University of Duhok

College of Sciences

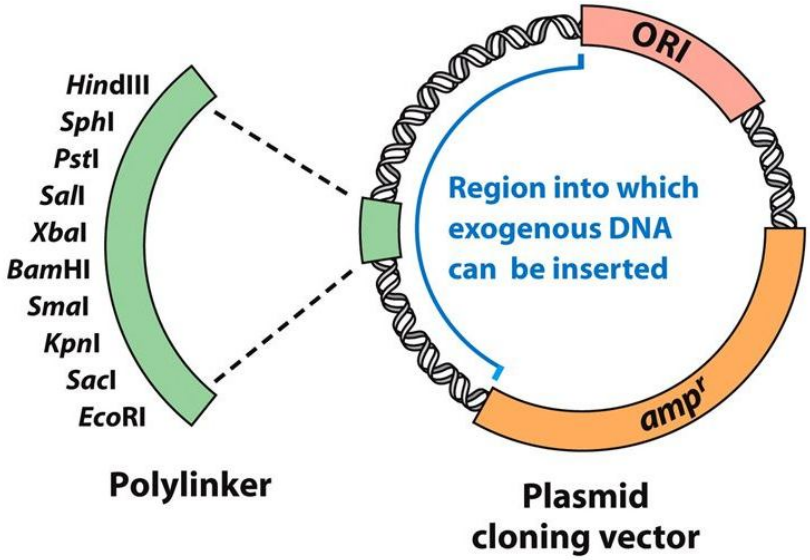
Department of Biology

4th Year Class

Lecture5. Cloning Vectors

Lecture outlines:

- Cloning Vectors?
- Key requirements for a cloning vector
- Essential Features of cloning vectors and their types.
- Why they are used?



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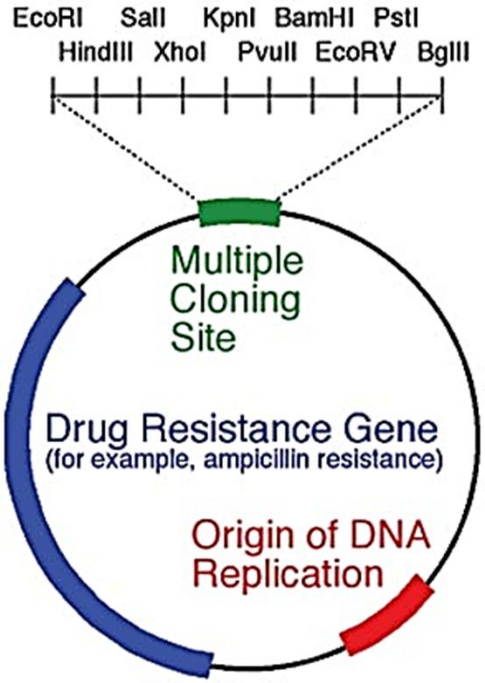
What are Cloning Vectors?

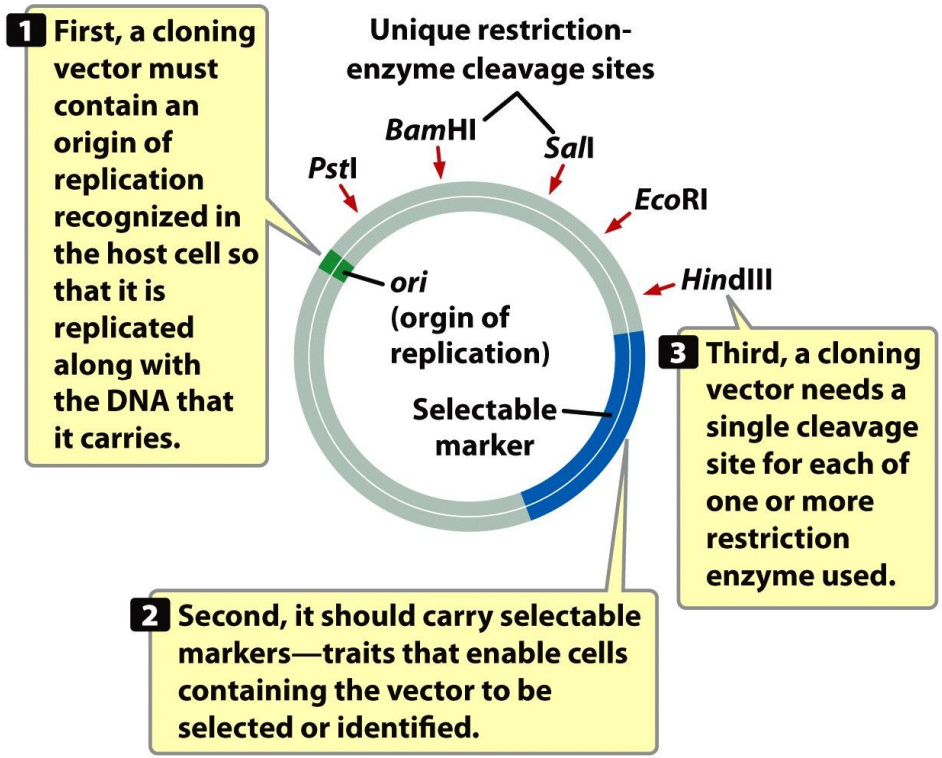
- A vector is a small piece of DNA molecule that is used to carry a foreign DNA into the host cell for cloning purposes.
- In molecular cloning, a vector is a DNA molecule used as a vehicle to artificially carry foreign DNA into another cell, where it can be replicated and/or expressed.
- Vectors can replicate autonomously and typically include features to facilitate the manipulation of DNA as well as a genetic marker for their selective recognition.



What are the key required elements for a cloning vector?

Cloning vectors are required to have some key features that are extremely necessary for their functions and make it easier to insert DNA and select for presence of vector in cell, these include:

1. Origin of replication (ORI).
2. Selectable marker gene (e.g. Antibiotic resistance gene).
3. Restriction sites to enable breakup of certain sequences with respect to restriction enzymes.



Cloning Vectors: Essential Features

All cloning vectors should have the following features in common:

a. It should be capable of self-replicating inside host cell.

b. It should possess a unique restriction site for RE enzymes to insert DNA of interest.

c. It should have a selectable marker to indicate which host cells received recombinant DNA molecule.

d. It should be small in size so that it can easily integrate into the host cell.

e. It should be capable of inserting a large segment of DNA.

f. It should be capable of working under the prokaryotic and eukaryotic systems.

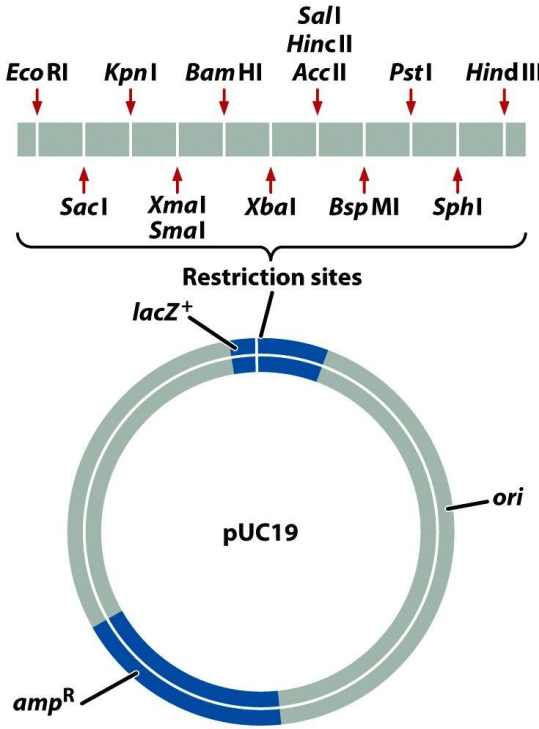
Types of cloning vectors

- There are different types of vectors available for cloning which are **plasmids**, **bacteriophages**, bacterial artificial chromosomes (**BACs**), yeast artificial chromosomes (**YACs**) and mammalian artificial chromosomes (**MACs**).
- The cloning vectors are limited to the size of insert that they can carry. Depending on the size and the application of the insert the suitable vector is selected for a particular purpose.

Name	Size	Cloning limit	Marker gene	Example
Plasmid	**4361 bp**	**0.1-10 kb**	**Ampicillin and tetracycline**	**PBR322**
Bacteriophase	**48502 bp**	**20kb**	**–**	**Lambda genome**
Cosmid	**7900 bp**	**30-50 kb**	**–**	**COS1**
Bacterial artificial Chromosome	**11827 bp**	**35-300 kb**	**chloramphenicol and lactose metabolizing gene**	**pUvBBAC**
Yeast Artificial Chromosome	**11400 bp**	**100-1000 kb**	**Similar to yeast**	**–**
Human Artificial	**–**	**No limit**	**–**	**–**

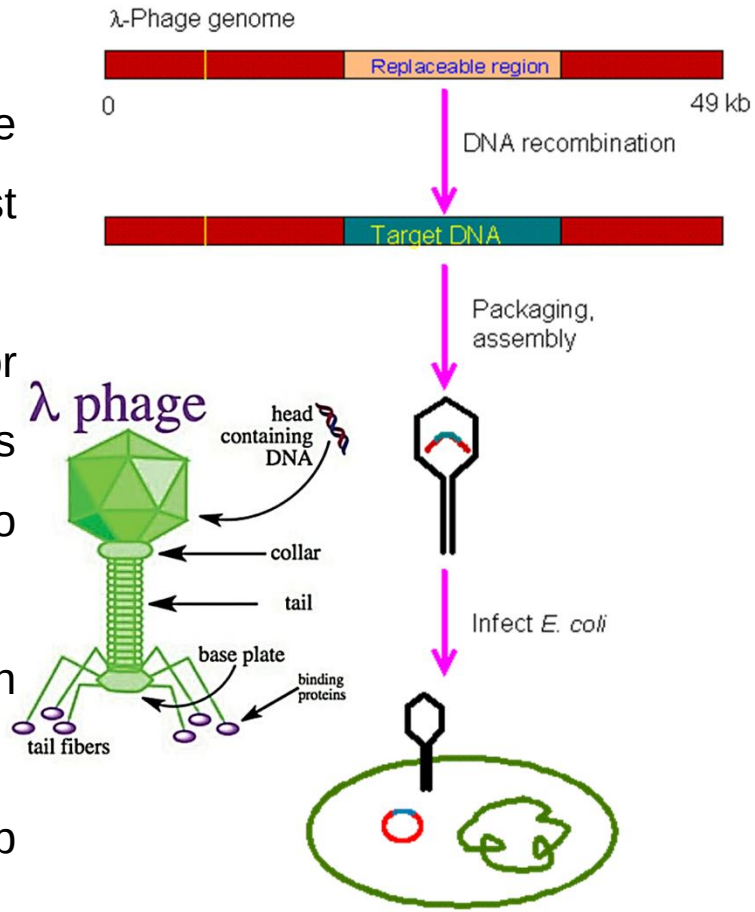
1. Plasmid vector

- Bacterial plasmids are small, circular DNA molecules that are separate from the rest of the chromosome.
- They replicate independently of the bacterial chromosome.
- Plasmids show the size ranging from 5.0 kb to 400 kb.
- Plasmids are inserted into bacterial cells by a process called transformation.
- Plasmids can accommodate an insert size of up to 10 kb DNA fragment.
- Generally plasmid vectors carry a marker gene which is mostly a gene for antibiotic resistance; thereby making any cell that contains the plasmid will grow in presence of the selectable corresponding antibiotic supplied in the media.



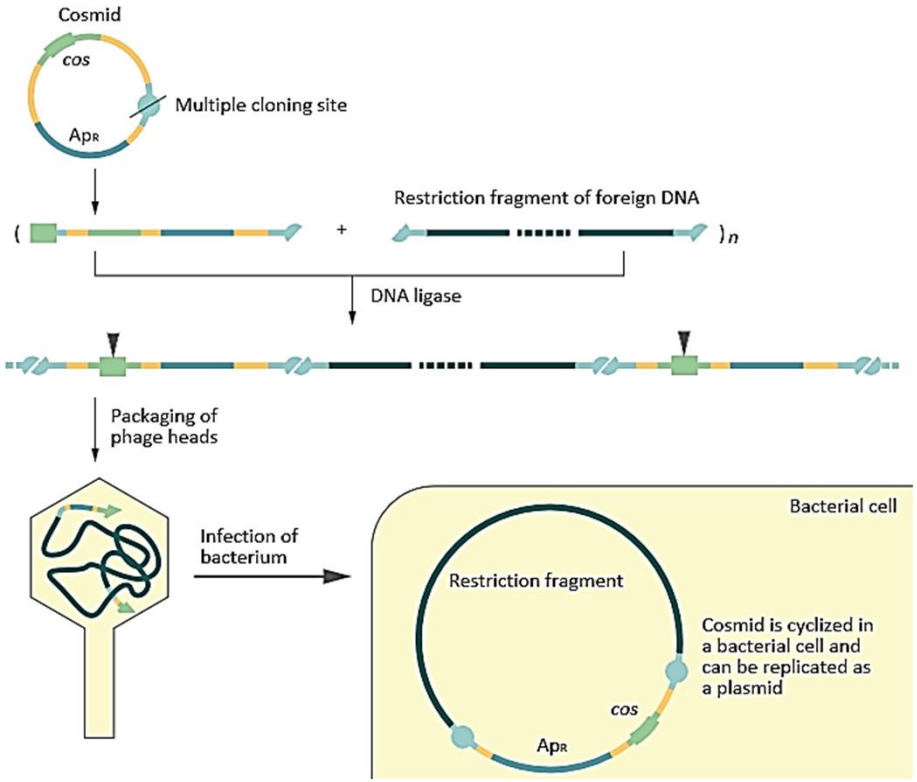
2. Bacteriophage

- The viruses that infect bacteria are called bacteriophage.
- These are intracellular obligate parasites that multiply inside bacterial cell by making use of some or all of the host enzymes to replicate.
- Bacteriophages have a very high significant mechanism for delivering its genome into bacterial cell known as transfection. Hence it can be used as a cloning vector to deliver larger DNA segments.
- Most of the bacteriophage genome is non-essential and can be replaced with foreign DNA.
- Using bacteriophage as a vector, a DNA fragment of size up to 20 kb can be transformed e.g. **lambda (λ-Phage)**.



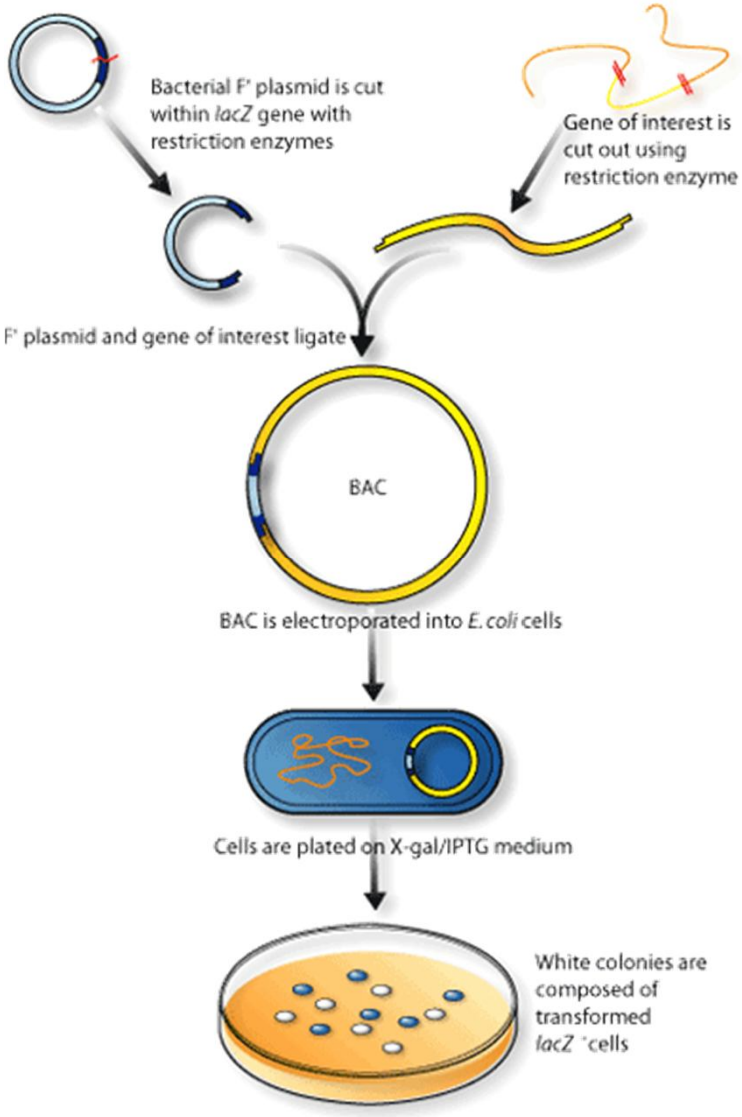
3. Cosmids

- Cosmids are hybrid DNA molecules of phages and plasmids that can carry DNA fragments up to 45kb.
- Constructed by combining plasmid origin of replication and selectable marker gene and bacteriophage (cos site) allow them to be packaged in a phage coat and to be transduce to a recipient by the lambda infection machinery.
- They can replicate like plasmids but can be packaged like phage lambda.
- It has no genes for viral protein, therefore viral particle are not formed in host.
- Host cell lysis are also absent, so infected cells grow into normal colonies.
- They are *E. coli*-based cloning vector.
- Used for constructing genomic library.



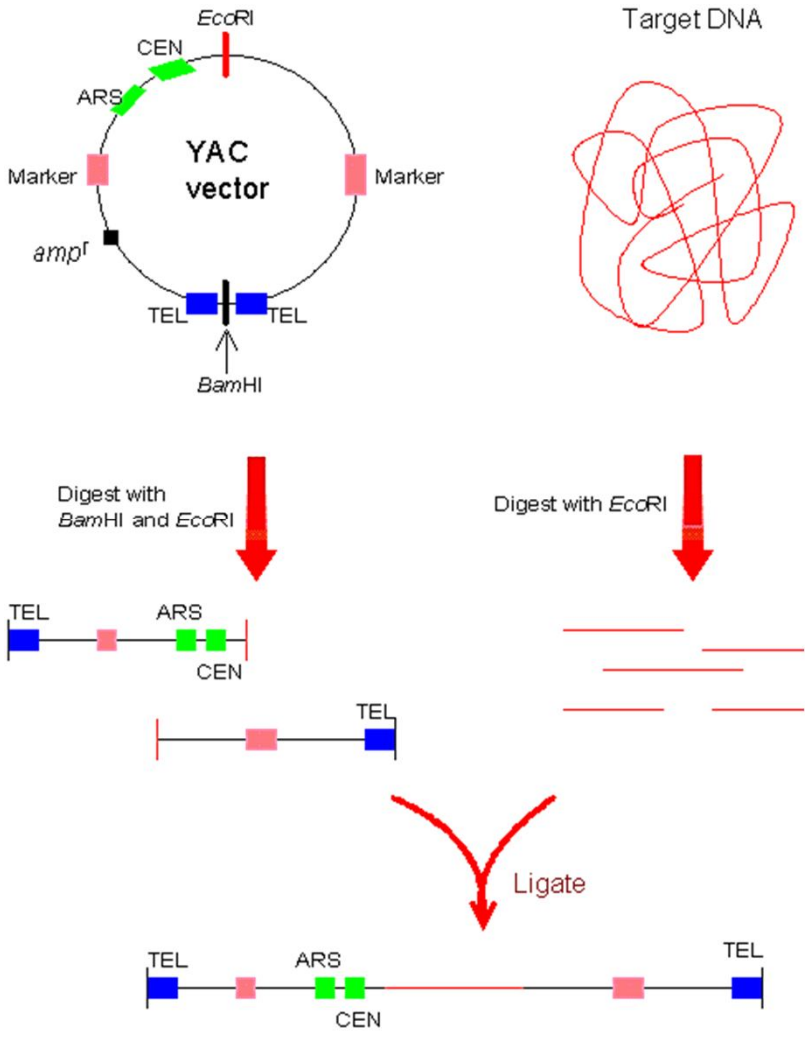
4. Bacterial artificial chromosomes (BACs)

- Bacterial artificial chromosomes (BACs) are simple plasmid which is designed to clone very large DNA fragments ranging in size from 75 to 300 kb.
- BACs basically have selectable marker such as antibiotic resistance genes and a very stable origin of replication (ori) that promotes the distribution of plasmid after bacterial cell division and maintaining the plasmid copy number to one or two per cell.
- BACs are basically used in sequencing the genome of organisms in genome projects (example: BACs were used in human genome project) and to study genetic disorders.
- Several hundred thousand base pair DNA fragments can be cloned using BACs.



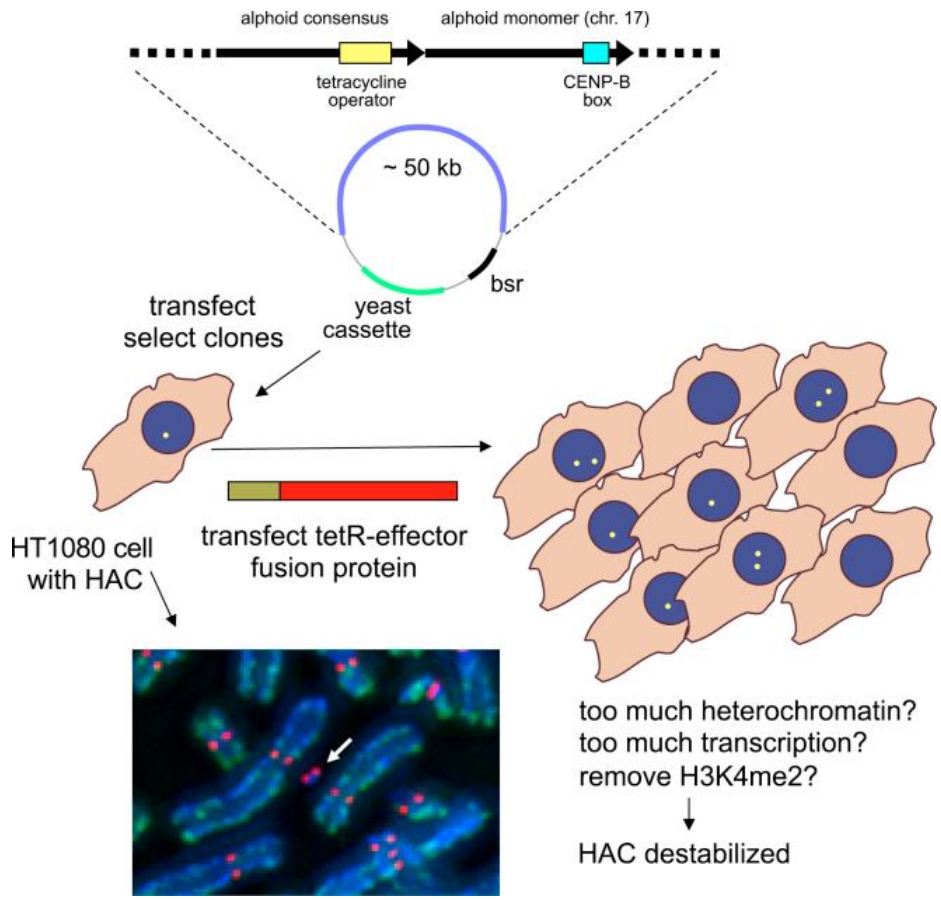
5. Yeast artificial chromosomes (YACs)

- Yeast artificial chromosomes (YACs) are yeast vectors that have been engineered to contain a centromere, telomere, origin of replication, and a selectable marker.
- They can carry up to 100 kb to 3000 kb of DNA.
- Since they are maintained in yeast (a eukaryote), they are useful for cloning eukaryotic genes that contain introns.
- Also, eukaryotic genes are more easily expressed in a eukaryotic host such as yeast.
- YACs have an advantage over BACs in expressing eukaryotic proteins that require post translational modifications.
- But, YACs are known to produce chimeric effects which make them less stable compared to BACs.



6. Human artificial chromosomes (HACs)

- Human artificial chromosomes (HACs) or mammalian artificial chromosomes (MACs) are still under development.
- HACs are micro-chromosomes that can act as a new chromosome in a population of human cells.
- HACs range in size from 6 to 10 Mb that carry new genes introduced by human researchers.
- HACs can be used as vectors in transfer of new genes, studying their expression and mammalian chromosomal function can also be elucidated using these microchrosomes in mammalian system.



Types of Vectors based on their use

Artificial plasmids vectors are classified into two broad types based on their use:

1. Cloning vectors

Cloning vectors are the DNA molecules that carry a specific gene of interest into the host cell and its main purpose is to make numerous copies of the inserted gene. A typical cloning vector consists of an origin of replication, a selectable marker, a reporter gene, and restriction sites.

2. Expression vectors

Expression vectors are associated with the actual expression of the gene into mRNA and protein in the target organism. Therefore, the expression vectors not only contain all the elements of a typical cloning vector, but also contain all the regulatory sequences, such as promoter, ribosomal binding site, transcription initiation site, translation initiation site, which are essential for getting maximum expression.

Uses of Vectors

Two primary uses are:

1. To isolate, identify and archive fragments of a larger genome
2. To selectively express proteins encoded by specific genes.

- Vectors were the first DNA tools used in genetic engineering, and continue to be cornerstones of the technology.