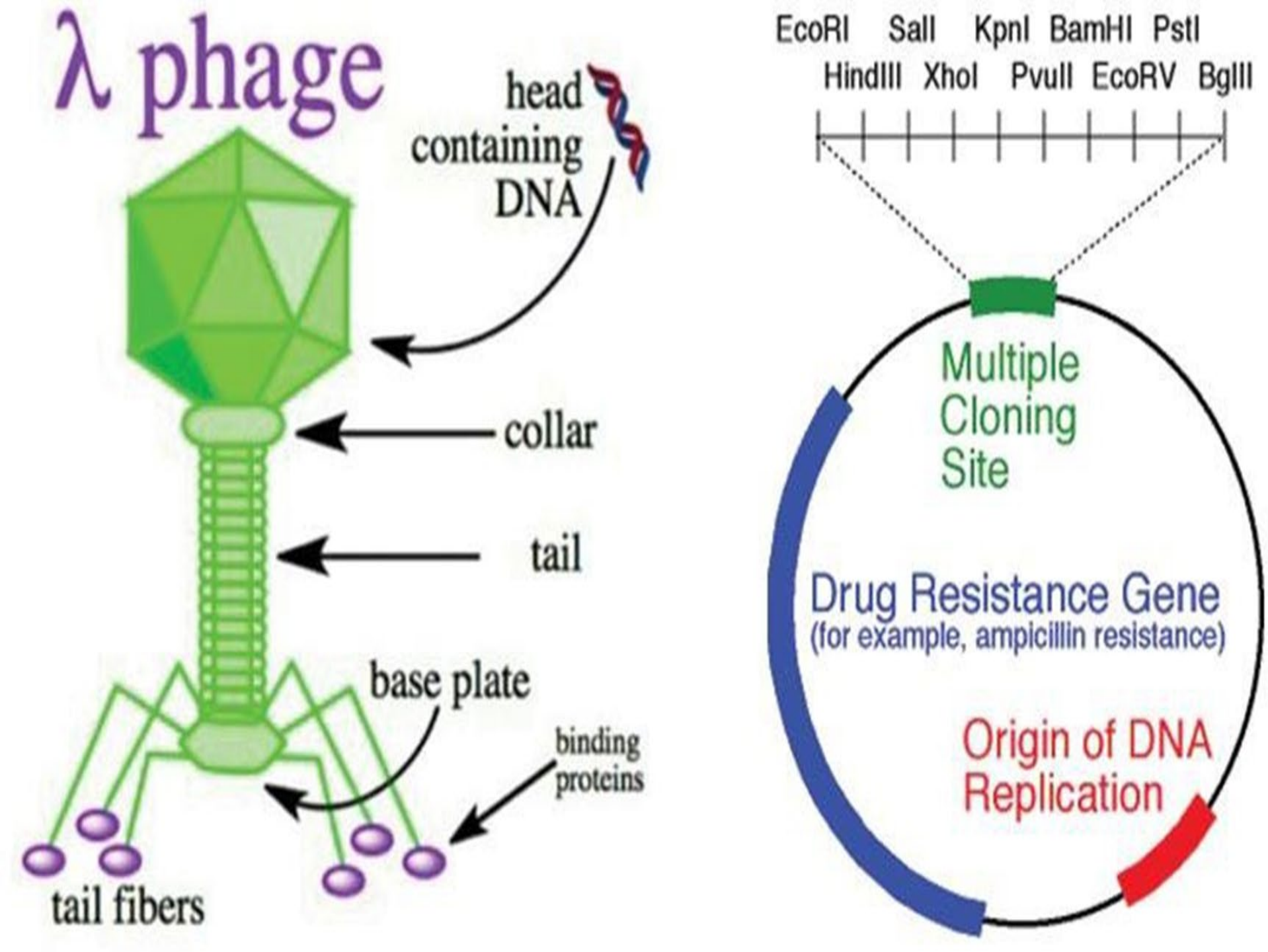
Phages.

One of the most common and diverse entities in biosphere.

Come in different shapes, but most with capsid and tail

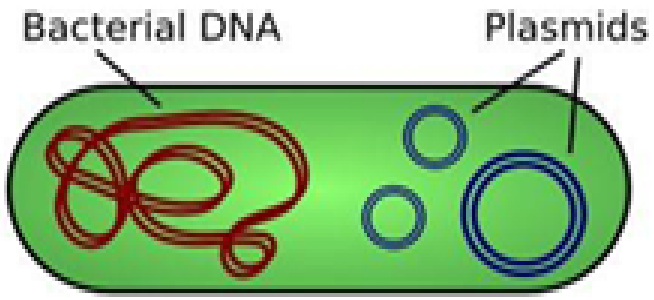
Caudovirales: 95%




λ phage
head containing DNA
collar
tail
base plate
binding proteins
tail fibers
EcoRI SalI KpnI BamHI PstI
HindIII XhoI PvuII EcoRV BglII
Multiple Cloning Site
Drug Resistance Gene
(for example, ampicillin resistance)
Origin of DNA Replication

What is a plasmid?

- A plasmid is a circular DNA molecule separate from the much larger bacterial chromosome.
- Every plasmid has an origin of replication, required for its replication within the cell.



pBR322

The nomenclature of plasmid cloning vectors

The name "pBR322" conforms with the standard rules for vector nomenclature:

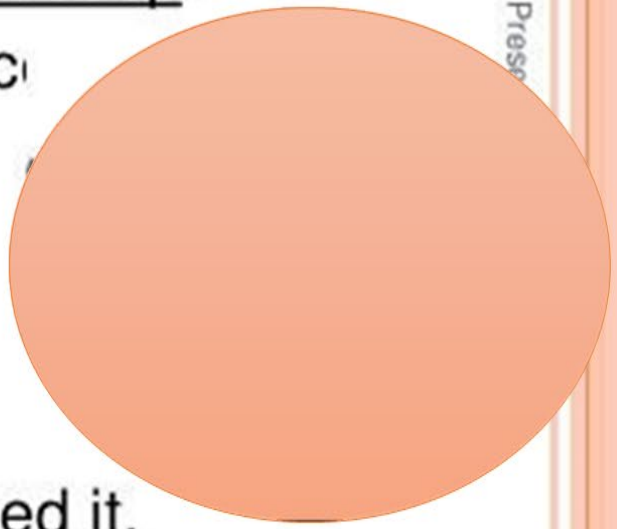
- "p" indicates that this is indeed a plasmid.

- "BR" identifies the laboratory in which the vector was originally constructed
(BR stands for Bolivar and Rodriguez, the two researchers who developed pBR322).

- "322" distinguishes this plasmid from others developed in the same laboratory
(there are also plasmids called pBR325, pBR327, pBR328, etc.).

pBR322:

- This was the first widely used plasmid vector .
- pBR322 has a relatively small size of 4.363bp.
- Also this vector has a reasonably high c
  (~15 copies per cell).



Nomenclature of pBR322:

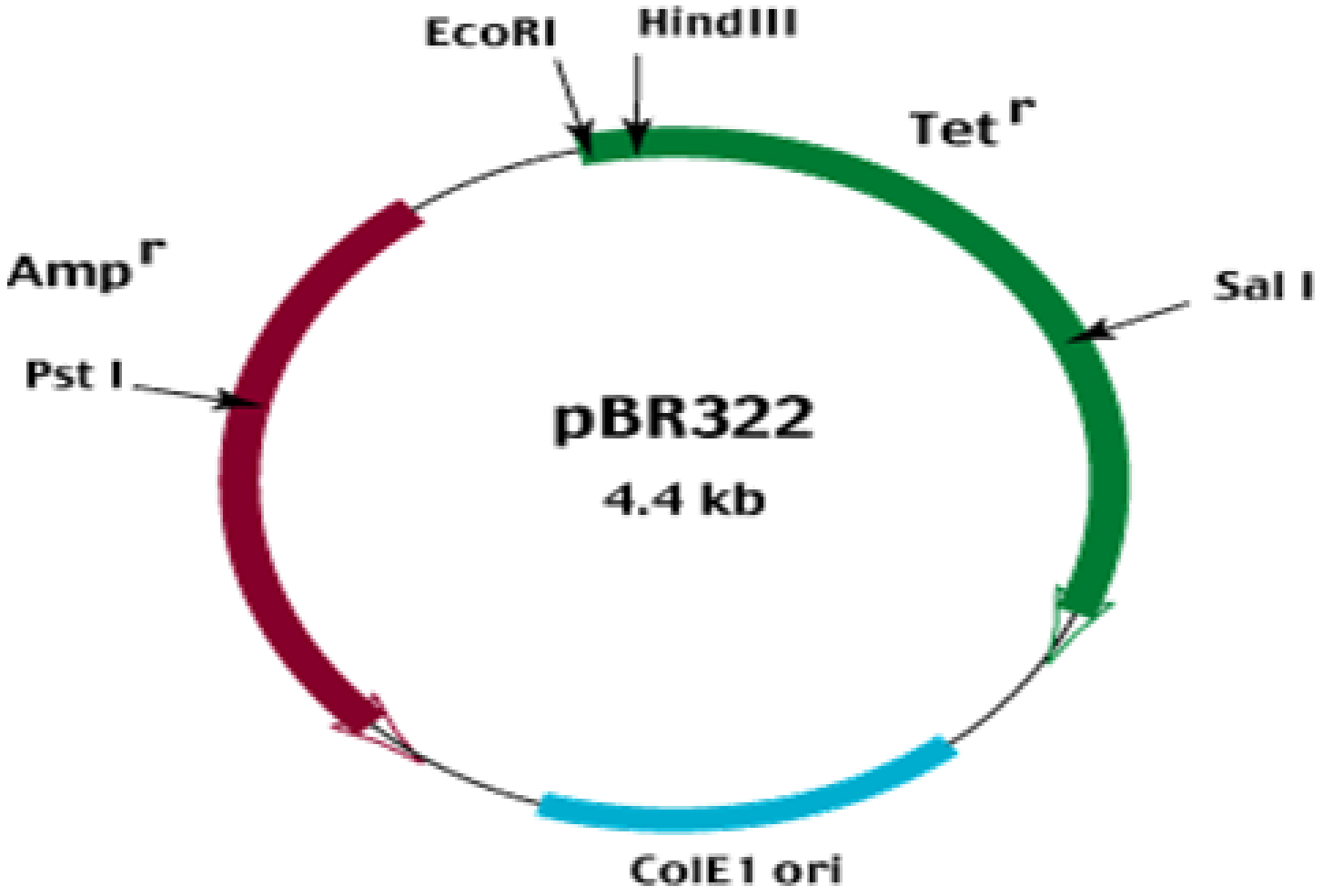
p- Plasmid

BR- Boliver and Rodriguez-

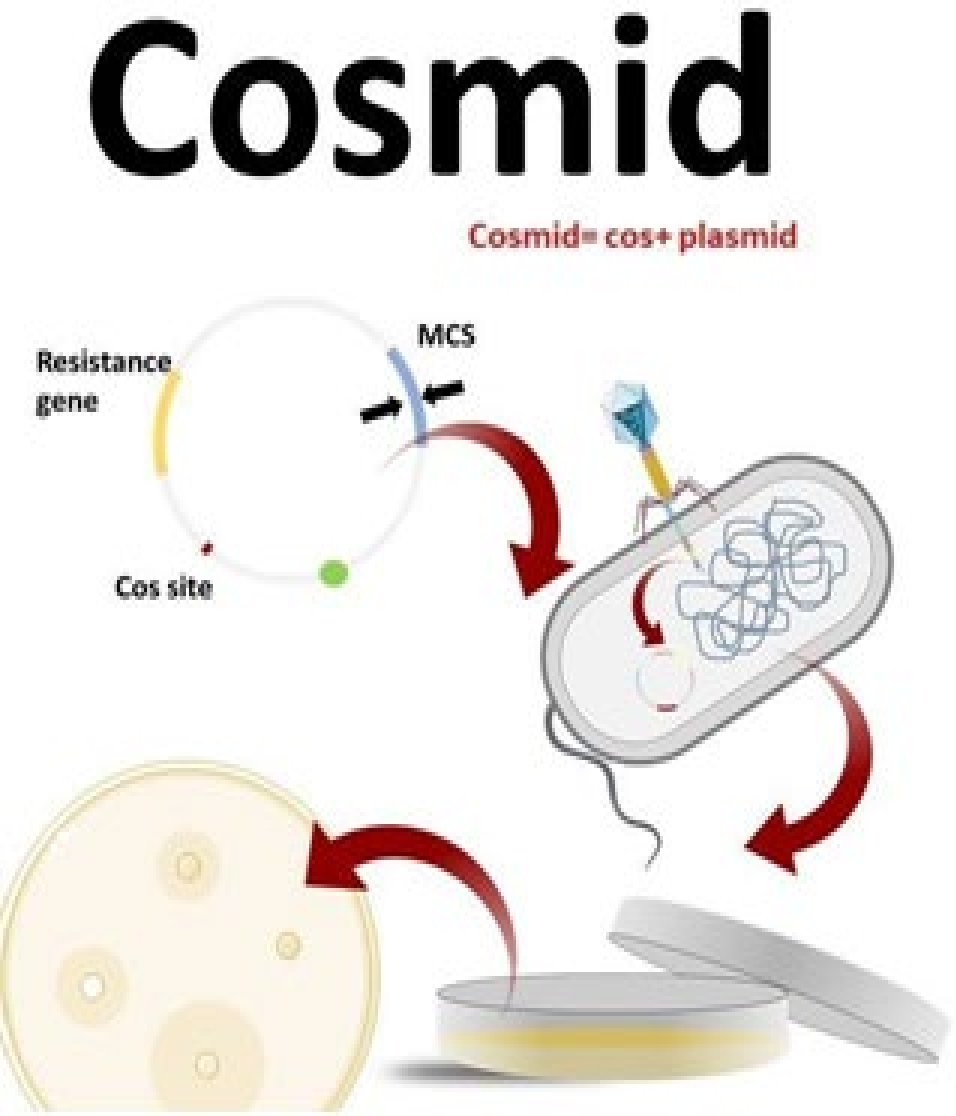
The two researchers who developed it.

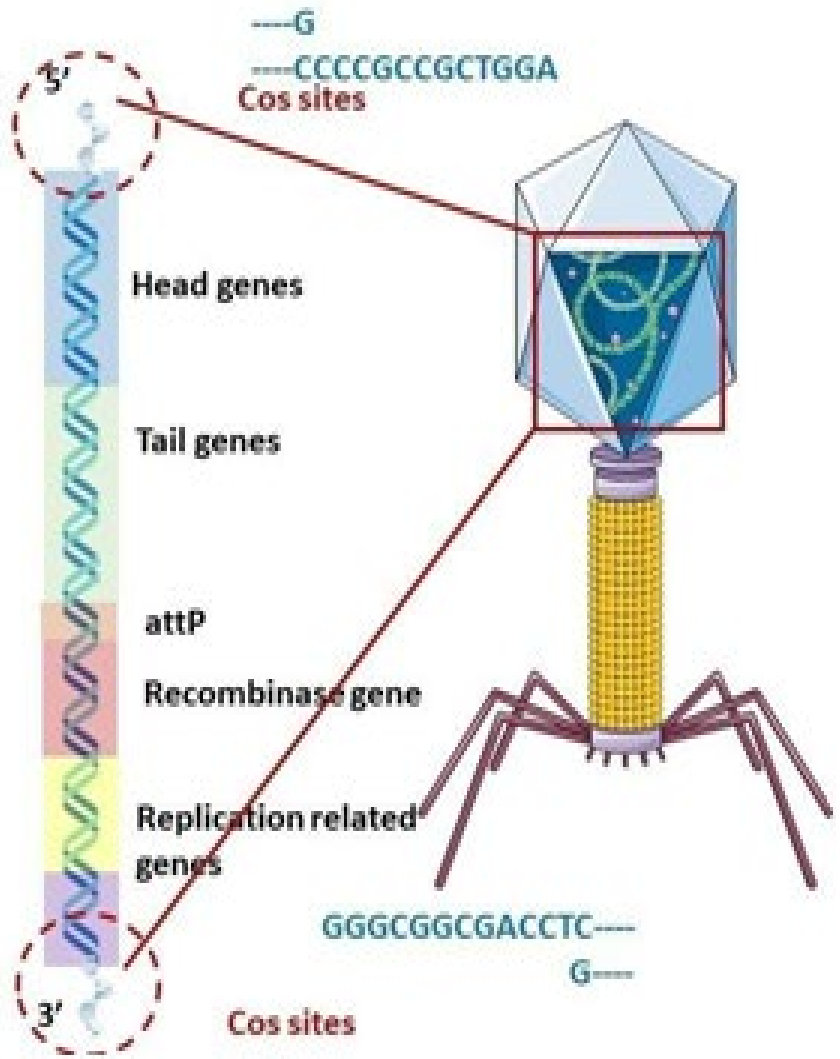
322- No. of developed in the same laboratory.

2- plasmids ex: pBR322 plasmid



3- Cosmids


Cosmid
Cosmid= cos+ plasmid
Resistance gene
MCS
Cos site


----G
----CCCCGCCGCTGGA
Cos sites
5'
Head genes
Tail genes
attP
Recombinase gene
Replication related genes
GGGCGGCGACCTC----
G----
3'
Cos sites

In Brief

- A cosmid is a plasmid that contain phage sequence that allows the vector to be packaged and transmitted to bacteria like phage vector.

Or

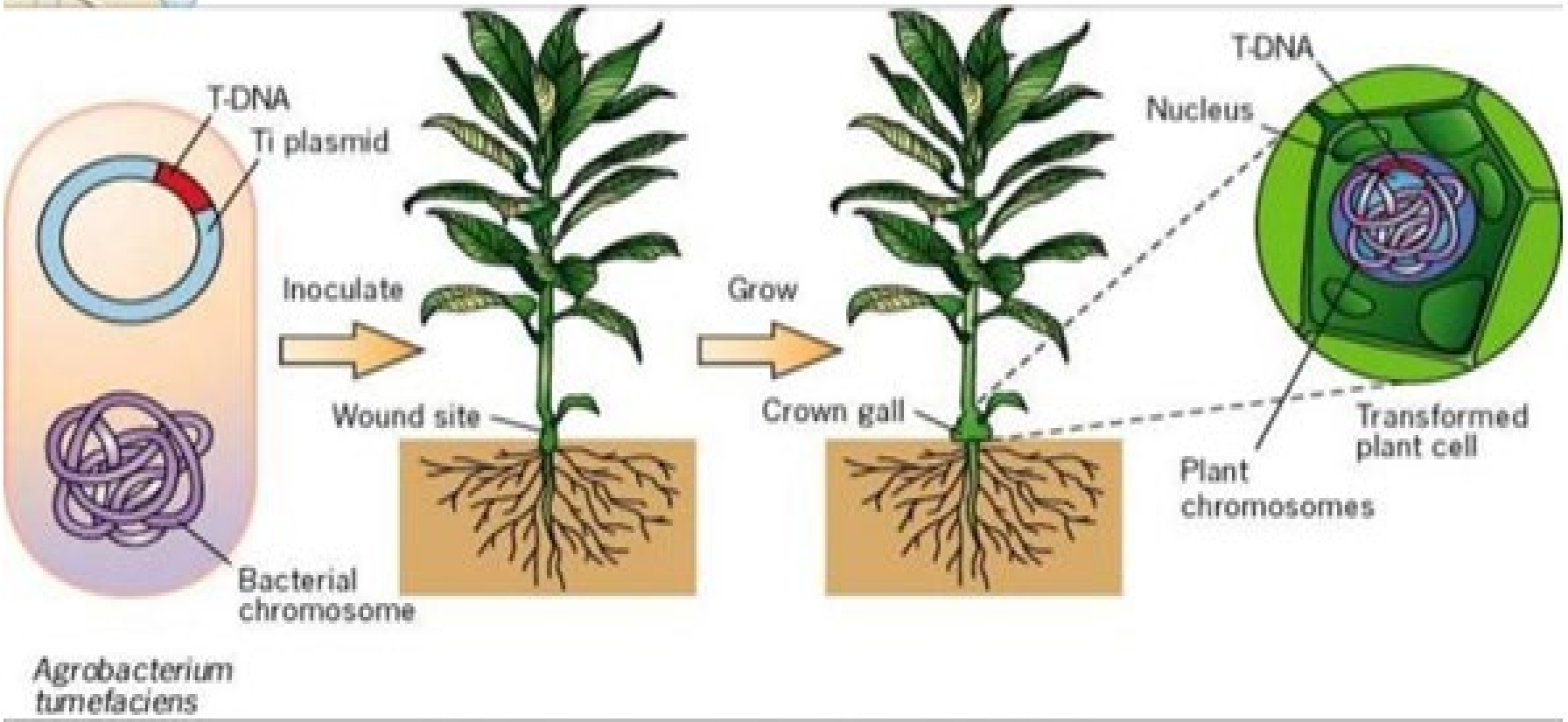
- A cosmid is a type of hybrid plasmid that contains a Lambda phage cos sequence. Cosmids (cos sites + plasmid = cosmids) DNA sequences are originally from the lambda phage.

Agrobacterium Tumefaciens Mediated Gene Transfer

- Agrobacterium tumefaciens, a soil bacterium known as‘nature’s own genetic engineer
- It has the natural ability to genetically engineer plants.
- It carries genetic information from bacteria to plant cell.
- genetic transformation of plant cells with a piece of DNA is called T-DNA
- It causes crown gall disease
- It can be use as a vehicle to transfer genes in plants.

Crown gall disease

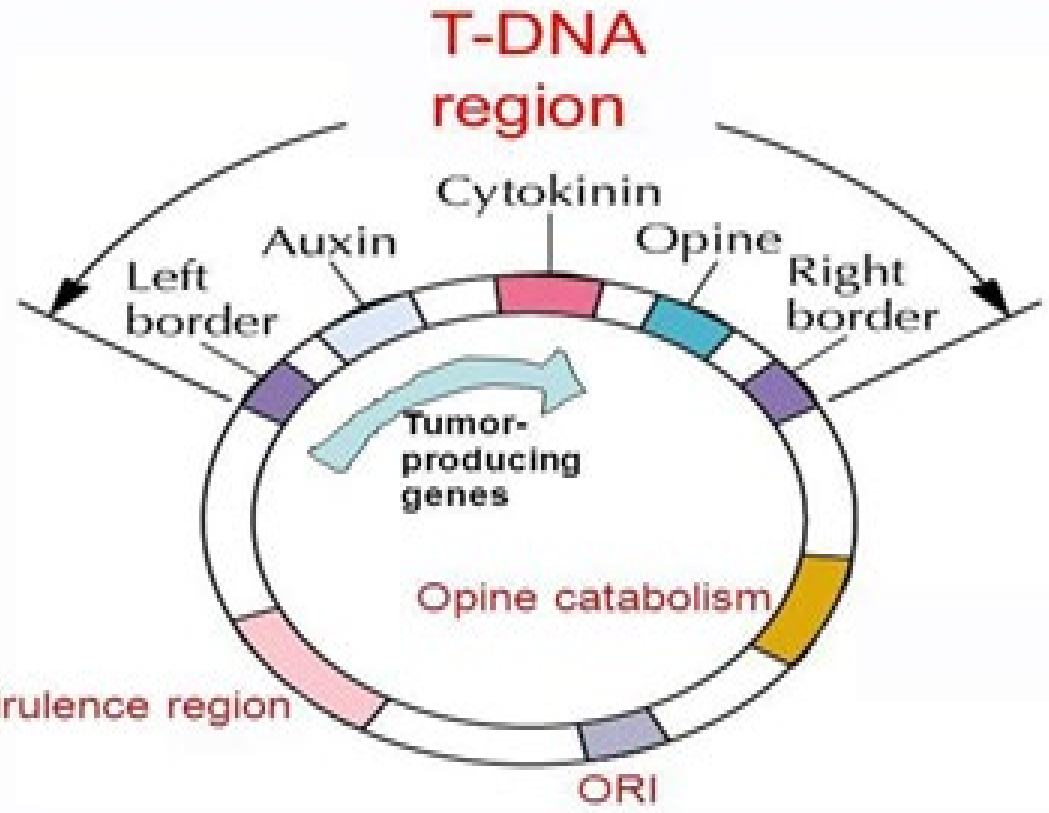
Agrobacterium mediated transformation, its mode of action and applications in crop improvement



Ti plasmid

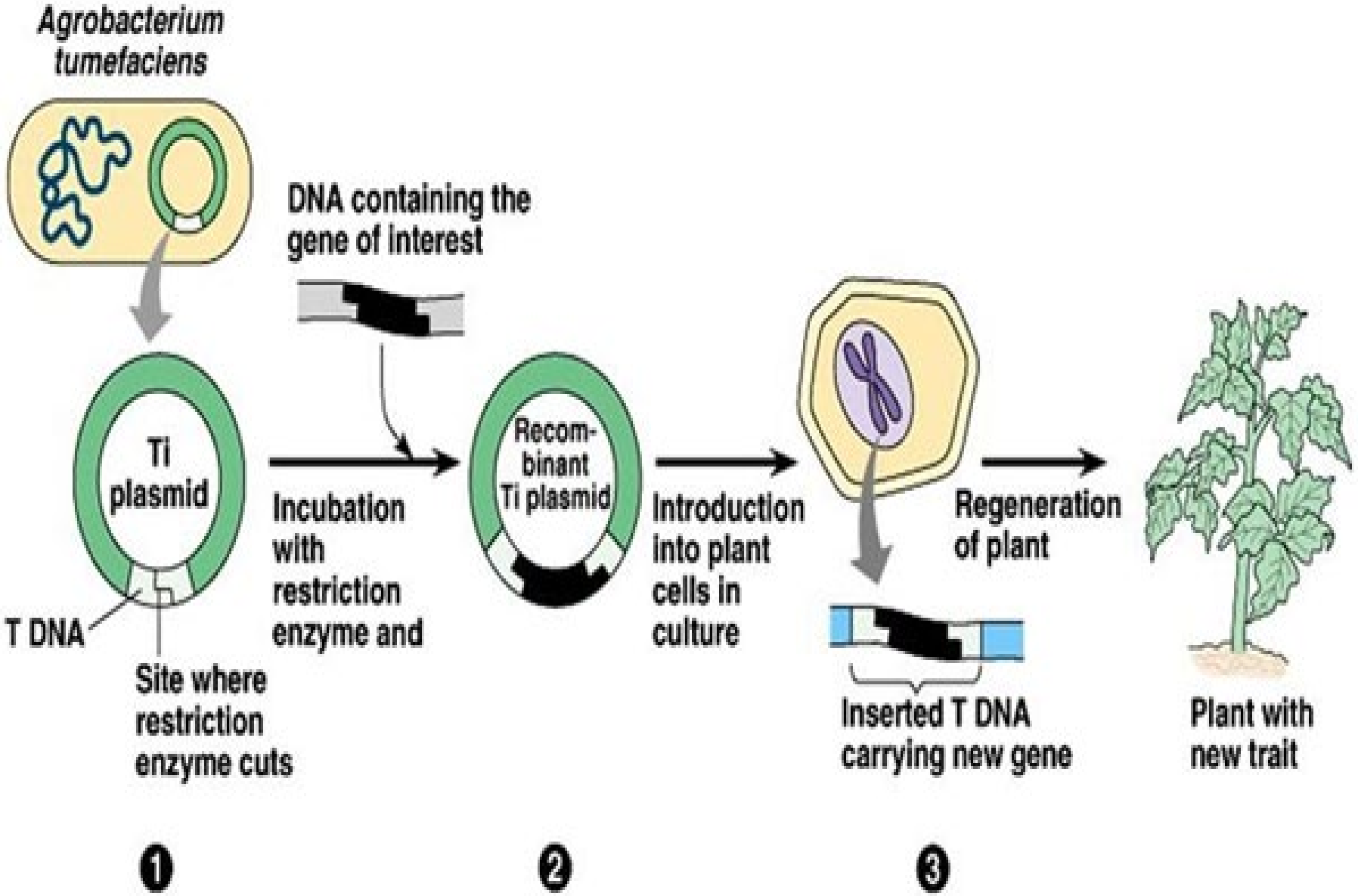
- A central role in crown gall formation and it is the portion of Ti plasmid that is integrated into the host genome.
  - Ti plasmid is responsible for the tumorous phenotype.
  - Ti plasmid features
    - contain T-DNA regions
    - contain a vir region
    - contain an origin of replication
    - contain genes for the catabolism of opines (a class of amino acid conjugates)

Ti Plasmid



DNA between L and R borders is transferred to plant as ssDNA;

T-DNA encoded genes can be substituted by target genes



THANK YOU