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Vectors are DNA molecules that are used as a vehicle to carry foreign DNA fragments into other cells where they can replicate and/or express. Among these most commonly used vector is a plasmid.

Characteristics of vectors:

- Self replicating, multiple copies. Replication origin site.
- Cloning site.
- Selectable marker gene.
- Low molecular weight.
- Easily isolates and purifies.
- Easily isolates into host cells.

The molecular cloning process involves the following steps:

Restriction enzymes – helps to cut the DNA of interest.
Ligation – DNA of interest is pasted to the vector DNA with the help of ligase.
Transformation – helps in the introduction of vector DNA into the host cell often by bacteria or yeast. Then the host cells copy the vector DNA along with their own DNA, creating multiple copies of interested DNA.
Isolation – the vector DNA is then isolated (separate) from the host cell and then purify.

Classification of vectors

a) Cloning vectors – use to clone foreign DNA.
b) Artificial vectors – they are engineered vectors so that any foreign DNA can be transcript in RNA and translate into proteins.

Important features of cloning vectors:-

- Ability to replicate into host cells.
- Unique restriction enzyme sites for insertional cloning.
- Genetic marker to select for host cells containing the vectors.
- Low molecular weight.

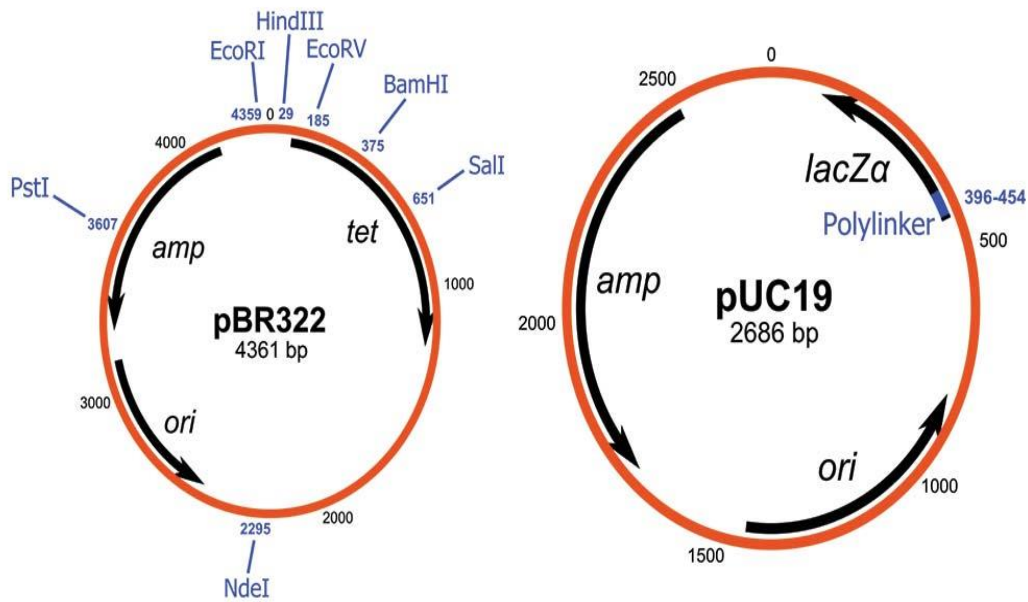


Figure: Schematic representation of the pBR322 plasmid, one of the first plasmids widely used as a cloning vector. The pUC plasmid has a high copy number, contains a multiple cloning site (polylinker), a gene for ampicillin antibiotic selection, and can be used for the blue-white screen.

Types of vectors

1) **Plasmids-** extra chromosomal, double-stranded DNA molecules, circular, self-replicating. Contain origin of replication, allowing for replication-independent and found in prokaryotes.

Classification of plasmids

a) Fertility plasmid- fertility plasmid is also known as F plasmid, contains a transferred gene that allows genes to be transferred from one cell to another through conjugation.
E.g. F plasmid of Escherichia coli

b) Col plasmid- col plasmid contains genes that make bacteriocins (also known as aa colicins), which are protein that kills other bacteria and thus defends the host bacterim.
E.g. ColE1 of *Escherichia coli*

c) Resistance plasmid – resistance plasmid or R plasmid contains genes that help the bacteria cell defend against the environment, factors such as poisons or antibiotics.
E.g. RP4 in Pseudomonas

d) Degradative plasmid- degradative plasmid helps the host bacterium to digest compounds that are commonly found in nature, such as camphor, xylene, toluene. These plasmids contain the gene for special enzymes that break down specifics compound.
E.g. Tol of Pseudomonas putida

e) Virulence plasmids- when a virulence plasmid is inside the bacterium, it turns that plasmid into pathogen which is an agent of antigens.
E.g. Ti plasmid of *Agrobacterium tumefaciens*

2) Bacteriophage lambda- phage lambda is a bacteriophage or phage i.e. bacterial virus that uses *E. coli* as a host cell. Its structure is as a typical phage- head, tail, fibers. Lamda viral genome contains 485kb linear DNA with 12base ssDNA. Sticky end at both ends and ends is complementary in sequence and can hybridize to each other.
E.g. phage lambda, M13phage

3) Cosmid- the Cosmid vectors are the combination of plasmid vector and the cos site which allows the target DNA to be inserted into the lambda head.
E.g. PJB8

4) Artificial chromosomes- artificial chromosomes are DNA molecules or fragments assembled in vitro from define constituents, which guarantee stable maintenance of large DNA fragments with the properties of natural chromosomes. Useful for genome sequencing programs, functional characteristics of entire genome regions, and for transduction of large DNA segment into human and non-human mammalian cells.

Types of artificial chromosomes:

a). Bacterial artificial chromosome (BAC)
b) Yeast artificial chromosome (YAC)

PLASMID

Plasmids are extrachromosomal DNA molecules. They are small, circular and have an ability to replicate autonomously. Replication of plasmid is not under the control of chromosomal DNA. They are mostly found in bacteria. Some of the eukaryotes like yeast and plants also contain plasmids.

Their ability to replicate independently makes plasmid a cloning vector in the recombinant DNA technology for transferring and manipulating genes.

Many antibiotic-resistant genes in bacteria are present in plasmids.

The size of plasmid varies from a few base pairs to thousands of bp.

Plasmids also get transferred from one bacterial cell to another by the process of conjugation.

Plasmids carrying a specific gene are introduced into bacterial cells, which multiply rapidly and the required DNA fragment is produced in larger quantities.

Plasmids are used to prepare a recombinant DNA with the desired gene to transfer genes from one organism to another. This is known as genetic engineering.

Joshua Lederberg coined the term plasmid.

Plasmid Structure

- Plasmids are extrachromosomal and not essential. They are useful but not necessarily present in every organism of the species

- Plasmids are not a part of the genome and the same plasmid can exist in different species and gets transferred from one another
- Plasmids have their own origin of replication (ORI) and they replicate along with the cell so that each daughter cell possesses a copy of the plasmid also
- Apart from the origin of replication, often it contains genes for antibiotic resistance, for the production of toxins and other useful genes, that may be required for the survival of cells

Plasmid Vector

Plasmids and bacteriophages are frequently used as a cloning vector in the DNA recombinant technology.

- The ease with which plasmids can be modified and replicated makes it a great tool in genetic engineering and biotechnology
- For genetic engineering purpose, plasmids are artificially prepared in the lab
- The lab-grown plasmids, which are used as a vector contain an origin of replication, cloning site and selection marker

Vector Element	**Description**
Origin of Replication (ORI)	DNA sequence where initiation of replication starts
Selectable Marker	For selecting bacteria containing desired plasmid, e.g. **antibiotic resistance genes** and other specific genes
Multiple Cloning Sites (MCS)	Recognition sites to insert foreign DNA fragment by using restriction enzymes, a few or single recognition site is preferred to avoid getting several fragments
Promoter Region	Promotes transcription of the target gene to get the desired protein
Primer Binding site	The sequence of DNA used as a start point for PCR amplification and sequence verification

- DNA is cut at the specific points by using restriction enzymes (molecular scissors), which make sticky ends of the DNA

Herbert Boyer and Stanley Norman Cohen together discovered recombinant DNA technology by recombining DNA segments as desired and inserting into the bacteria cell to get the desired protein

- The desired genes are then inserted by using DNA Ligase
- The recombinant DNA molecule is then introduced to the host bacteria cell by the process of **transformation**
- The recombinant plasmid then multiplies using host DNA polymerase

- The first plasmid used as a cloning vector was **pSC101** of ***Salmonella typhimurium***. They showed that a gene from a frog can be expressed in the bacterial cell
- *E.coli.* plasmid is frequently used as a cloning vector

pBR322 Plasmid

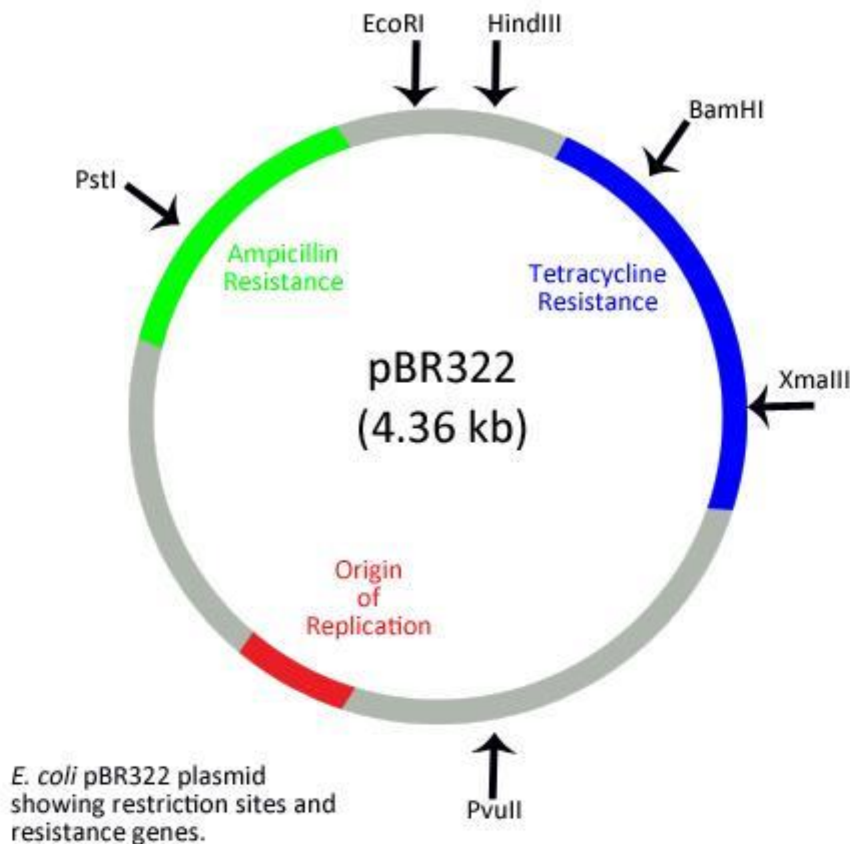
The main characteristics of pBR322 are:

- **Restriction sites:** BamH I, Hind III, Sal I, Pvu I, Pvu II, Pst I, EcoR I, Cla I
- **Selectable marker:** antibiotic resistance genes for ampicillin (amp^R) and tetracycline (tet^R)
- **ORI:** the origin of replication
- **ROP:** It codes for proteins, which are involved in the process of replication of plasmid

Different antibiotic resistance genes act as a restriction site and to ligate foreign DNA and for the selection of transformants. The gene, where the foreign DNA is inserted becomes inactive.

Alternative selectable marker: Mostly these have an ability to produce some colour after reacting with a chromogenic substance. The alternative markers are used for the ease of differentiating recombinants from non-recombinants, e.g. gene coding for β-galactosidase.

When a foreign gene is inserted between the gene coding for β-galactosidase**,** the recombinant cell does not produce the enzyme β-galactosidase due to inactivation of the gene. In the presence of a chromogenic substrate, non-recombinants form blue colour colonies and recombinants form colourless colonies.



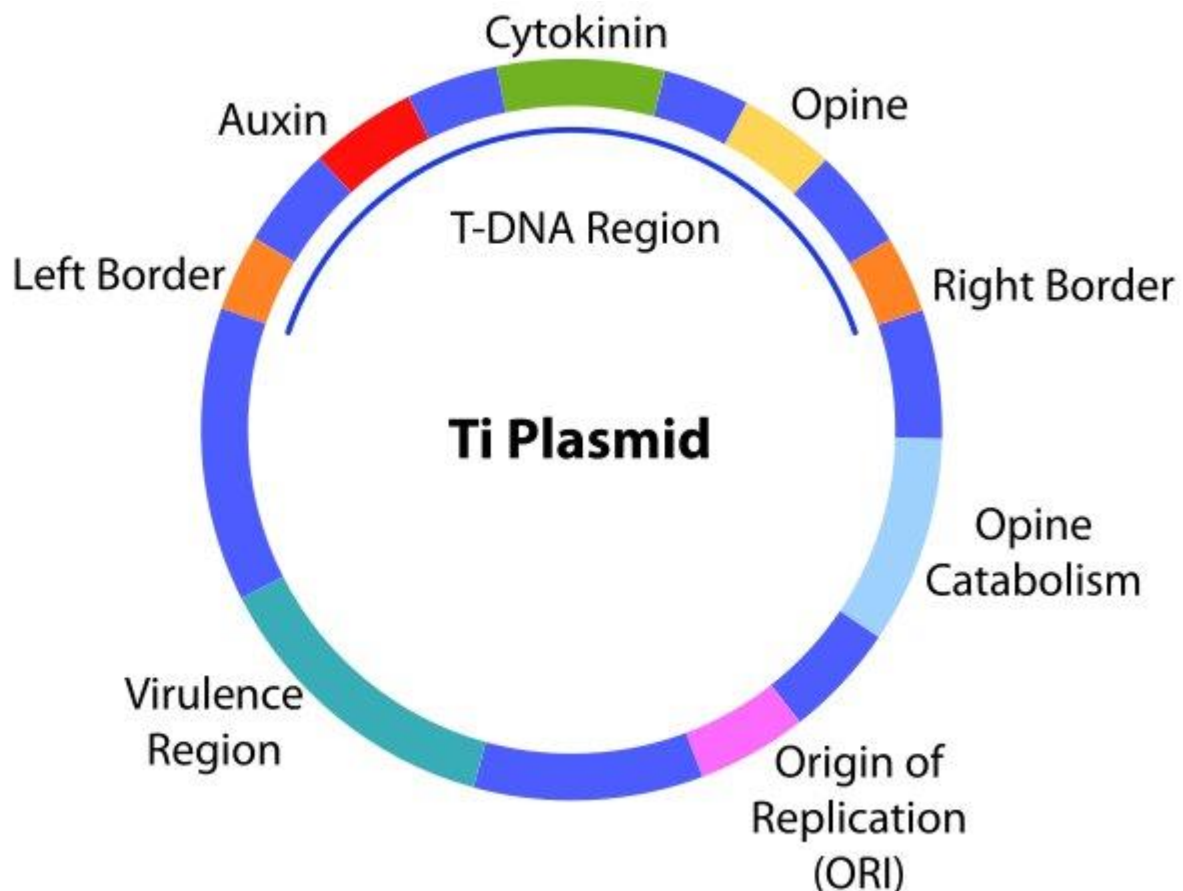
E. coli pBR322 plasmid showing restriction sites and resistance genes.

Ti Plasmid

The Tumour inducing or Ti plasmid is present in the bacterium *Agrobacterium tumifaciens.*

It is widely used now as a cloning vector to deliver desirable genes to the host plant to get **transgenic plants.** The main characteristics of Ti plasmid are:

- Size of the plasmid is ~ 250kbp
- There are different kinds of Ti plasmids based on the different genes they possess, which code for different opines, e.g. leucinopine, nopaline, octopine, etc.
- It is a pathogenic species to many dicotyledonous plants. It causes crown gall disease in plants.
- It contains one or more T-DNA region
- *Agrobacterium tumifaciens* has an ability to transform the normal cells to tumour cells by inserting a DNA piece known as T DNA and it starts producing chemicals, that are required by the bacterium
- After inserting the desired gene into Ti plasmid, it loses its pathogenic ability but is still able to insert the desired gene into the plant cell
- It contains *vir* or virulence genes**,** which transfer T-DNA region to plant cells and gets integrated into the plant genome
- Ti plasmid can be modified as per the requirement to insert the desired genes
- *Agrobacterium tumifaciens* is called “nature’s genetic engineer”



Lambda Phage

λ phages are viruses that can infect bacteria. The major advantage of the λ phage vector is its high transformation efficiency, about 1000 times more efficient than the plasmid vector.

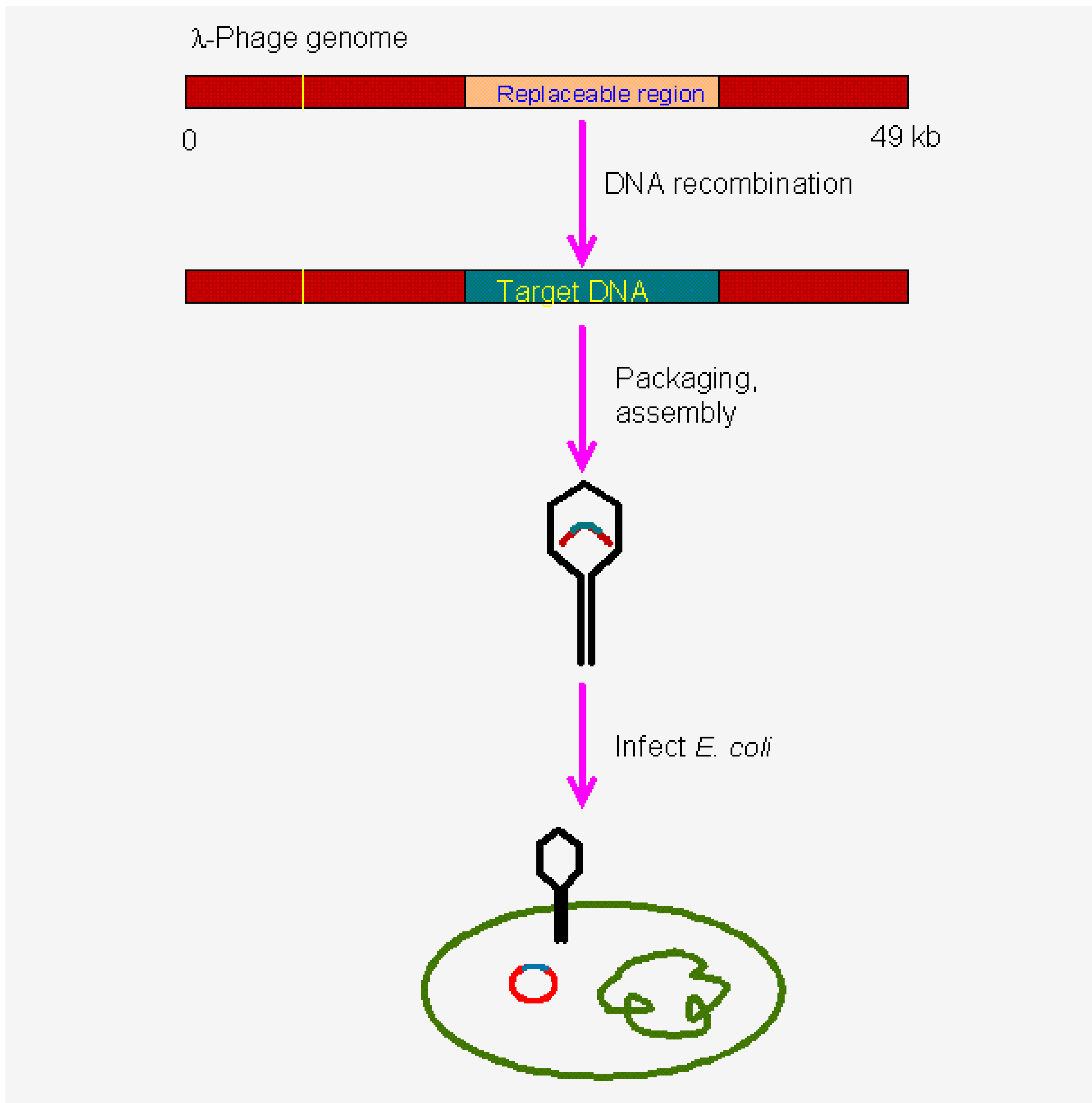


Figure 9-A-4. Schematic drawing of the DNA cloning using λ phages as vectors. The DNA to be cloned is first inserted into the λ DNA, replacing a nonessential region. Then, by an **in vitro assembly system** (described below), the λ virion carrying the recombinant DNA can be formed. The λ genome is 49 kb in length which can carry up to 25 kb foreign DNA.

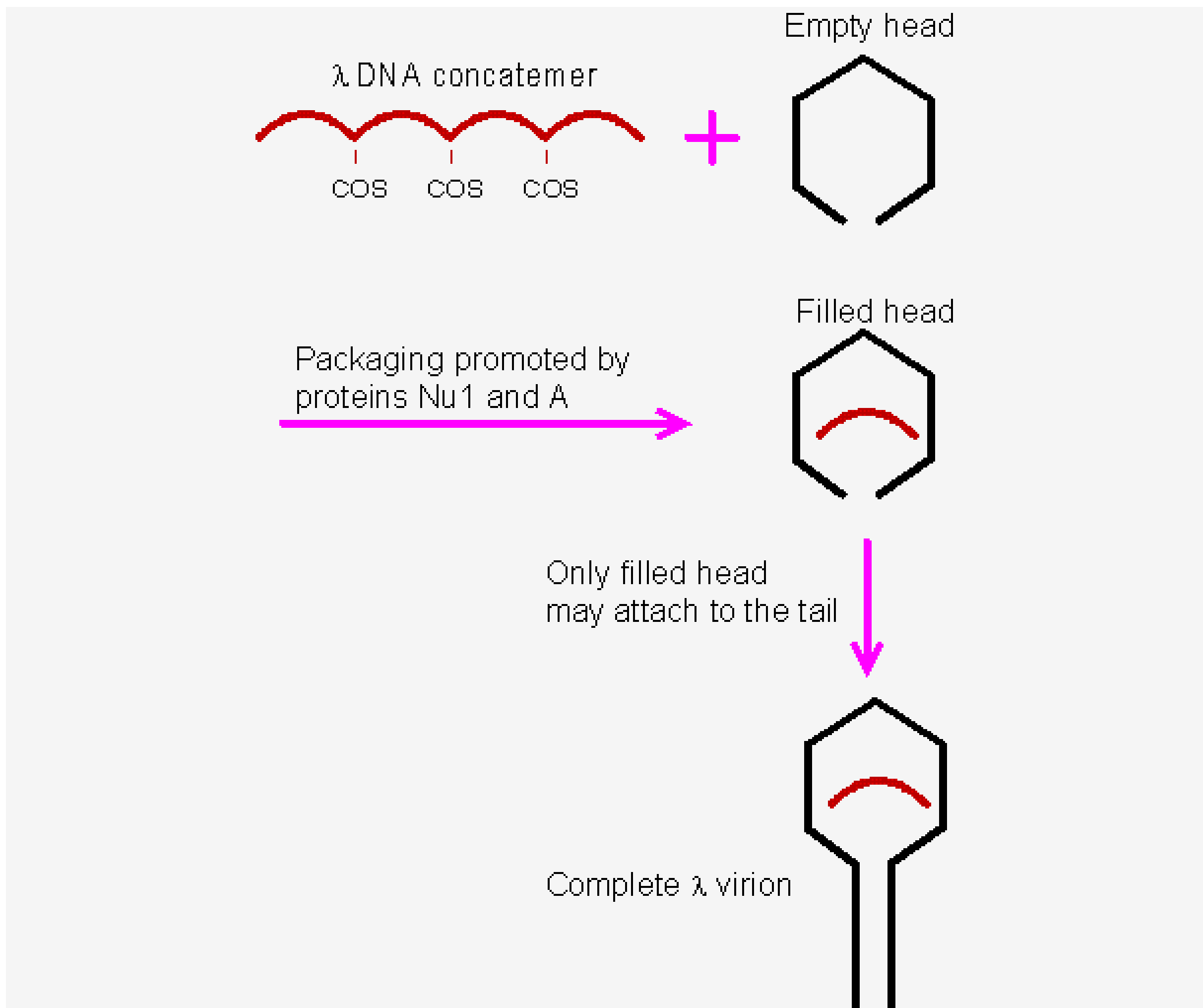


Figure 9-A-5. The assembly process of the λ virion.

The extreme ends of the λ DNA are known as **COS sites**, each is single stranded, 12 nucleotides long. Because their sequences are complementary to each other, one end of λ DNA may base-pair with the other end of a different λ DNA, forming concatemers. The two ends of a λ DNA may also bind together, forming a circular DNA. In the host cell, the λ DNA circularizes because ligase may seal the join of the COS sites.

In the assembly process of λ virions, two proteins Nu1 and A can recognize the COS site, directing the insertion of the λ DNA between them into an empty head. The filled head is then attached to the tail, forming a complete λ virion. The whole process normally takes place in the host cell. However, to prepare the λ virion carrying recombinant λ DNA, the following **in vitro assembly system** is commonly used.

Proteins Nu1 and A are encoded by the genes in the λ genome. If the two genes are mutated, λ DNA cannot be packaged into the pre-assembled head. Because tails attach only to filled heads, the cell will accumulate separate empty heads

and tails, which can then be extracted. When the extract is mixed with recombinant λ DNA and proteins Nu1 and A, the complete λ virion carrying recombinant λ DNA will be assembled.

Cosmid

The cosmid vector is a combination of the plasmid vector and the COS site which allows the target DNA to be inserted into the λ head. It has the following advantages:

- High transformation efficiency.
- The cosmid vector can carry up to 45 kb whereas plasmid and λ phage vectors are limited to 25 kb.

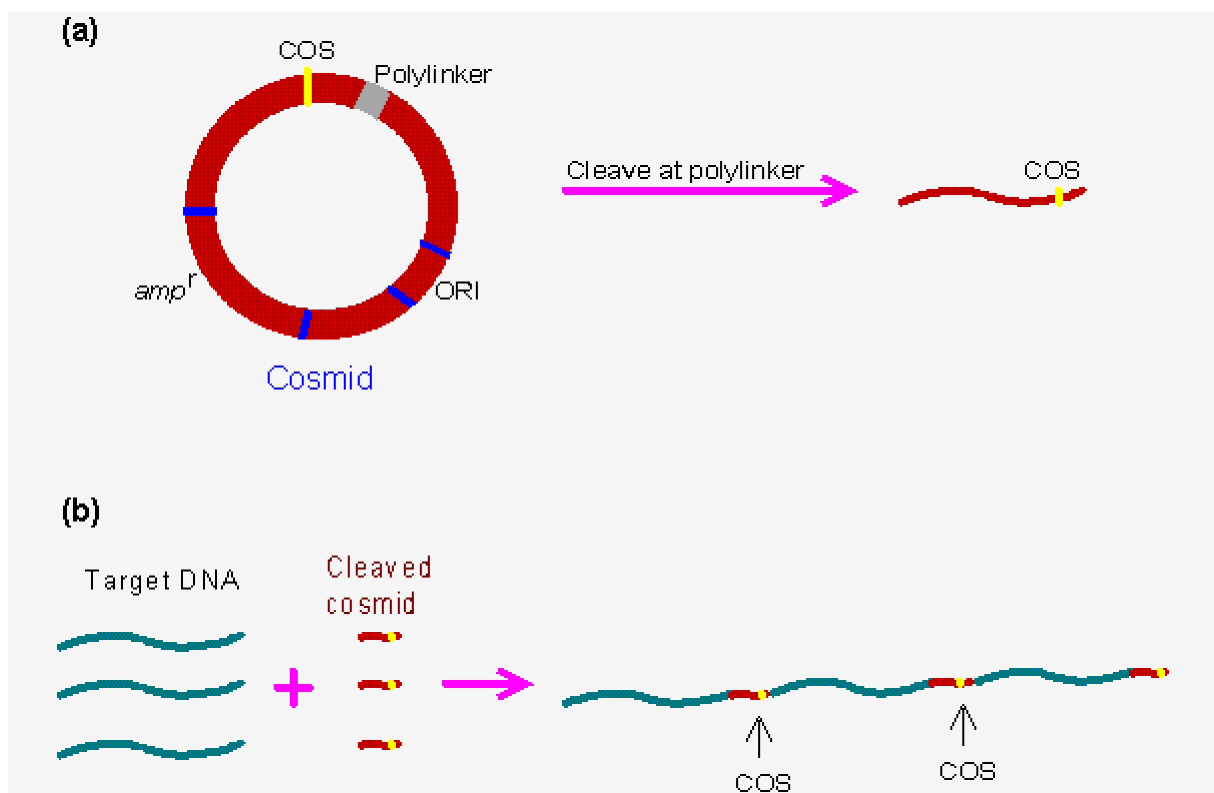


Figure 9-A-6. Cloning by using cosmid vectors. **(a)** In addition to amp^r, ORI, and polylinker as in the plasmid vector, the cosmid vector also contains a COS site. **(b)** After cosmid vectors are cleaved with restriction enzyme, they are ligated with DNA fragments. The subsequent assembly and transformation steps are the same as cloning with λ phages.

YAC

The yeast artificial chromosome (YAC) vector is capable of carrying a large DNA fragment (up to 2 Mb), but its transformation efficiency is very low.

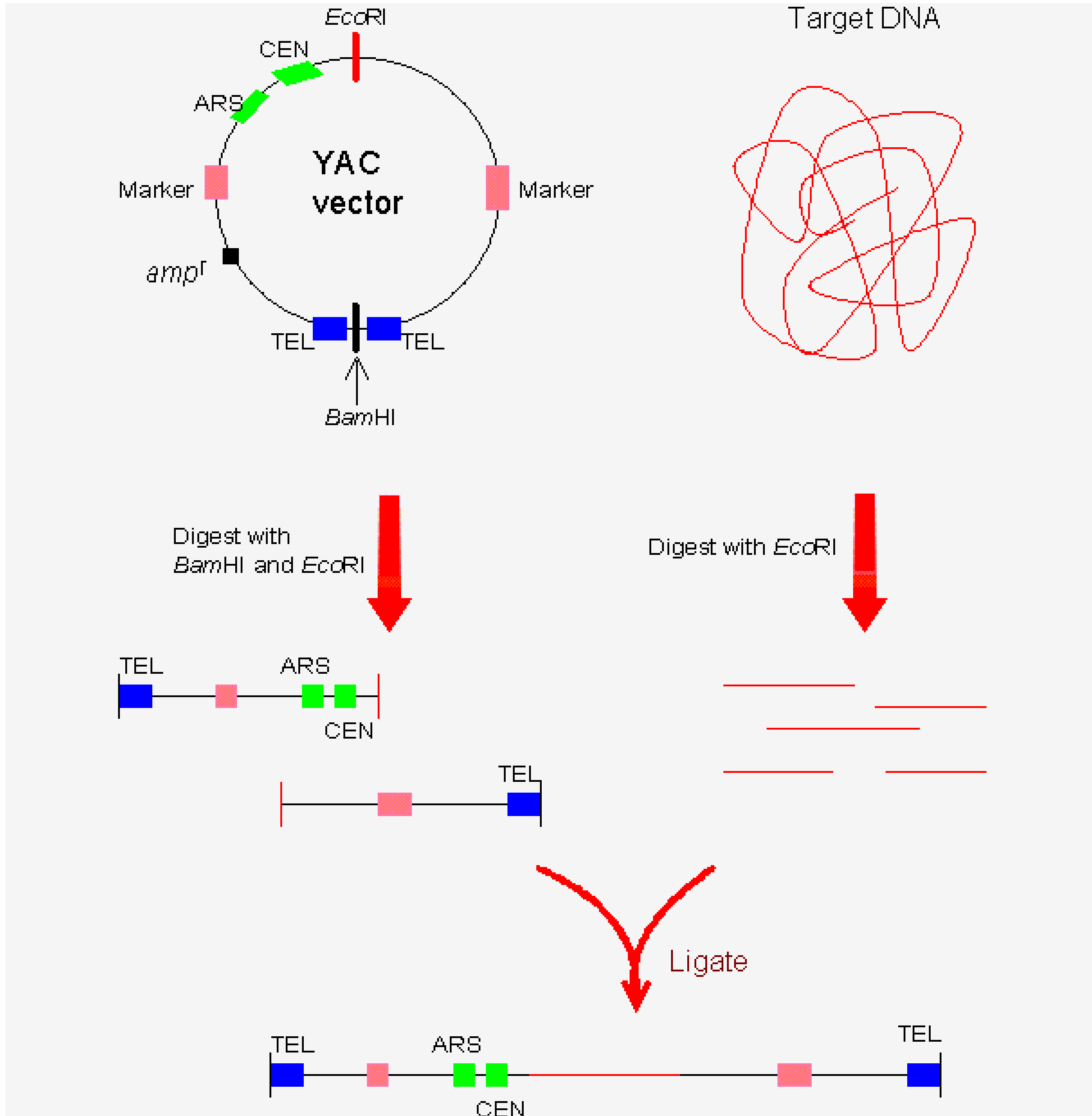


Figure 9-A-7. Cloning by using YAC vector.

Essential components of YAC vectors

- Centromers (CEN), telomeres (TEL) and autonomous replicating sequence (ARS) for proliferation in the host cell.
- *amp*^{r} for selective amplification and markers such as TRP1 and URA3 for identifying cells containing the YAC vector.

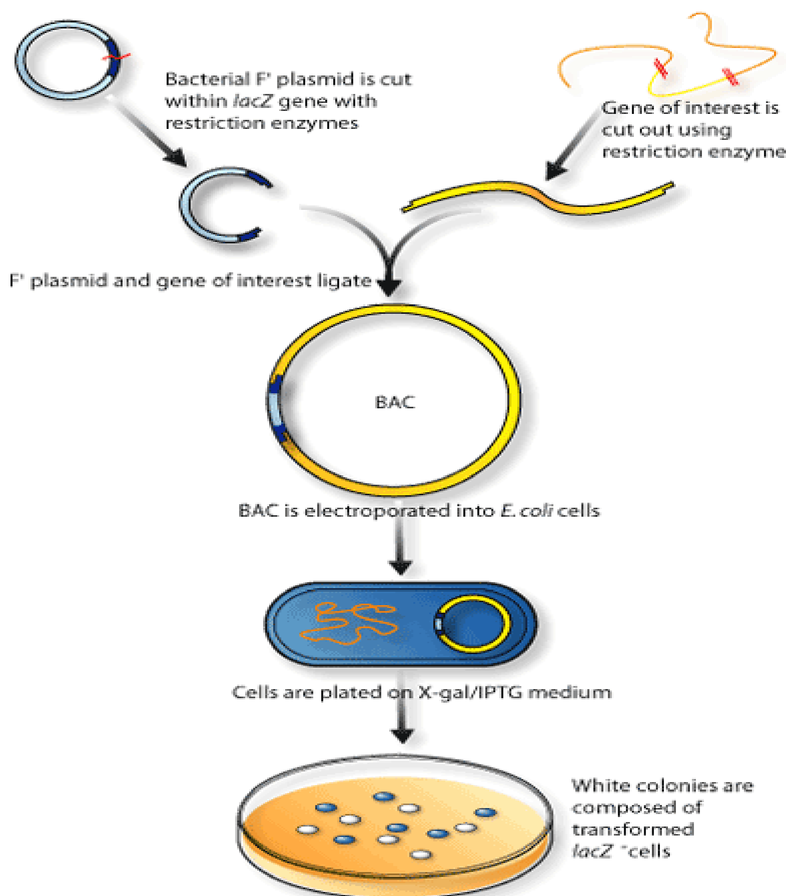
- Recognition sites of restriction enzymes (e.g., EcoRI and BamHI)

Procedure

1. The target DNA is partially digested by EcoRI and the YAC vector is cleaved by EcoRI and BamHI.
2. Ligate the cleaved vector segments with a digested DNA fragment to form an artificial chromosome.
3. Transform yeast cells to make a large number of copies.

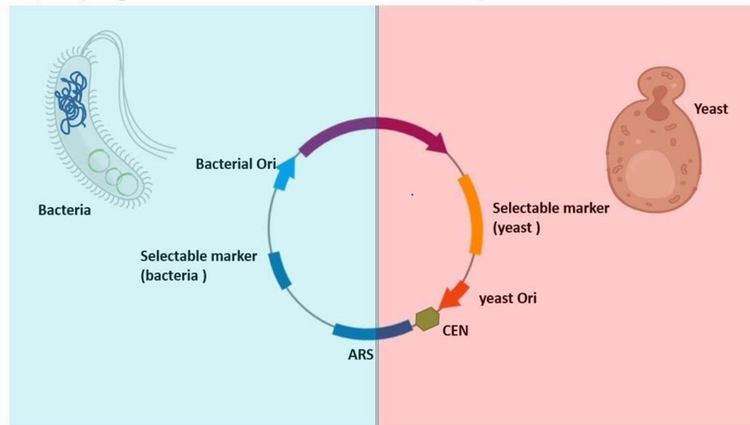
BAC Vector

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.



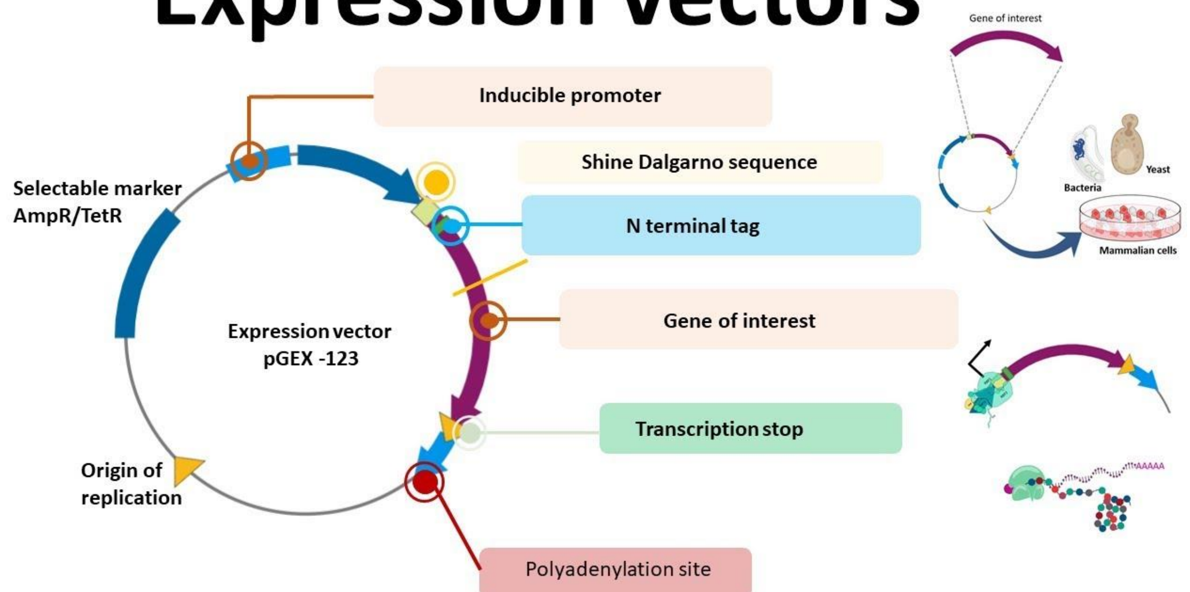
Shuttle vector

A shuttle vector is a vector constructed so that it can propagate in two different host species. Therefore, DNA inserted into a shuttle vector can be tested or manipulated in two different cell type



EXPRESSION VECTOR

An **expression vector**, otherwise known as an **expression** construct, is usually a plasmid or virus designed for gene **expression** in cells. The **vector** is used to introduce a specific gene into a target cell, and can commandeer the cell's mechanism for protein synthesis to produce the protein encoded by the gene.



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