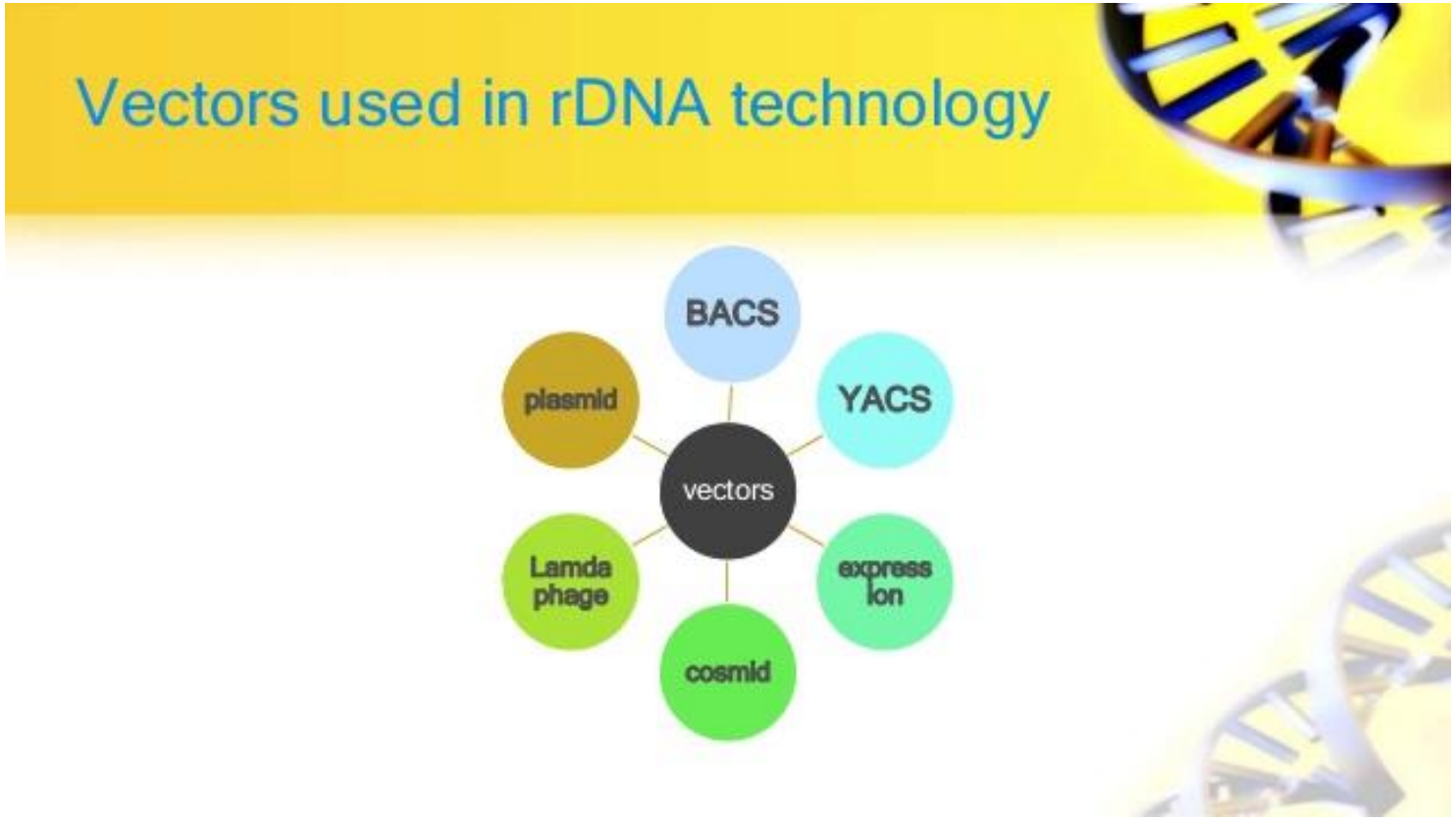
# VECTOR in RDT




Sayanti Kar

# Vectors:

Vectors can be defined in three ways:

- **In epidemiology:**

An organism or vehicle that transmits the causative agent or disease-causing organism from the reservoir to the host.

- **In molecular biology:**

A vehicle (e.g. a plasmid) used to transfer the genetic material such as DNA sequences from the donor organism to the target cell of the recipient organism.

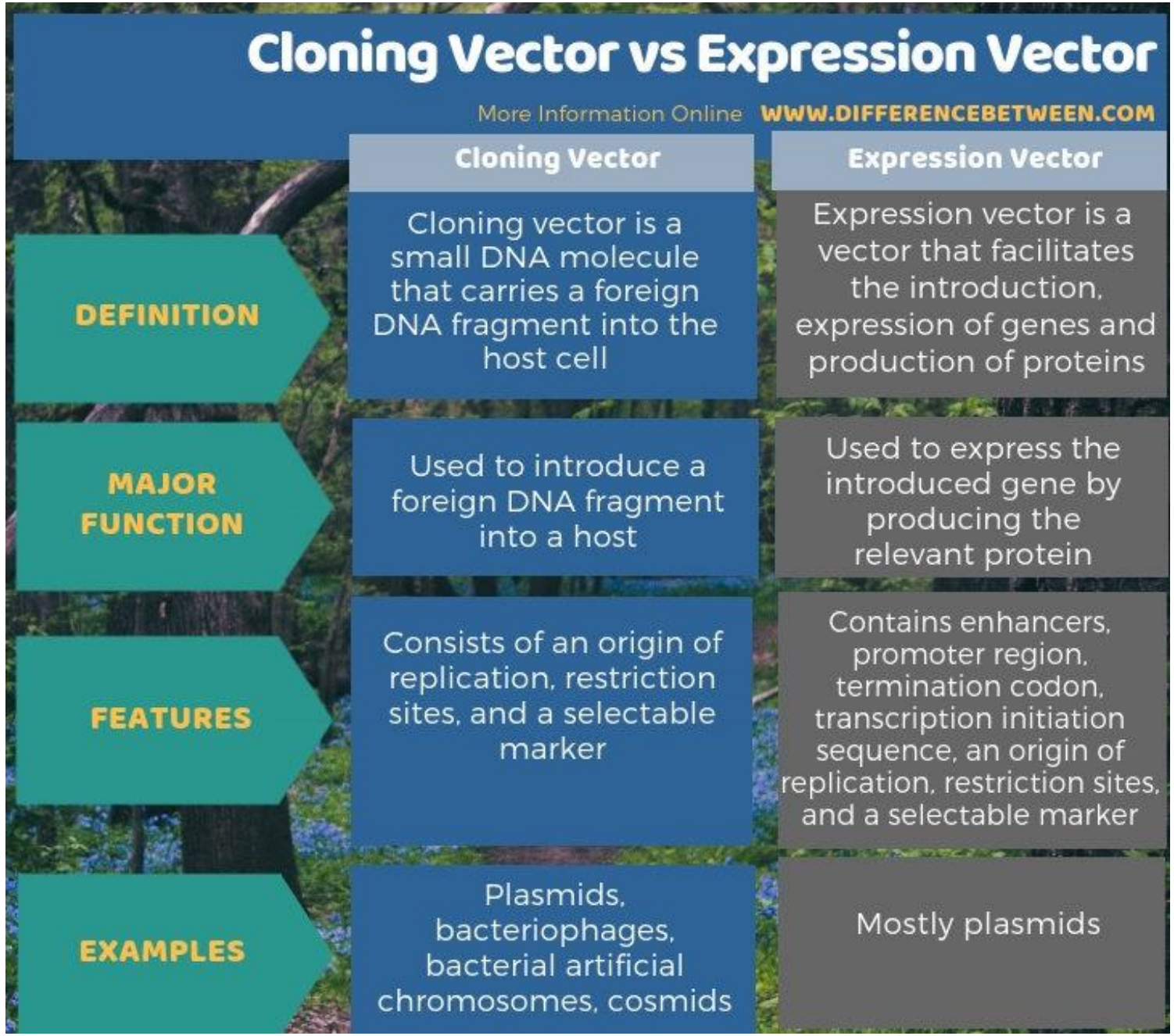
- **In biology:**

A biotic agent that disperses reproductive structures of another organism, as a bee transmitting pollen to the stigma of a flower.

# Cloning…

- is the process of producing genetically identical individuals of an organism either naturally or artificially. In nature, many organisms produce **clones** through asexual reproduction.

- **Cloning vectors** are used to clone foreign DNA whereas **Expression Vectors** are Engineered so that any foreign DNA can be transcribed in RNA and translated into protein.


Cloning Vector vs Expression Vector
More Information Online
WWW.DIFFERENCEBETWEEN.COM
Cloning Vector
Expression Vector
DEFINITION
Cloning vector is a small DNA molecule that carries a foreign DNA fragment into the host cell
Expression vector is a vector that facilitates the introduction, expression of genes and production of proteins
MAJOR FUNCTION
Used to introduce a foreign DNA fragment into a host
Used to express the introduced gene by producing the relevant protein
FEATURES
Consists of an origin of replication, restriction sites, and a selectable marker
Contains enhancers, promoter region, termination codon, transcription initiation sequence, an origin of replication, restriction sites, and a selectable marker
EXAMPLES
Plasmids, bacteriophages, bacterial artificial chromosomes, cosmids
Mostly plasmids

# The Important Features of a Cloning Vector are as follows….

- The ability to Replicate in host cells
- Unique restriction enzyme sites for insertional cloning (MCS)
- Genetic Marker to select for the host cells containing the vector (SELECTABLE MARKER)
- Low molecular weight

# Vectors for *E.coli*

- Plasmids are usually occurring circular, extrachromosomal, autonomously replicating DNA, present in many prokaryotic and a few eukaryotic organism.

- Plasmids range in size 1kb to over 300kb

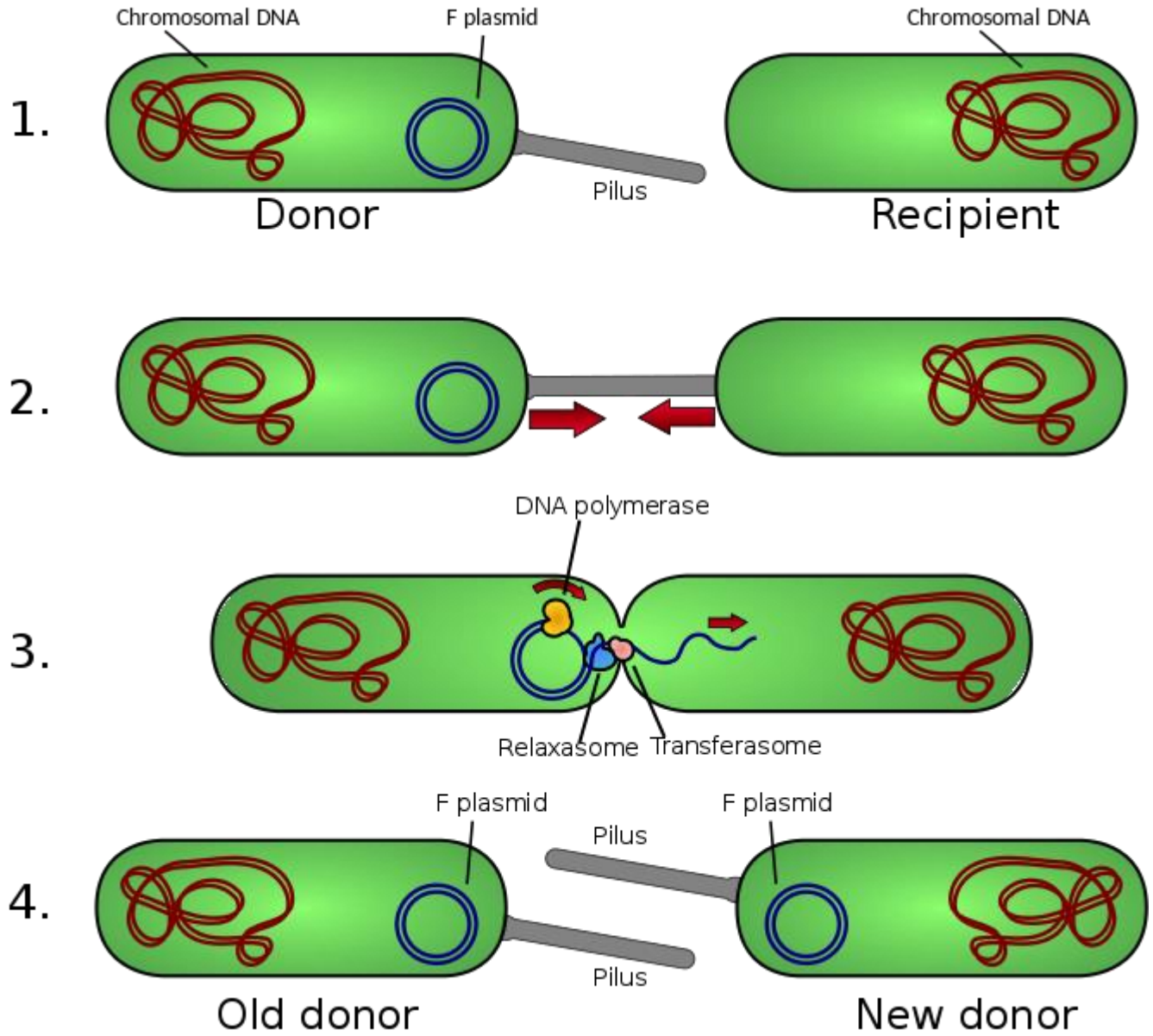
- **Relaxed Plasmids** are maintained at multiple copies per cell, while **Stringent Plasmids** are present in a single copy

# General Types of Plasmids

- Conjugative and Non-Conjugative

Conjugative plasmids contain a set of transfer or *tra* genes which promote sexual conjugation between different cells.

Non-conjugative plasmids are incapable of initiating conjugation, hence they can be transferred only with the assistance of conjugative plasmids


1.
Chromosomal DNA
F plasmid
Chromosomal DNA
Pilus
Donor
Recipient
2.
3.
DNA polymerase
Relaxasome
Transferasome
4.
F plasmid
Pilus
F plasmid
Pilus
Old donor
New donor

# Incompatibility

- A microbe can harbour different types of plasmids, but different plasmids can only exist in a single bacterial cell if they are compatible.

- If two plasmids are not compatible, one or the other will be rapidly lost from the cell.

Plasmids are incompatible if they have the same reproduction strategy in the cell; this allows the plasmids to inhabit a certain territory within it without other plasmids interfering.

# Types of Plasmids.

- There are five main **types of plasmids**:

- fertility F-**plasmids:** which contain *tra* genes. They are capable of conjugation and result in the expression of sex pili. Bacteria that have the F-plasmid are known as F positive ($F^+$), and bacteria without it are F negative ($F^-$).

- resistance **plasmids:** which contain genes that provide resistance against antibiotics or poisons. veruse of antibiotics to treat other infections, like urinary tract infections, may lead to the proliferation of drug-resistant strains.

**virulence plasmids:** which turn the bacterium into a pathogen, which is an agent of disease. *E. coli* is found naturally in the human gut and in other animals, but certain strains of *E. coli* can cause severe diarrhea and vomiting.

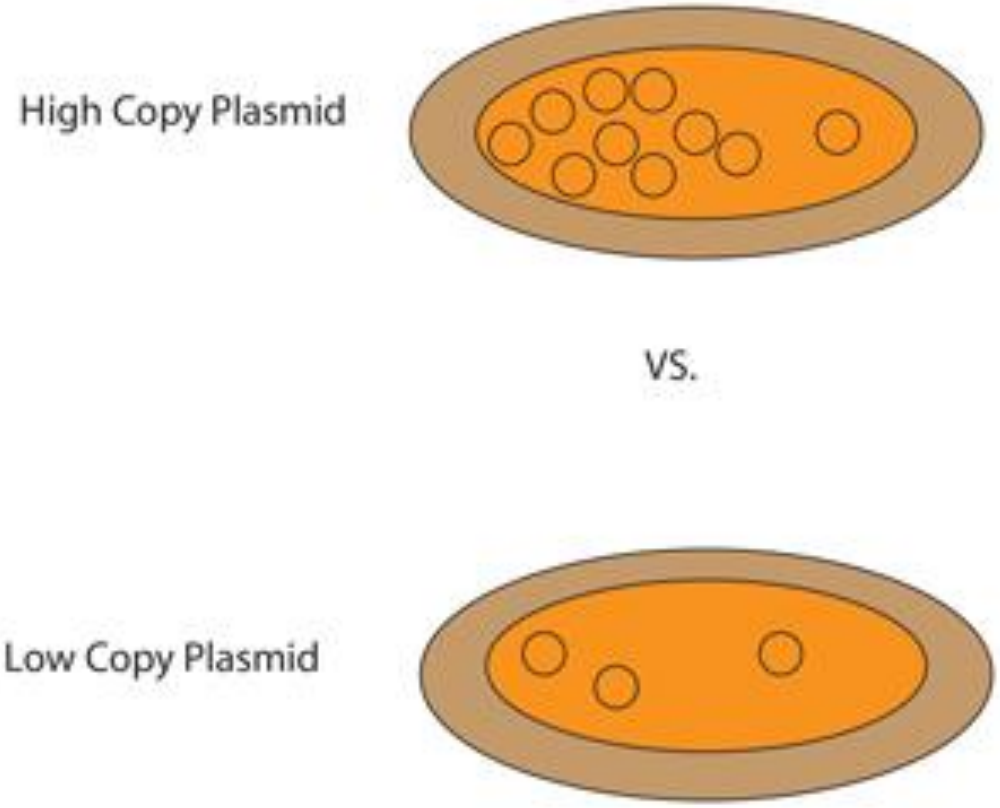
**degradative plasmids:** which enable the digestion of unusual substances, e.g. toluene and salicylic acid. the host bacterium to digest compounds that are not commonly found in nature, such as camphor, xylene, toluene, and salicylic acid. These plasmids contain genes for special enzymes that break down specific compounds. Degradative plasmids are conjugative.

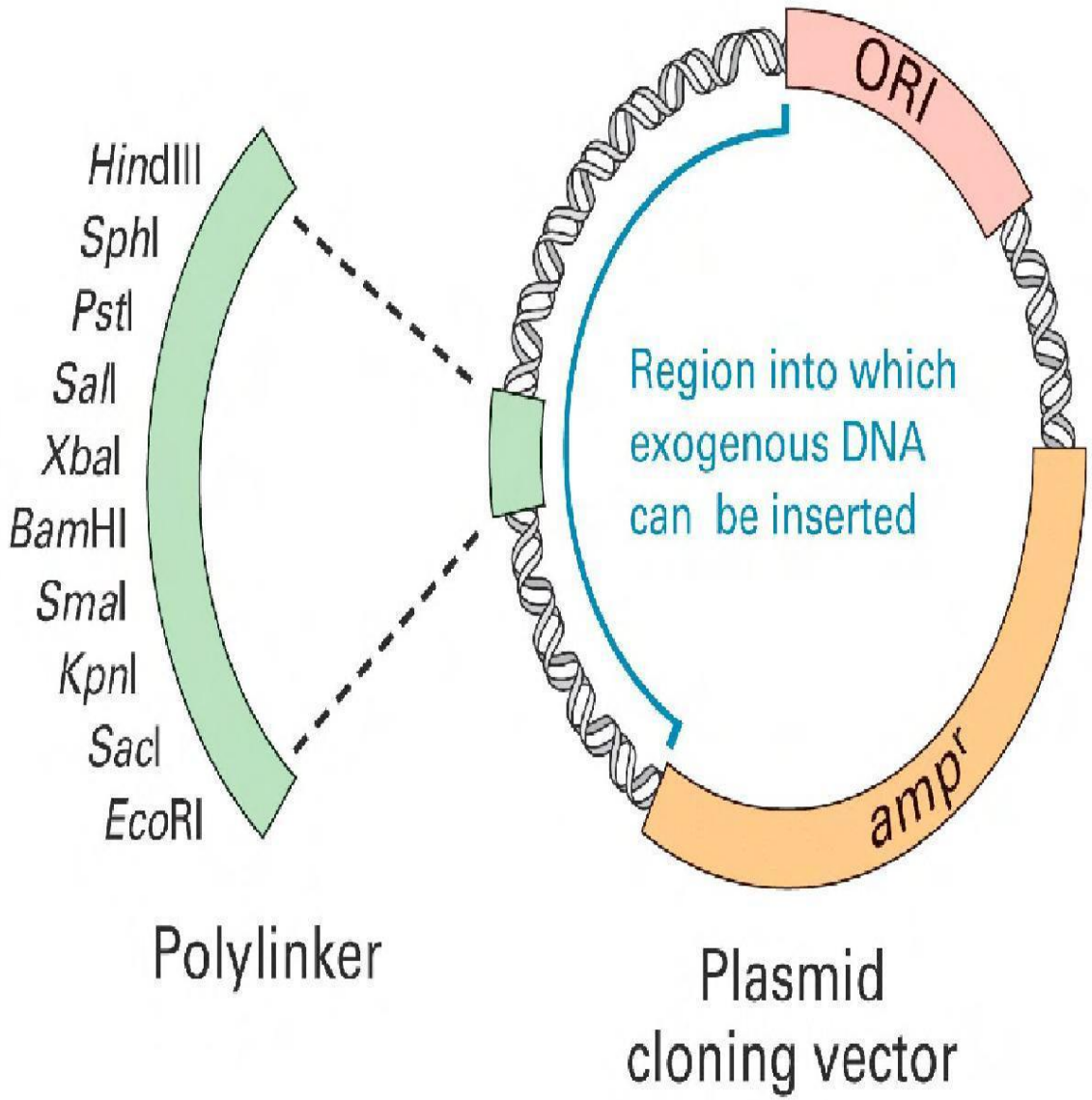
**Col plasmids:** which contain genes that code for bacteriocins (colicins), proteins that can kill other bacteria. which are proteins that kill other bacteria and thus defend the host bacterium. Bacteriocins are found in many types of bacteria including *E. coli*, which gets them from the plasmid ColE1.

# Plasmid Copy No.

- An average or expected number of values of **Plasmid** DNA per host cells

The copy number influences the plasmid stability, i.e. the maintenance of the plasmid within the cells during cell division. A positive effect of a high copy number is the greater stability of the plasmid when the random partitioning occurs at cell division.




HindIII
SphI
PstI
SalI
XbaI
BamHI
SmaI
KpnI
SacI
EcoRI
Polylinker
ORI
Region into which
exogenous DNA
can be inserted
ampr
Plasmid
cloning vector

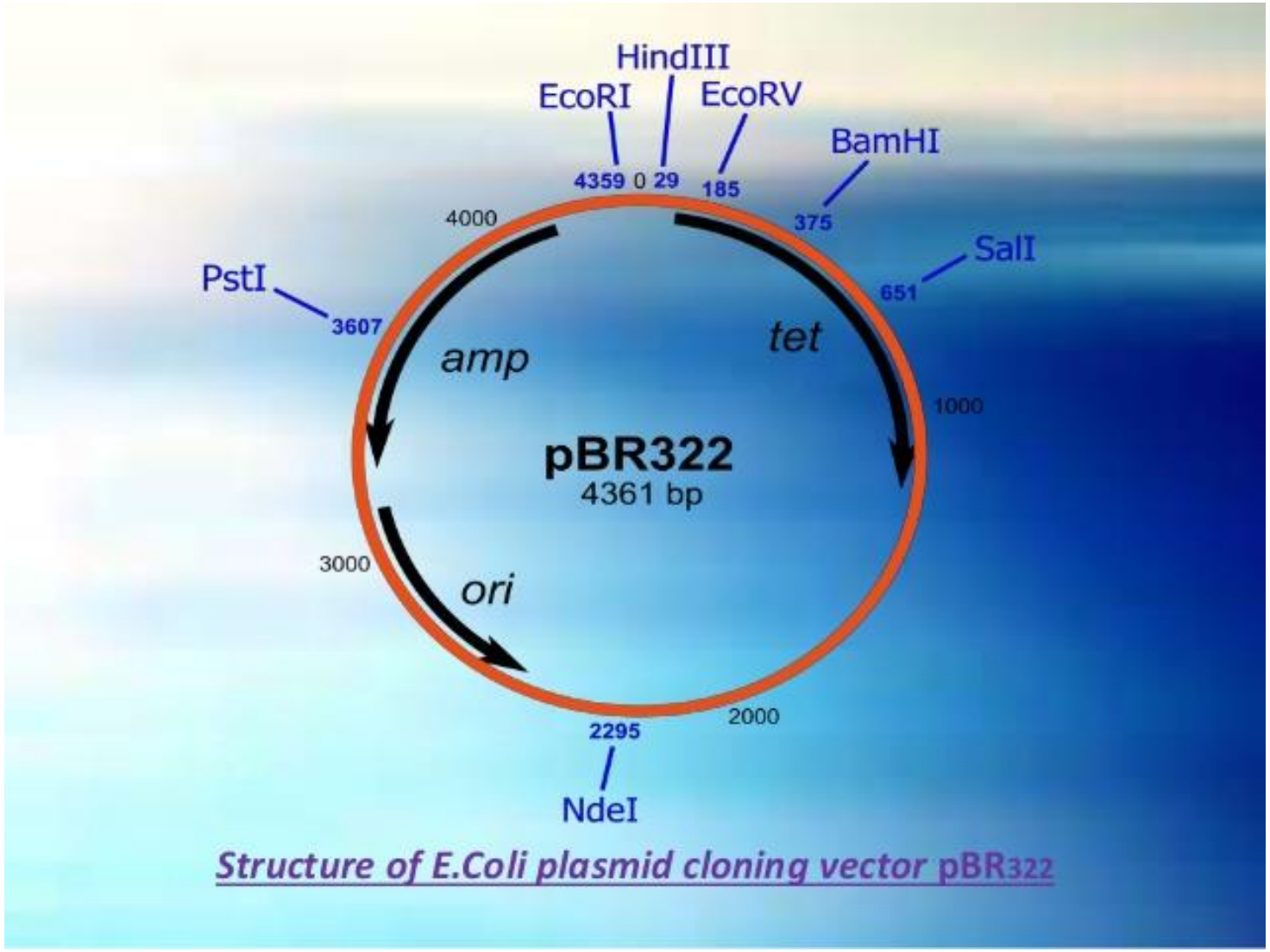
# AGENTS USED AS VECTORS

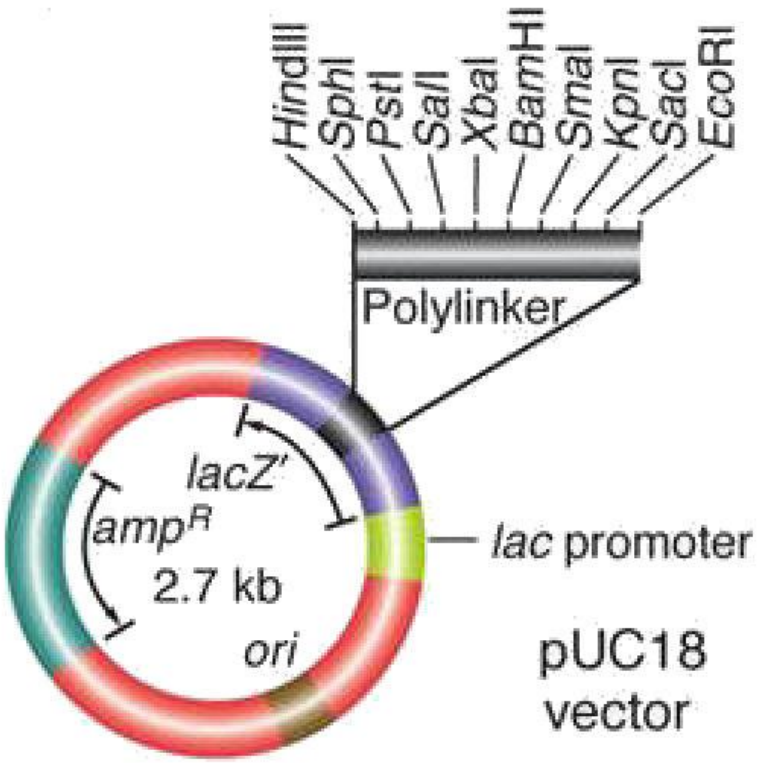
- PLASMIDS
- BACTERIOPHAGES
- COSMID
- ARTIFICIAL CHROMOSOME VECTORS

In 1973, Cohen described first successful construction of recombinant vector.

Plasmid PSC101 - Ecoli

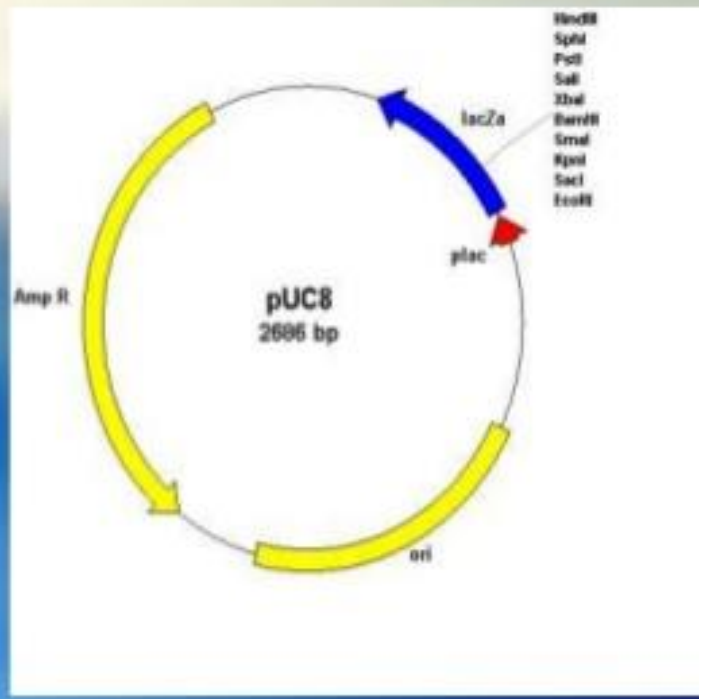
# CLONING VECTORS BASED ON PLASMID DNA






A **multiple cloning site** (**MCS**), also called a **polylinker**, is a short segment of DNA which contains many (up to ~20) restriction sites - a standard feature of engineered plasmids.

# $pUC_8$



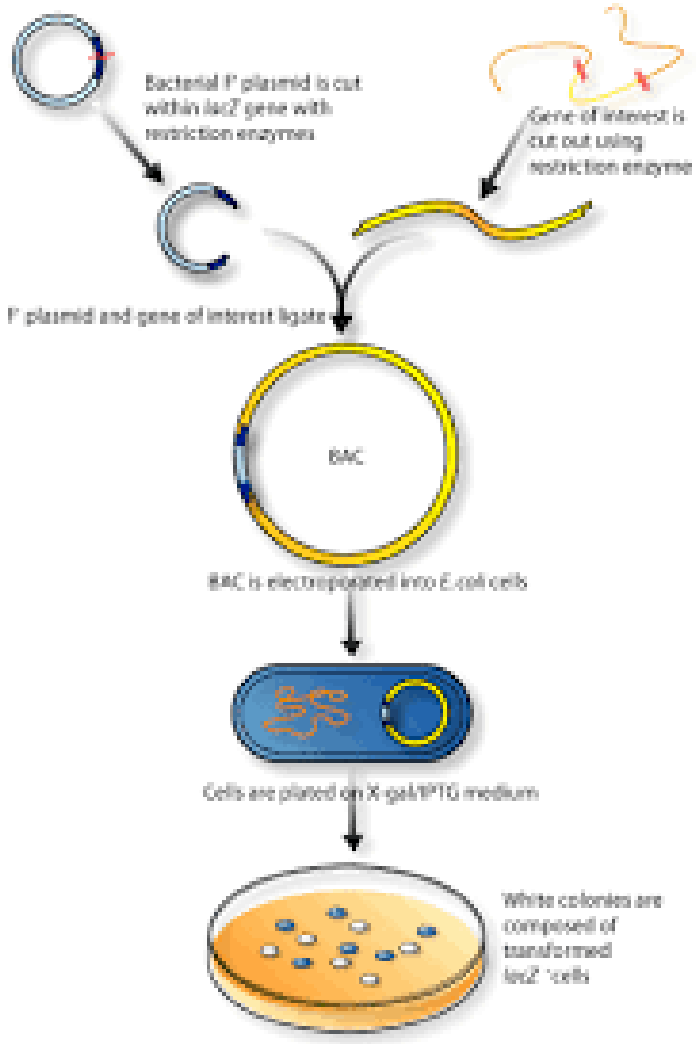

- ✓ Popular Ecoli cloning vector.
- ✓ Derivative of pBR322.
- ✓ Two parts derived:-
  - ➢ Ampicillin resistance gene.
  - ➢ ColEI – origin of replication.
- ✓ 2700 base pairs.
- ✓ lac Z gene derived from Ecoli.
- ✓ Polylinker sequence having unique restriction sites

lies in lac region.

# BAC (Bacterial Artificial Chromosome)

- A bacterial artificial chromosome (BAC) is an engineered DNA molecule used to clone DNA sequences in bacterial cells (for example, E. coli).
- BACs are often used in connection with DNA sequencing.
- Segments of an organism's DNA, ranging from 100,000 to about 300,000 base pairs, can be inserted into BACs. The BACs, with their inserted DNA, are then taken up by bacterial cells. As the bacterial cells grow and divide, they amplify the BAC DNA, which can then be isolated and used in sequencing DNA.

A **bacterial artificial chromosome** (**BAC**) is a DNA construct, based on a functional fertility plasmid (or F-plasmid), used for transforming and cloning in **bacteria**, usually E. coli.




A large piece of DNA can be engineered in a fashion that allows it be propagated as a circular artificial chromosome in bacteria--so-called bacterial artificial chromosome, or BAC

# Cloning Vectors Based on Viral DNA

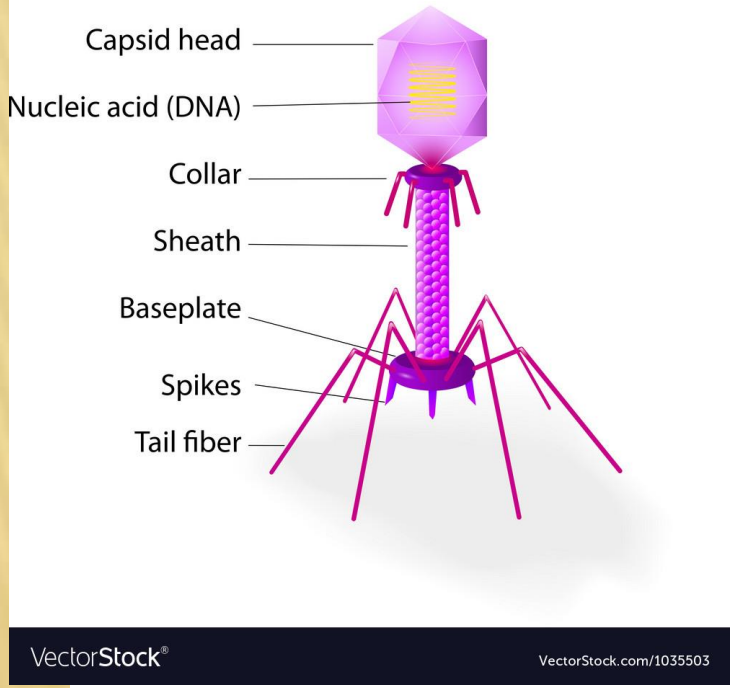
- Viral vectors are those in which the gene or genes of interest are incorporated into the genome of a virus.

- Virus infect cells with high efficiency, the cloned gene can be introduced into the cells at a significantly higher frequency than by simple transformation.

# BACTERIOPHAGE

- Virus that infect bacteria is known as bacteriophage.
- It was discovered by Frederick.W.Twort in Great Britian (1915) and Felix d' Herelle in France(1917).
- D' Herelle coined the term bacteriophage meaning 'bacterial eater' to describe the agent's bacteriocidal activity.

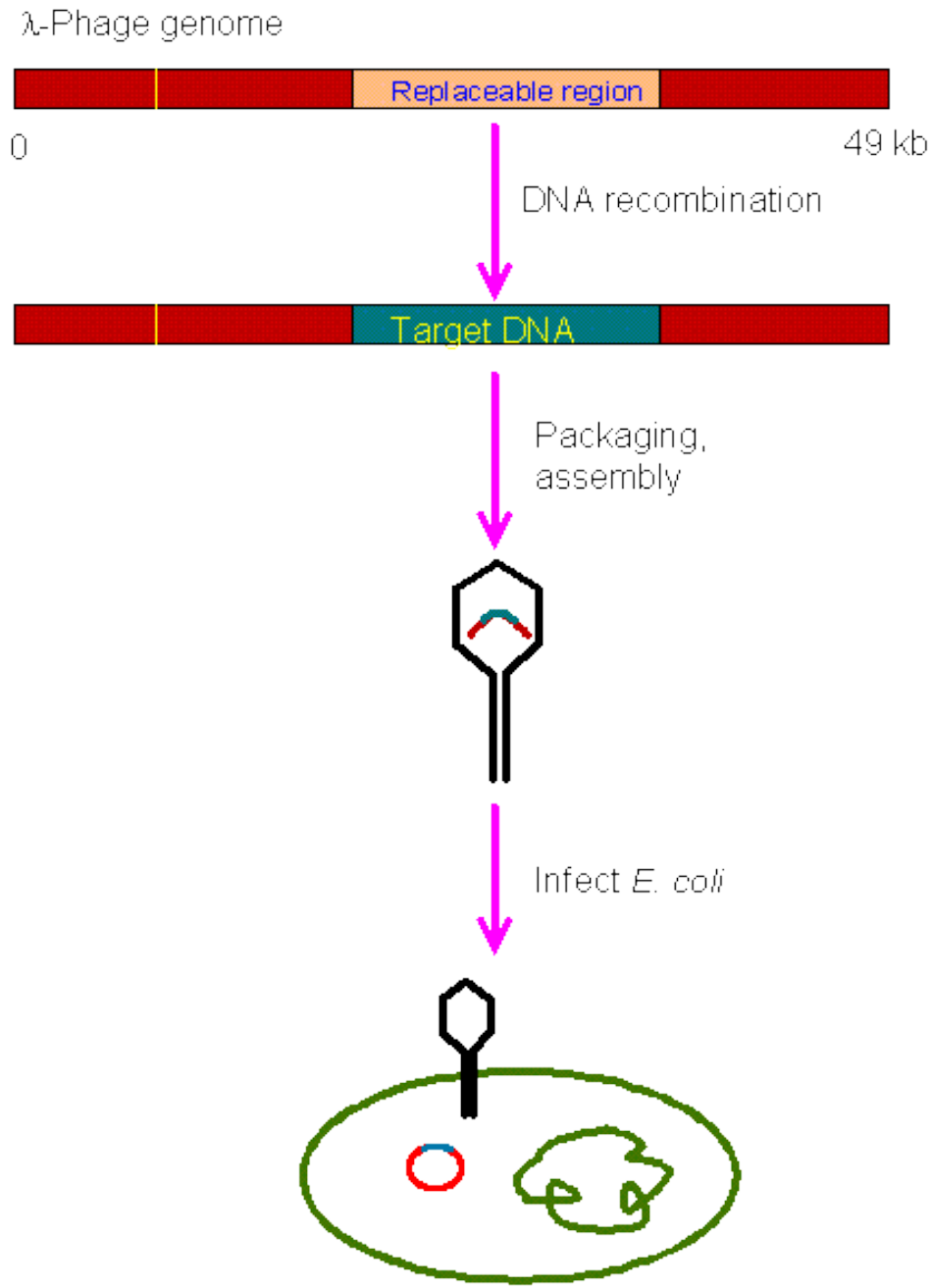
**Structure of bacteriophage**



- Phages are very simple in structure, consisting merely of a **DNA** (or occasionally ribonucleic acid (RNA)) molecule carrying a number of genes,surrounded by a protective coat or **capsid** made up of protein molecules.
- They can undergo *two life cycle*

  **Lytic cycle**

  **Lysogenic cycle**


λ-Phage genome
Replaceable region
0
49 kb
DNA recombination
Target DNA
Packaging,
assembly
Infect E. coli

# WHY BACTERIOPHAGE AS A VECTOR?

- It can accept **very large pieces of foreign DNA.**
- Genetic engineers have constructed numerous derivatives of phage vectors that contain only one or two sites for a variety of **restriction enzymes.**
- Phage that have a *stuffer fragment* are called **substitution vectors** because they are designed to have a piece removed and substituted with something else.
- Examples are Lambda phage, M13 phage, T4,T7 phage, P1 phage etc.

# COSMID

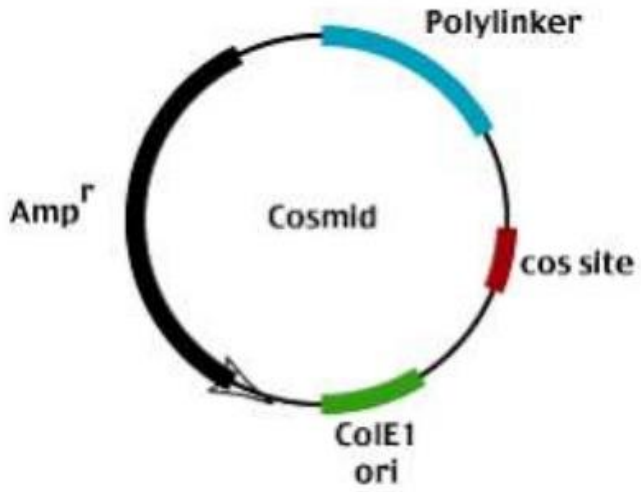
## In Brief

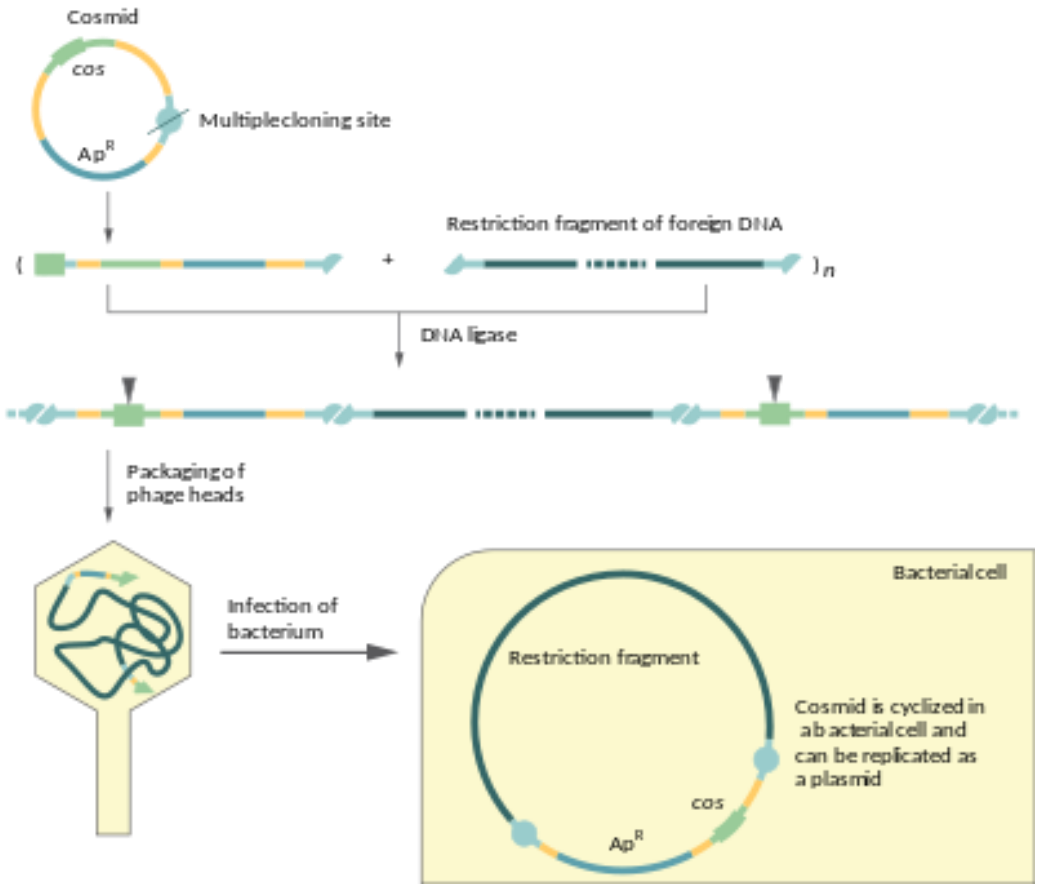
- A cosmid is a plasmid that contain phage sequence that allows the vector to be packaged and transmitted to bacteria like phage vector.

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.

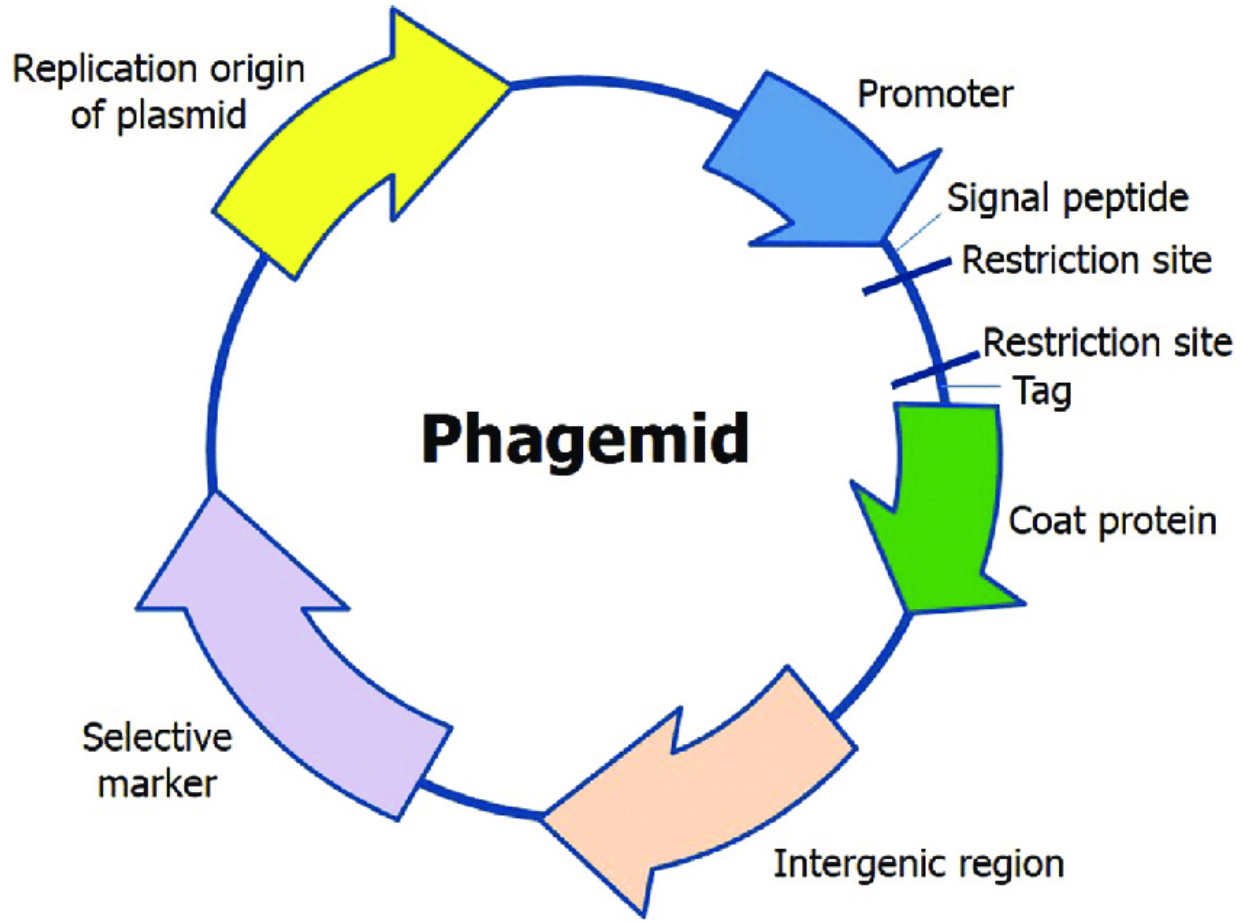
**Cos sequences are ~200 base pairs long and essential for packaging.**




Cosmids are predominantly plasmids with a bacterial *oriV*, an antibiotic selection marker and a cloning site, but they carry one, or more recently two, cos sites derived from bacteriophage lambda.

A **phagemid** or **phasmid** is a DNA-based cloning vector, which has both bacteriophage and plasmid properties. These vectors carry, in addition to the origin of plasmid replication, an origin of replication derived from bacteriophage



Cosmid and Phagemid are cloning vectors used in recombinant DNA technology. Cosmids are hybrid vectors that contain special sticky ends known as cos sites. *in-vitro* packaging require these cos sites. While phagemids are plasmids that contain an f1 origin of replication extracted from f1 phage. Both cosmid and phagemid can undergo independent replication or *in vitro* packaging to bacterial cells. These are the important differences between cosmid and phagemid.

**Cosmid can carry DNA inserts about three times as large as those carried by Lambda itself ( 45kb)**

# Cloning Vectors for Yeast

- Shuttle Vectors are capable of propagating between two different organisms. They can be maintained in *E.coli* as well as *S.cerevisiae.*
- One of the most common types of shuttle vectors is the yeast shuttle vector
- **Yeast episomal plasmids**

The word "**episomal**" indicates that a YEp can replicate as an independent **plasmid**, but also implies that integration into one of the **yeast** chromosomes can occur.

- Yeast artificial chromosome (YAC) is a human-engineered DNA molecule used to clone DNA sequences in yeast cells. YACs are often used in connection with the mapping and sequencing of genomes.
- Segments of an organism's DNA, up to one million base pairs in length, can be inserted into YACs. The YACs, with their inserted DNA, are then taken up by yeast cells. As the yeast cells grow and divide, they amplify the YAC DNA, which can be isolated and used for DNA mapping and sequencing.

Yeast artificial chromosome (YAC) is a human-engineered DNA molecule used to clone DNA sequences in yeast cells

YACs are plasmid shuttle vectors capable of replicating and being selected in common bacterial hosts such as *Escherichia coli*, as well as in the budding yeast *Saccharomyces cerevisiae*.

# YEAST ARTIFICIAL CHROMOSOMES

- YAC is an artificially constructed chromosome that contains a
  - Centromere
  - Telomeres
  - Autonomous replicating sequence (ARS) element required for replication and preservation of YAC in yeast cells
- ARS elements are thought to act as replication origins

- First described in 1983 by Murray and Szostak

- The Major limitation of most of the vectors is the size limit of DNA that can be cloned into them.

- YAC vector allow the cloning, within yeast cells, of the fragment of foreign genomic DNA that can approach 500 kb in size.

# Circular map of plasmid vector pYAC3