Topic : CLONING VECTOR

Course : M.Sc. Botany Part – II

Paper: PAPER XVI

(Biotechnology and Bioinformatics)

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CLONING VECTOR

- ✓ A small piece of DNA that can be stably maintained in an organism, and into which a foreign DNA fragment can be inserted for cloning purposes.
- ✓ Vectors can transfer donar DNA fragment with gene of interest to host cell (recipient) and are capable of replicating in the host cell.
- ✓ Cloning vectors include plasmids, bacteriophages, cosmids, phasmids, Bacterial Artificial Chromosomes (BACs) & Yeast Artificial Chromosomes (YACs).

CHARACTERISTIC FEATURES OF CLONING VECTORS

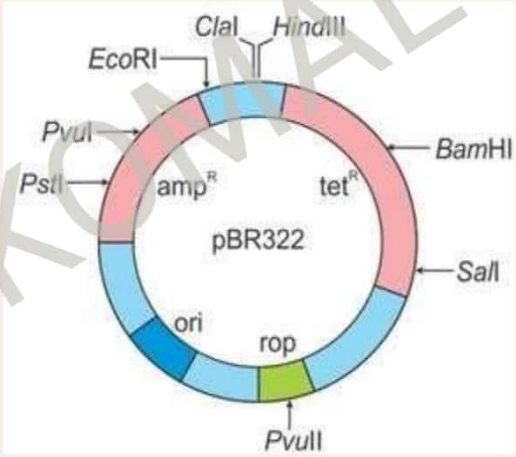
- ✓ An Ori (Origin of Replication)
- ✓ A selectable marker gene like tet^R, amp^R, kan^R
- ✓ Multiple cloning sites
- ✓ Unique restriction site which must be present in one of the marker gene
- ✓ Selectible marker which can be antibiotic resistance genes

VARIOUS TYPES OF VECTORS AND THEIR INSERT SIZE

CLONING VECTORS	INSERT SIZE (kb)
Plasmid	0.5-8
Bacteriophage	5-25
Cosmids	35-45
BAC	50-300
YAC	200-1000

PLASMIDS

- Plasmids are extrachromosomal, double - stranded , self replicating circular DNA.
- Present naturally in almostallbacteria.
- The size of plasmids varies from 1-200kb
- Act as DNAcarrier up to 8kb.



Examples:

pBR322

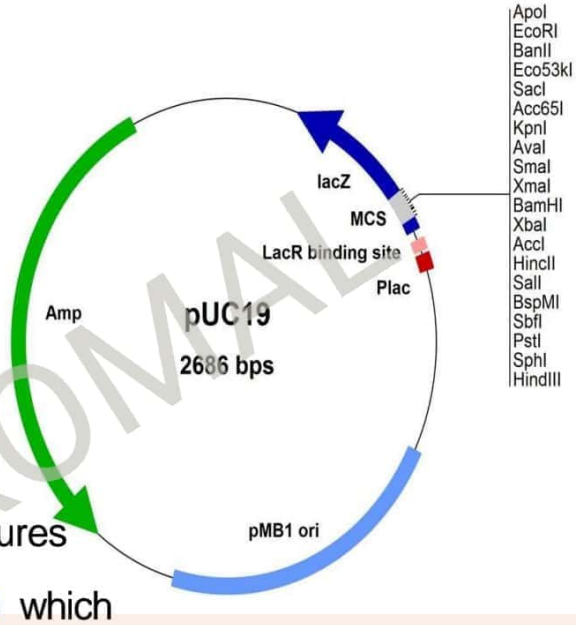
First plasmid vector constructed by Bolivar & Rodriguez from E.coli plasmid and it was named after them.

Characteristics features:

1. Small in size about 4363bp, so handled easily.
2. Origin of replication.
3. Carries two antibiotic resistant genes– ampicillin resistance and tetracyclin resistance for selection of transformants.
3. It has multiple restriction sites (*Eco*RI, *Bam*HI, SalI, etc.).

pUC vectors

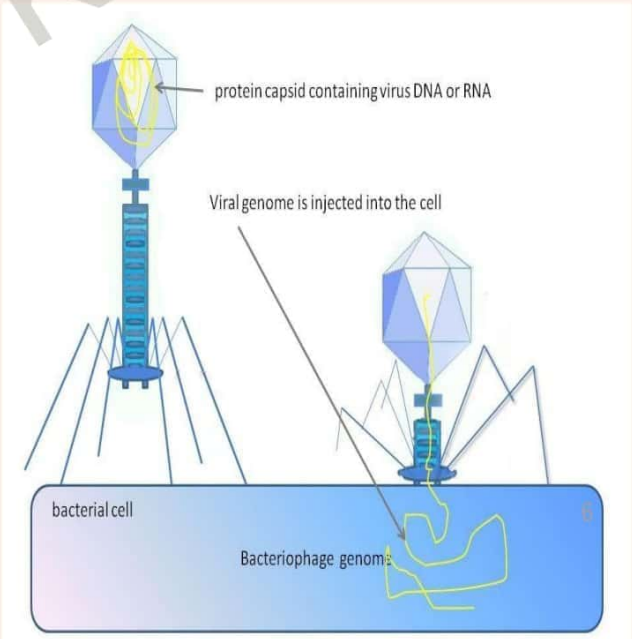
Messings and coworkers in1983 developed the pUC vectors at the University of California. pUC 19 is one of the popular plasmid vectors of pUC family which contains the following charecterists:



- It is a sophisticated vector with several enhanced features
- Consists of polylinker or multiple cloning site (MCS) which carry sites for several restriction endonucleases extending the range of enzymes that can be used to generate a restriction fragment suitable for cloning.
- Contains DNA sequence for lac Z' gene coding for β-galactosidase that permits rapid visual detection of an insert via blue-white selection method.
- Has high copy number in contrast to pBR322 as the former.

BACTERIOPHAGE

- ✓ Bacteriophage is a virus that infect bacterial cell and replicates using the machinery of the infected cell.
- ✓ They are advantageous over plasmids as they infect cells with high efficiency than transformation by plasmids
- ✓ They carry a larger fragment of size up to 25kb in contrast to limit of 8 kb in plasmids.
- ✓ Two bacteriophages eg- λ phage and M13 have been studied as cloning vectors.
- ✓ M13 phage has the ability to isolate single stranded form of the cloned gene which is of utmost importance in both sequencing and mutagenesis whereas λ phage has the ability to clone double stranded gene.



COSMIDS

- ✓ Cosmids can be defined as the hybrid vectors derived from the plasmids which contains cos sites of phage λ (cosmid = cos site + plasmid).
- ✓ It was developed by Collins and Hohn.
- ✓ Cosmids are plasmids that incorporate cohesive end site (cos) of bacteriophage λ which contains elements required for packaging DNA into λ particles.
- ✓ It is used to clone large DNA fragments between 35 to 45 Kb.

PHAGEMIDS

- ✓ Phagemids are plasmids containing properties of plasmid and phage.
- ✓ They contain f1 origin of replication (ori) from filamentous 'f1' phage.
- ✓ They can be maintained as plasmids as well as can be packaged as single stranded DNA in viral particles.

ARTIFICIAL CHROMOSOMES

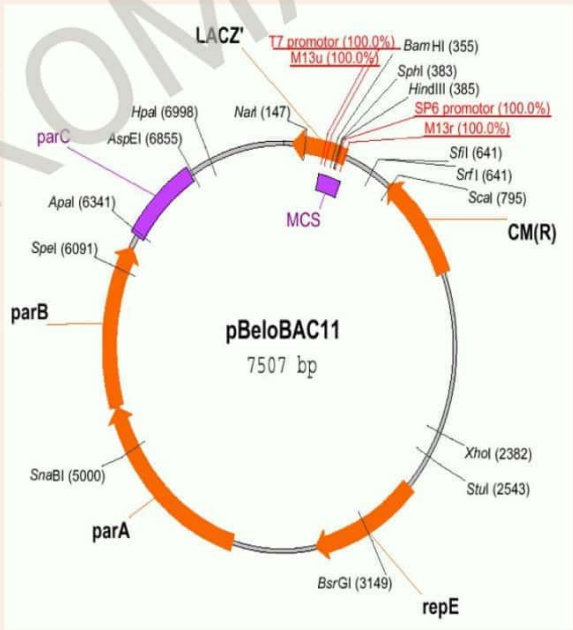
Artificial chromosomes are cloning vectors that can accommodate very large pieces of DNA, producing recombinant DNAmolecules resembling small chromosomes.

Example: BACs & YACs

BACTERIAL ARTIFICIAL CHROMOSOMES

Bacterial artificial chromosomes (BAC) are cloning vectors containing the origin of replicationfrom a natural plasmid found in E. coli, a multiple cloning site, and one or more selectable markers.

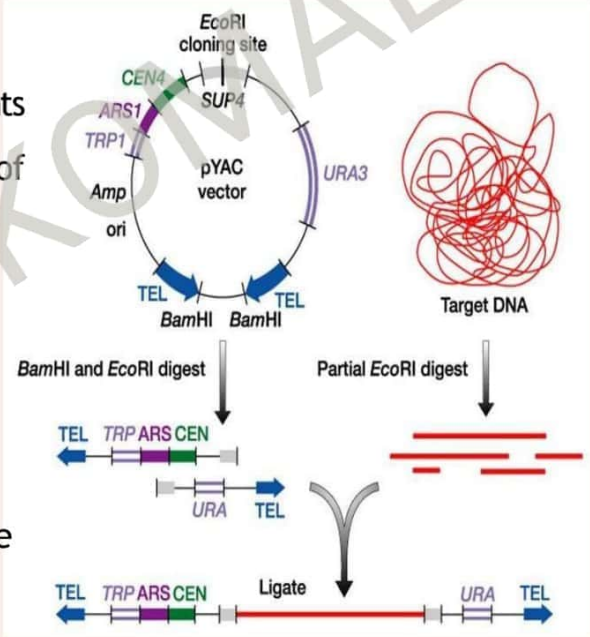
pBeloBAC11, is a BAC vector similar to a plasmid vector with one or more selectable markers (chloramphenicol resistance), a multiple cloning sites, but uses an origin derived from the F factor (plasmid) which limits the copy number of the BAC to one per E. coli cell.



YEAST ARTIFICIAL CHROMOSOMES

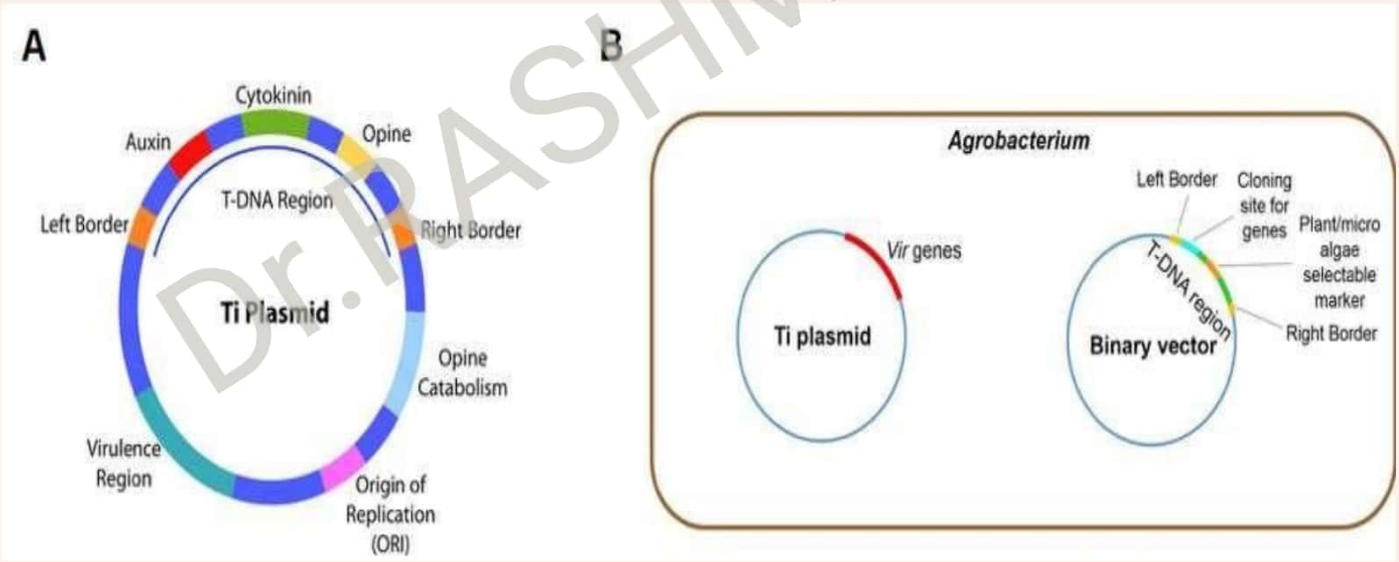
- ✓ Yeast artificial chromosomes (YACs) are cloning vectors that enable artificial chromosomes to be made and replicated in yeast cells.
- ✓ YAC vectors have been used to clone very large DNA fragments (between 0.2 and 2.0 Mb) Eg. in creating physical maps of large genomes such as the human genome.
- ✓ These vector have following features :

1. Autonomously Replicating Sequence (ARS) for plasmid replication.
2. Centromere (CEN) for uniform distribution of chromosome to daughter cells during cell division.
3. Telomeres (TEL) for protection of ends of chromosomes.
4. TRP1 and URA3 for selection of recombinants



Ti-PLASMIDS

- Tumor inducing (Ti) plasmid is found *Agrobacterium tumefaciens.*
- *These are gram negative soil bacteria known as* the Natures Genetic Engineer .
- It causes crown gall disease in dicot plants due to its natural ability to transfer a portion of its plasmid called T-DNA which integrates into the plant chromosomal DNA. This inherent property of bacteria can be exploited for cloning wherein T-DNA can be replaced with gene to be cloned and then can be delivered efficiently into the plants.
- The transfer of the gene can be done through protoplast culture of single cell culture in plants.



Shuttle Vectors

- Prokaryotic vectors cannot work and exist in eukaryotic cells.
- Therefore some vectors have been constructed which may exist in both prokaryotic and eukaryotic cells.
- Such vectors possess two origin of replication i.e. ori^{E} and ori^{EUK} so they are called shuttle vectors.
- ori^{E} functions in E.coli cells and ori^{EUK} functions in eukaryotic cells like Yeast.
- these vectors also contain antibiotic resistant gene amp^{R} as selectable marker.
- Eg : Yeast episomal plasmid(YEP)

Expression vectors

- In addition to the cloning of the desired genes RDT is helpful in producing high amount of protein encoded by the DNA insert.
- The introduced noble(new) gene must be expressed, so expression of cloned genes is carried out by inserting a promoter sequence and a terminator sequence.
- A translation initiation sequence is also introduced into the vector.
- Expression vectors contain these signals for protein sysnthesis.
- Eg : pSOMI