Department of Biology
Genetic engineering
4th stage
Second semester
Assist. Prof. Dr. Lubna Altaie **Lecture 2**

Cloning Vectors

A cloning vector is a small piece of DNA, taken from a virus, a plasmid, or a cell of higher organism, that can be stably maintained in an organism, and into which a foreign DNA fragment can be inserted for cloning purposes. The vector therefore contains features that allow for the convenient insertion or removal of a DNA fragment to or from vector,

Cloning is generally first performed using Escherichia coli, and cloning vectors in E. coli include plasmids, bacteriophages (such as phage λ), cosmids, and bacterial artificial chromosomes (BACs), while cloning vectors in yeast include yeast artificial chromosomes (YACs).

The host/ vector system is important to molecular biology. The vast majority of molecular cloning experiments utilize the bacterium ***Escherichia coli*** for the propagation of cloned DNA fragments. The advantages of *E. coli* that acceptance as the genetic engineering organism of choice are:

- It is easy to grow in simple, inexpensive growth medium.
- The organism has a rapid doubling time of about 20‑30 minutes.
- Laboratory strains of *E. coli* are generally safe.
- Its genetics are well understood and it has a fully mapped and sequenced genome.
- Extra-chromosomal copies of DNA (plasmids and bacterio-phage DNA) can be used to carry foreign DNA fragments.

Main characteristics of the vector:-

A vector must possess the following characteristics to make it useful for molecular cloning:

- The ability to self-replicate (have origin of replication).
- Contain at least one or more restriction sites for the insertion of the DNA fragments wants to be cloned.

- A selectable marker is a gene coding desirable feature used for identification of transformed from non-transformed cells. These markers may be antibiotic resistance genes such as ampicillin and tetracycline resistance gene. Or may be gene product that are give fluorescence or color reactions when react with substrate such as lacZ gene encode for B-galactosidase when react with X-Gal present in media give colored colonies (blue for non-transformed cells/ white for transformed cells).

Small size of the vector is more desirable that increase the efficiency of transformation and easy to manipulate.

Vectors that commonly used in cloning experiments are:

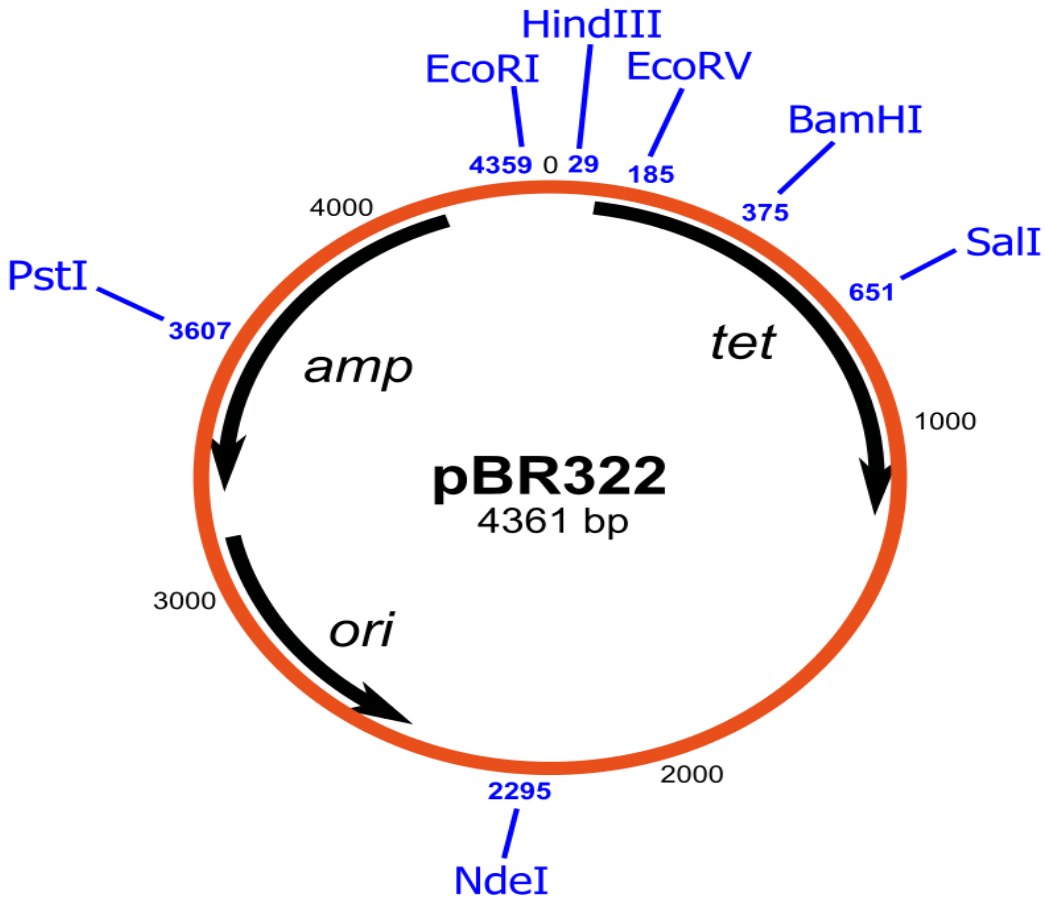
1. **Plasmids**

Plasmids are naturally occurring extra-chromosomal DNA fragments that are stably inherited from one generation to another. Plasmids are carrying a gene that encodes resistance to antibiotics, certain toxins or heavy metals, or that produces DNA restriction and modification enzymes. The copy number of the plasmids can vary from 1-2 or multiple copies (10-200)/cell.

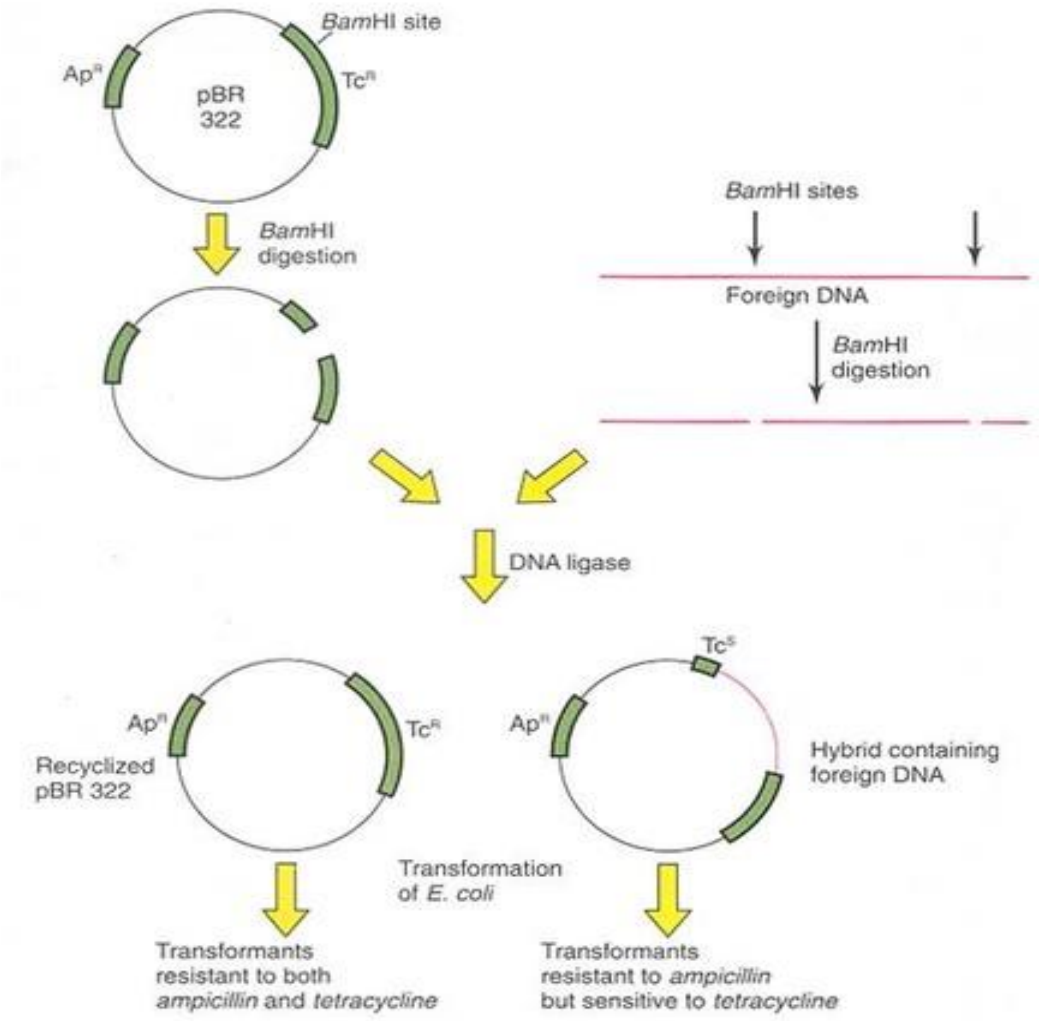
Plasmids are used to clone small pieces of DNA no more than 10 Kb in a site called **polylinker** is a short segment of DNA which contains many restriction sites that plasmid can cut with any of restriction enzymes and the desirable DNA can then be ligated into this site.

pBR322

The plasmid pBR322 was the first and commonly used vector in *E. coli* created in 1977.It is a small plasmid (4361 bp) contain15-20 copy number / cell, also pBR322 contains origin of replication, and it has two antibiotic resistance genes to ampicillin and tetracycline. The plasmid has unique restriction sites for more than 40restriction enzymes.



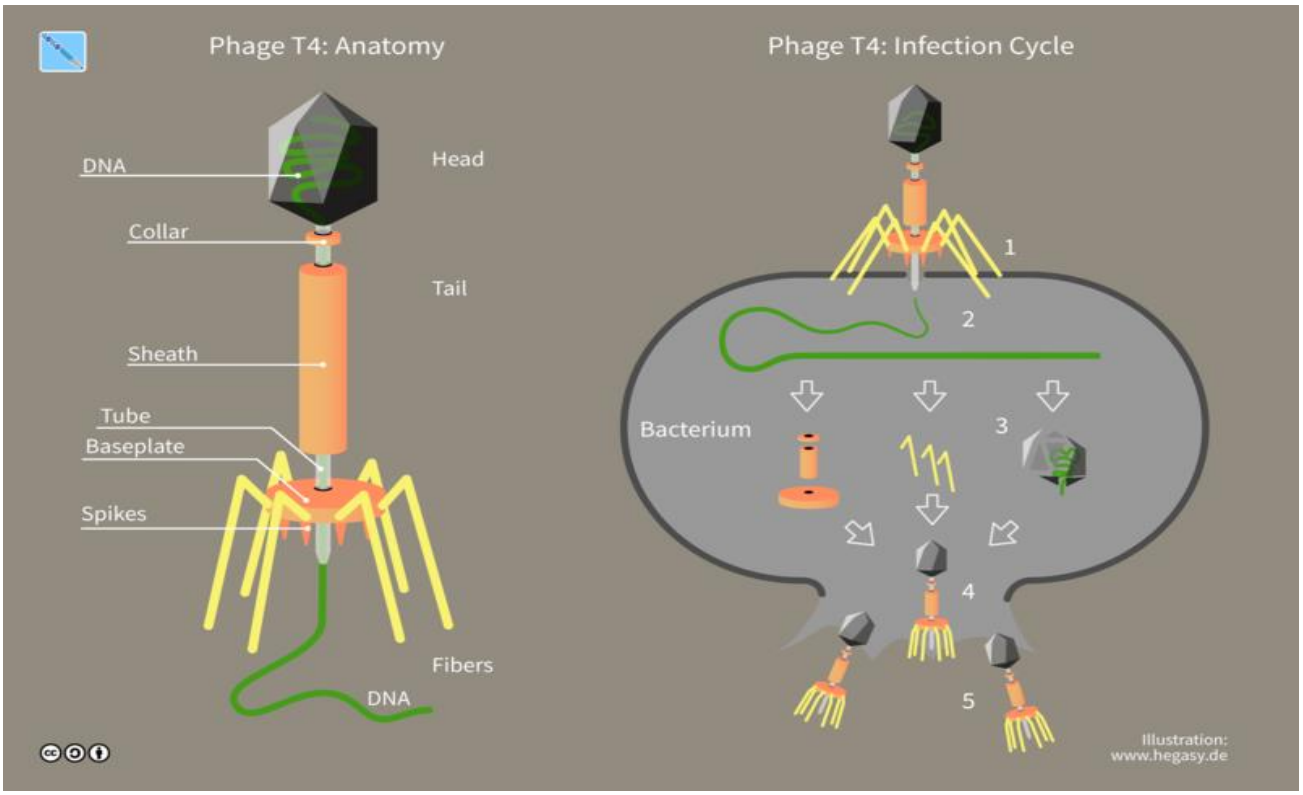
The antibiotic resistance genes in pBR322 allow for selection of recombinants (transformed cells) in a process called **insertional inactivation**. If we want to clone a DNA fragment into the BamHI site of pBR322, the insert DNA will interrupt the gene (nonfunctional) responsible for tetracycline resistance, but the gene for ampicillin resistance will not be altered. Transformed cells are grown on medium containing both ampicillin and tetracycline. The cells that die due to presence of tetracycline select as recombinants contain the foreign DNA fragment.



Bacteriophages

A bacteriophage, also known as a phage, is a virus that infects and replicates within bacteria and archaea. Phages are vectors larger than plasmids can inserts up to 40 Kb. **lambdaphage (*λ*)**, is bacteriophage that infects the bacterial species *E. coli*. This virus has a temperate life cycle that allows it to either reside within the genome of its host through lysogeny or enter into a lytic phase.

*λ*DNA has origin of replication and contains few unique restriction sites into which foreign DNA fragments could be cloned.

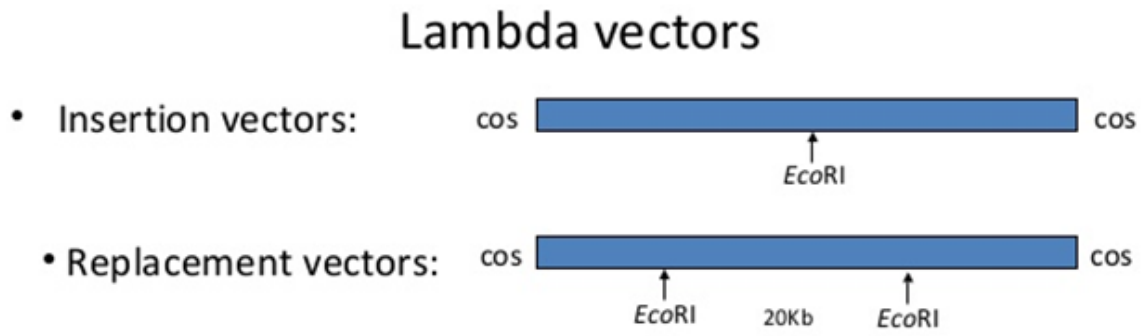


<u>Two basic types of λ vector are:</u>

Insertional vector: foreign DNA (8-10 Kb) is inserted into specific restriction site and can be introduced without removal of the stuffer fragment. These are useful in cloning of small DNA fragments such ad cDNA therefore used in cDNA libraries.

Replacement vector: foreign DNA replaces the stuffer fragment of vector. Replacement vectors are used for cloning of large DNA fragment (about 20 Kb) and are used in genomic libraries of higher eukaryotes.

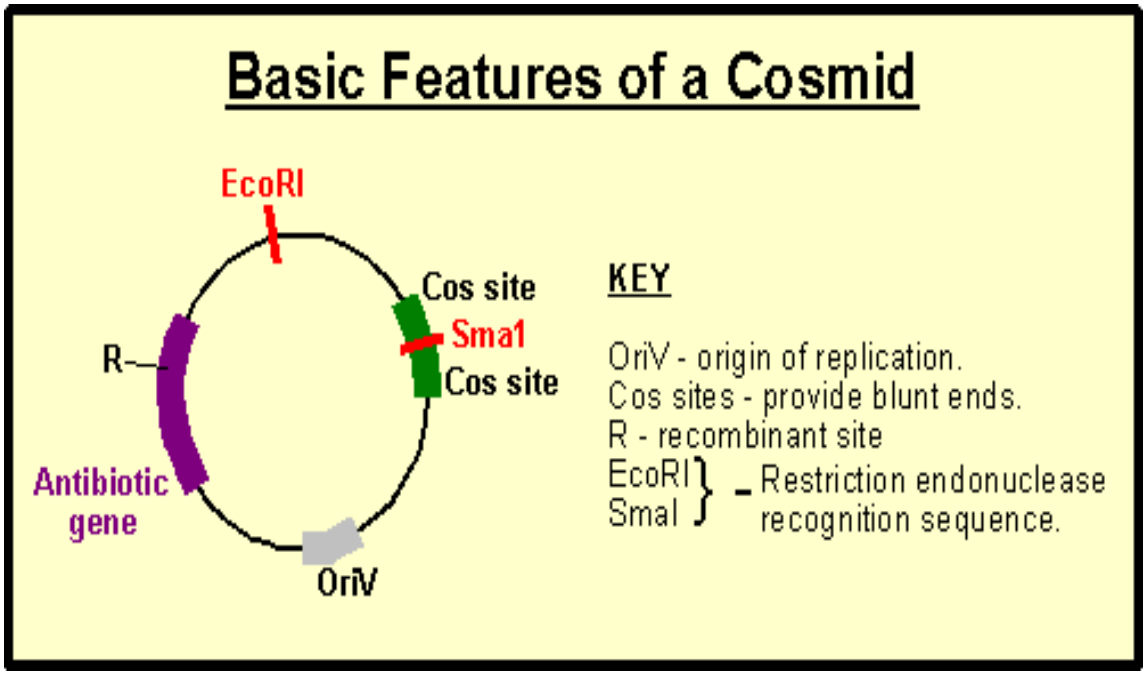
Stuffer fragment : A section of DNA contained within the genome of lambda vector that is replaced by the DNA to be cloned.



2. Cosmids

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. They are often used as a cloning vector in genetic engineering. Cosmids can be used to build genomic libraries. They contain 37 to 52 (normally 45) kb of DNA.

As plasmids, cosmids contain an origin of replication, a selectable marker and a unique restriction site into which DNA fragments can be ligated.



Artificial chromosome

1. YACs

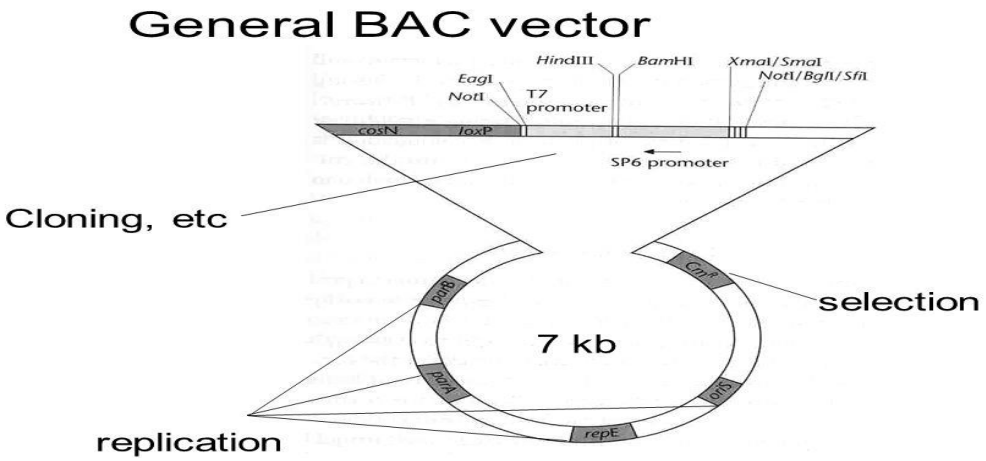
Yeast artificial chromosomes (YACs) are genetically engineered chromosomes derived from the DNA of the yeast, Saccharomyces

cerevisiae, which is then ligated into a bacterial plasmid. By inserting large fragments of DNA, from 100–1000 kb.

There are difficulties associated with working with YACs such as very large DNA molecules are very fragile and tend to breakage, leading to problems of rearrangement.

2. BACs

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. BACs are capable of carrying 150- 350 Kb of inserted DNA sequence.



BACs contain origin of replication sequence derived from *E. coli* plasmid, also contain multiple unique restriction sites and a selectable antibiotic resistance makers. Additionally, the BAC contains a λ cos site that used for specific cleavage during restriction mapping.

The DNA inserted into a BAC appears to be very stable. It can survive for many hundreds of generations in *E. coli* cells, and appears to be less likely to rearrangements and deletions.

Vector	host	Insert size
plasmid	*E. coli*	10 kb
λ phage	*E. coli*	5–25 kb
λcosmids	*E. coli*	35–45 kb
BACs	*E. coli*	≤300 kb
YACs	*Saccharomyces cerevisiae*	200–2000 kb