Vectors: The carriers of DNA molecules

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DNA vectors and their properties one of the most important elements in gene cloning is the vector, which in conjunction with the passenger DNA forms the recombinant DNA which can be propagated in suitable host cells. In order to perform its function, a vector must possess the following properties:

- ✓ They should be capable of autonomous replication in at least one host organism.
- ✓ They should be of small size, since this aids the preparation vector DNA and reduces the complexity of analyzing recombinant molecules. They should be capable of amplifying the cloned sequence by occurring in multiple copies. High copy number facilitates in maximizing expression of cloned genes.
- ✓ There should be a unique cleavage site for a range of restriction endonucleases. Occurrence of multiple cleavage sites reduces the likelihood of functional recombinant DNA formation.
- ✓ They should possess one or more genetic markers enabling easy selection of cloned molecules.
- ✓ They should permit detection by simple genetic tests, of the presence of passenger DNA inserted at cloning site.
- ✓ They should have appropriate transcriptional and translational signals located adjacent to cloning sites for better expression of cloned DNA sequences.
- ✓ They should have host specificity when there is biological containment for a vector. A variety of different cloning vectors have been developed by using the items mentioned above as guidelines. They are as follows: plasmids, phages, cosmids, phasmids, shuttle vectors, expression vectors and single stranded DNA

Plasmids

Plasmids are self replicating, double stranded, circular DNA molecules that are maintained in bacteria as independent extra chromosomal entities. These are also found in some yeast but not in higher eukayotes. Plasmids are widely distributed throughout the prokaryotes, vary in size from less than 1×10^6 to greater than 200×10^6 Da and are generally dispensable.
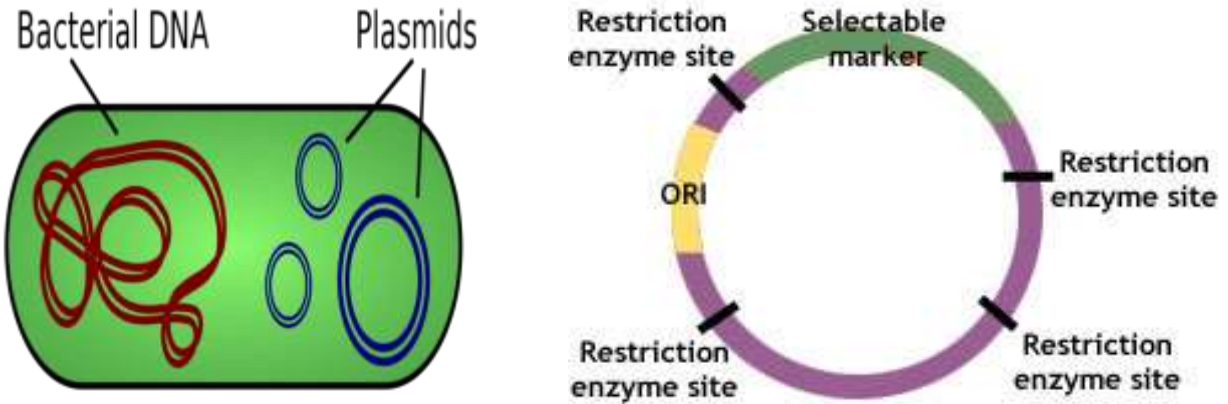
Plasmids can be grouped into two major types: **conjugative** and **nonconjugative**. In conjugative plasmids transfer genes (tra) and mobilizing genes (mob) are present whereas in non-conjugative plasmids tra genes absent. The non-conjugative plasmids can be mobilized by another conjugative plasmid present in the same cell, if the mob gene is intact.
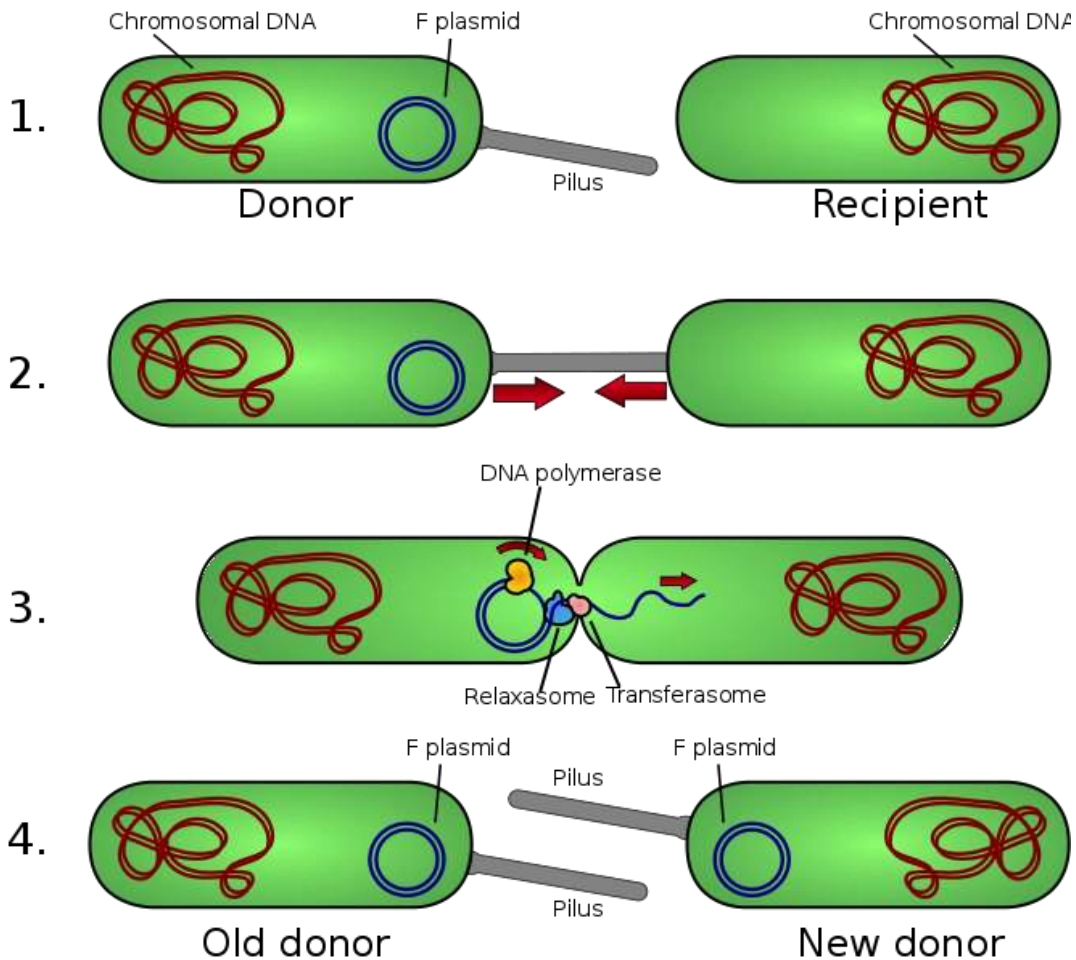
Non-conjugative differ from conjugative plasmids by the absence of tra gene Plasmids can also be categorized on the basis of their being maintained as multiple copies per cell (relaxed plasmids or high copy number plasmids) or as limited copies per cell. The replication of stringent plasmids is coupled to chromosome replication, hence their low copy number. Generally conjugative plasmids are of low molecular weight and present in multiple copies per cell. An exception is the conjugative plasmids RBK which has a molecular weight of 25x106 daltons and is maintained as relaxed plasmid.

Plasmids that carry specific sets of genes for the utilization of unusual metabolites are called as degradative plasmids. Some plasmids will not have any apparent functional coding genes and are called cryptic plasmids. Some of the plasmids do not coexist in the same host cell in the absence of selection pressure and are called incompatible plasmids. Some plasmids are capable of promoting their own transfer to a wide range of host. These plasmids are called as promiscuous plasmids. Plasmids can also be grouped into narrow-host range plasmids and wide host range plasmids based on their nature of infectivity. Based on the origin of plasmids, they can be grouped into naturally occurring plasmids and synthetic plasmids.

pBR322 plasmid

Plasmid pBR322 is the one of the best studied and most often used "general purpose" plasmids. The BR of the pBR322 recognizes the work of the researchers F. Bolivar and R. Rodriguez, who created the plasmid and 322 is a numerical designation that has relevance to these workers. pBR322 is 4362 base pair long and completely sequenced. pBR322 carries two antibiotic resistance genes. One confers resistance to ampicillin (Ampr) and the other confers resistance to tetracycline (Tetr) There are eleven known enzymes which cleave pBR 322 at unique sites. For three of the enzymes, Hind III, Bam HI and Sal I, the target site lies within the Tetr genes and for another two, Pst I and Pru I, they lie in Ampr genes. Thus cloning in pBR 322 with the aid of these enzymes results in insertional inactivation where the inserted DNA disrupts the function of the gene containing the cloning site. Where the cloning site is within in an antibiotic resistance gene, such insertional inactivation results in transformants sensitive to the appropriate antibiotic. Thus, insertional inactivation helps in the selection of recombinants.
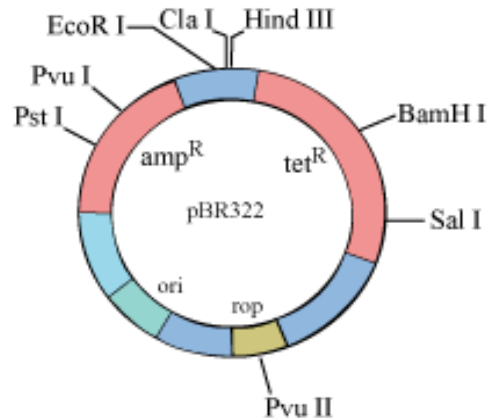
pUC19 plasmid

Plasmid pUC19 is 2686 bp long and contains an ampicillin resistance (Ampr) gene, a regulatable segment of â-galactosidase gene (lacZ) of the lactose operon of E. coli, lac I gene that produces a repressor protein that regulates the expression of lacZ gene, a short sequence with multiple cloning sites (EcoRI, SacI, KpnI, XmaI, SmaI, BamHI, XbaI, SalI, HincII, AccI, BspMI, PstI, SphI and HindIII) and the origin of replication from pBR322. The presence of lac Z and lacI genes allows to select the recombinants based on the â- galactosidase production in the presence of isopropyl- â D-thiogalactopyranoside (IPTG), an inducer of the lac operon. (UC in pUC stands for University of California).
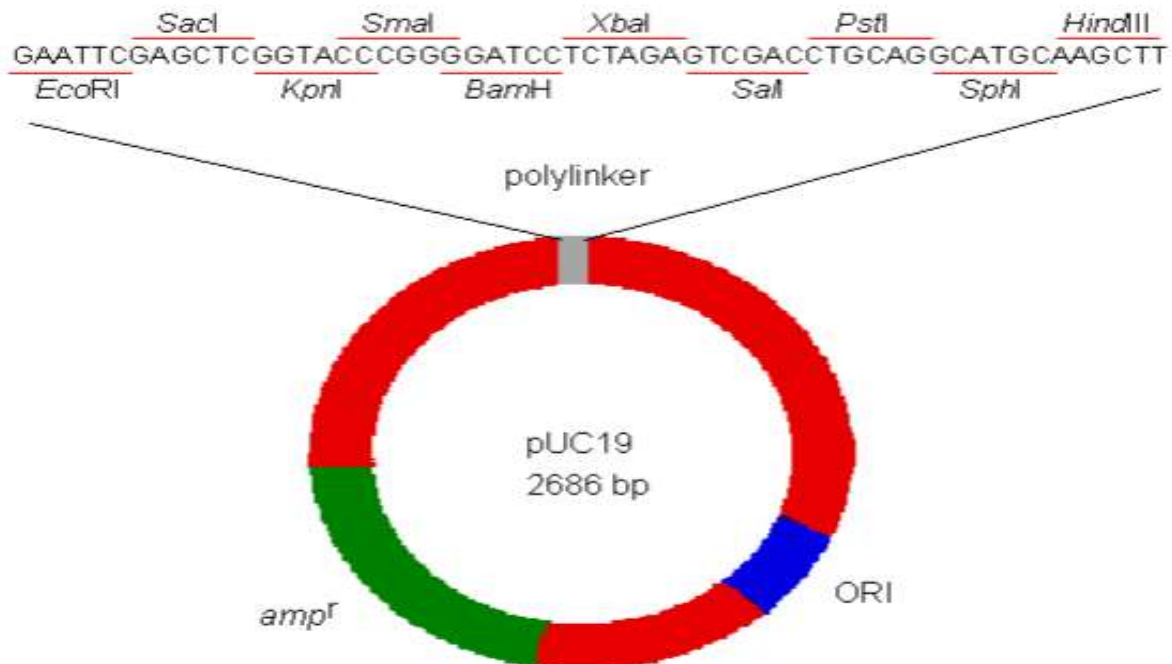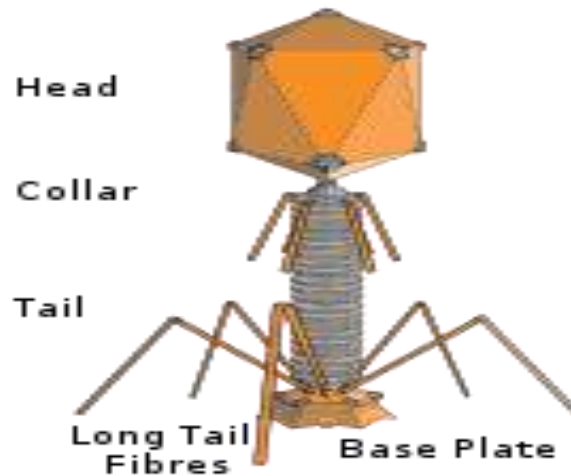

Phages

Derivatives of phage have been developed as cloning vectors since the early days of gene technology. The phage derivatives are considered to be the most suitable cloning vehicles for cloning genomic eukaryotic DNA because of the following advantages over the plasmids.

- ✓ Thousands of phage plaques can be obtained in a single petridish.
- ✓ Selection by DNA-DNA hybridisation is possible
- ✓ In vitro packaging into empty phage head is possible thus increasing phage infectivity
- ✓ Size selection of the packaged DNA is possible
- ✓ Millions of independently cloned virus particle can be constituted to form a gene library.

Bacteriophage is a genetically complex but very extensively studied virus of E. coli. The DNA of phage, in the form in which it is isolated from the phage particle is a linear duplex molecule of 48502 bp (~49kb) in length. The DNA isolated from virus particles is a double

stranded linear molecule with short complementary single stranded projections of 12 nucleotides at its 5' ends. These cohesive termini, also referred to as cos sites, allow the DNA to be circularized after infection of the host cell.

The genetic map of phage ë comprises approximately 40 genes which are organized in functional clusters. Genes coding for head and tail are proteins (genes A-J) are on the left of the linear map. The central region contains genes, such as int, xis, exo etc. which are responsible for lysogenisation i.e., the process leading to the integration of viral DNA and other recombination events. Much of this central region is not essential for lytic growth. Genes to the right of the central region comprise six regulatory genes, two genes (O and P) which are essential for DNA replication during lytic growth and two more genes (S and R) which are required for the lysis of the cellular membranes.

**The structure of a typical tailed bacteriophage**

Types of phage vectors

Wild type phage DNA itself cannot be used as a vector since it contains too many restriction sites. Further, these sites are often located within the essential regions for phage's growth and development. From these wild phages, derivatives with single target sites and two target sites have been synthesized. Phage vectors which contain single site for the insertion of foreign DNA have been designated as insertional vectors; vectors with two cleavage sites, which allow foreign DNA to be substituted for the DNA sequences between those sites, are known as replacement vectors. Apparently if too much non-essential DNA is deleted from the genome it cannot be packaged into phage particles efficiently. For both types of vector, the final recombinant genome must be between 39 and 52 kb of the wild type phage genome, if they are to be packaged into infectious particles. Insertion vectors must therefore be at least 39 kb in length to maintain their viability. This places an upper limit of about 12 kb for the size of foreign DNA fragments which can be inserted. Replacement vectors have a larger capacity because the

entire non-essential region can be replaced, allowing the cloning of the fragments upto 22 kb. Several types of vectors have been developed which allow direct screening for recombinant phages and are useful for cloning specific DNA fragments. A list of phage vectors with their characteristics is given below.

Cosmids

Plasmids containing phage cos sites are known as cosmids. Cosmids can be used to clone large fragments of DNA by exploiting the phage in vitro packaging system. Since cosmids have advantages of both plasmids and phage vectors they can be delivered to the host by the more efficient infection procedures rather than by transformation. Cloning with cosmid vectors has widened the scope of plasmid cloning in the following ways.

✓ The infectivity of plasmid DNA packaged in phage head is at least three orders of magnitude higher than that of pure plasmids DNA.
✓ The process almost exclusively yields hybrid clones so that a subsequent selection for recombinant DNA becomes unnecessary.
✓ In contrast to normal plasmid transformations, the system strongly selects for clones containing large DNA inserts. It is therefore, particularly well suited for generating genomic libraries.
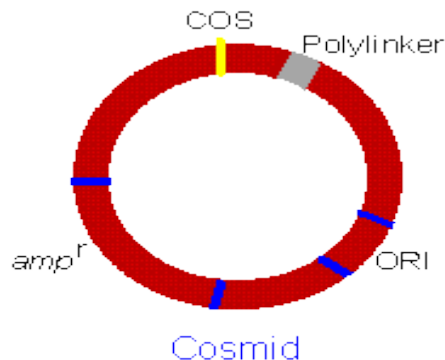

Phasmids

Phasmids, also called as phagemids, are hybrids formed between small multicopy plasmids and bacteriophages. A phasmid can be propagated as a plasmid or lytically as a phage. Lytic functions of phasmid can be switched off by propagation in the appropriate lysogene where the plasmid origin of replication is used for maintenance. The phasmid may replicate as phage if propagated in a non-lysogenic strain. In the case of phasmids based on ë, such as ë1130, the temperature sensitive gene, cI857 carried by the vector may be used to switch between replication modes, simply by growing the host at the permissive (plasmid mode) or restrictive (phage mode) temperature.

Phasmids are particularly useful in the generation and analysis of mutations exhibiting non-selectable or lethal phenotypes, such as those affecting the replication of plasmids. Phasmids

may also be used as phage replacement vectors and for directing the high level expression of protein from cloned sequences by replication in the phage mode.

Bacterial Artificial Chromosomes (BAC)

BACs are based on bacterial mini-F plasmids, which are small pieces of episomal bacterial DNA that give the bacteria the ability to initiate conjugation with adjacent bacteria. They have a cloning limit of 75-300 kb.
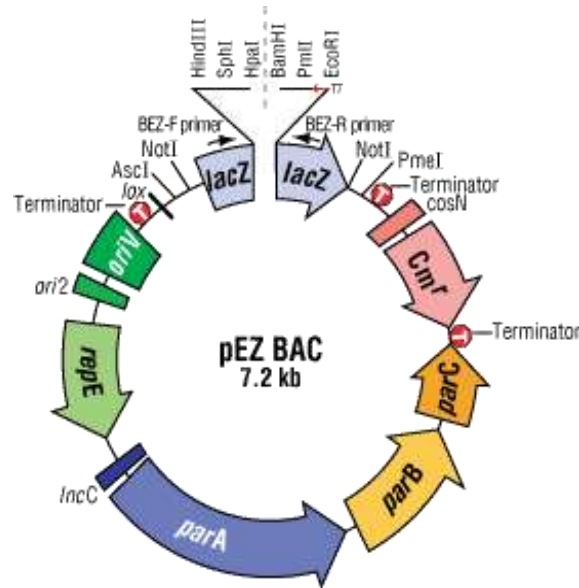

Yeast Artificial Chromosomes (YAC)

YACs are artificial chromosomes that replicate in yeast cells. They consist of:

- ✓ Telomeres, which are ends of chromosomes involved in the replication and stability of linear DNA.
- ✓ Origin of replication sequences necessary for the replication in yeast cells.
- ✓ A yeast centromere, which is a specialized chromosomal region where spindle fibers attach during mitosis.
- ✓ A selectable marker for identification in yeast cells.
- ✓ Ampicillin resistance gene for selective amplification.
- ✓ Recognition sites for restriction enzymes.

The procedure for making YAC vectors is as follows:

1. The target DNA is partially digested by a restriction endonuclease, and the YAC vector is cleaved by restriction enzymes.
2. The cleaved vector segments are ligated with a digested DNA fragment to form an artificial chromosome.
3. Yeast cells are transformed to make a large number of copies. They are the largest of the cloning vectors, with a cloning limit of 100-1000 kb, however they have very low efficiency.

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Sticky ends which can insert the foreign DNA
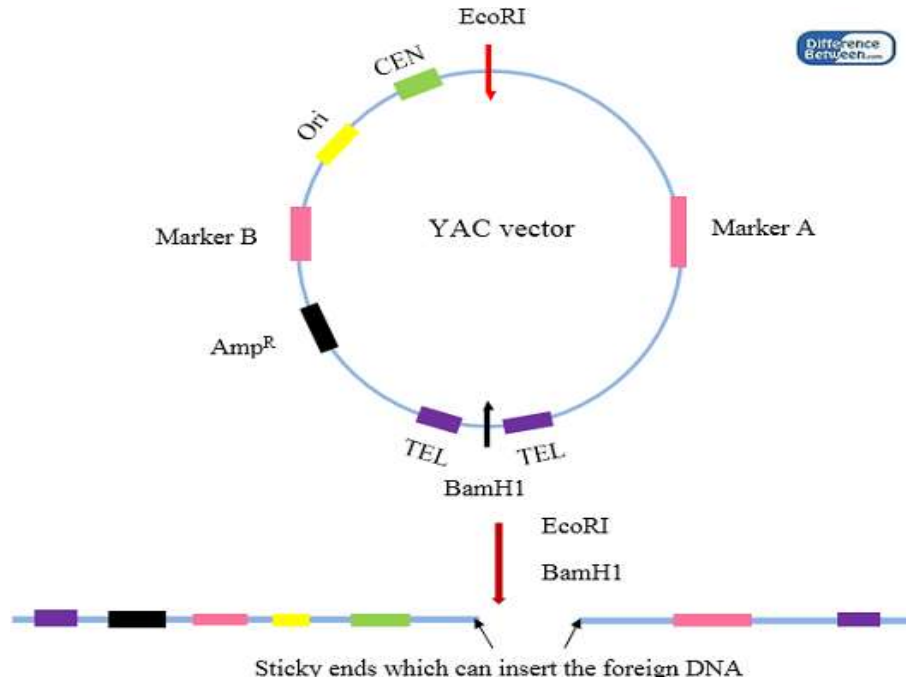
Shuttle vectors

Shuttle vectors normally comprise an E. coli plasmid or part of such plasmid (e.g., pBR 322), ligated in vitro to a plasmid or virus replicon from another species. Shuttle vectors can be made, for example, for E. coli/B. subtilis, E. coli/yeast or E. coli/mammalian cells. The shuttle vector strategy permits the exploitation of the many manipulative procedures, such as amplification, available in E. coli (or other genetically well characterized species such as *B. subtilis or S. cerevisiae*) backgrounds. The ability to transfer cloned genes across species boundaries is of potential value in the genetic manipulations of industrially important species and this can be achieved by using shuttle vectors.

Expression vectors

In DNA cloning experiments all the genes cloned are not expressed fully because of weak promoters in vector DNA. This can be dramatically improved by placing such genes downstream of strong promoters. An additional problem in maximizing expression of cloned genes in E. coli which is frequently encountered with genes from a heterologous source is that the gene carries no translation start signal which can be efficiently recognized by the E. coli translation system. This problem may arise for heterologous genes cloned into any host. Thus, even though the gene can be transcribed from a promoter within the vector, the resulting mRNA is poorly translated and little or no protein product will be synthesized. In such cases alternative strategies available are fusing the gene to amino terminal region of vector gene that is efficiently translated in the host or coupling the gene to a DNA fragment carrying both strong promoter and a ribosomal binding site. Vectors with this additional feature are called expression vectors.