Cloning Vectors

Introduction

A cloning vector is a small piece of DNA, taken from a virus, a plasmid, or the cell of a higher organism, that can be stably maintained in an organism, and into which a foreign DNA fragment can be inserted for cloning purposes. Vectors can replicate autonomously (independent of host genome replication) in host cells and facilitate the manipulation of the newly created recombinant DNA molecule. The major step in recombinant technology includes the preparation of large number of identical DNA molecules. These recombinant DNA molecules are composed of vector plus an inserted DNA fragment which is introduced into host cells. This single recombinant DNA molecule replicates independently producing a large number of recombinants that include the fragment of DNA originally linked to the vector. Therefore, the vector must contain the features that allow the manipulation by inserting or deleting the specific DNA fragment in or out of the vector.

The basic procedure of molecular cloning involves a series of steps.

First, the vector and the DNA fragments to be cloned are generated by using the same restriction endonuclease enzymes.

Second, Vector and DNA fragments produced by digestion with restriction enzymes are ligated to each other by the enzyme ligase.

Third, this recombinant DNA molecule is transferred to a host cell where it replicates and produces large number of identical copies known as clones.

Finally, the cloned DNA segments can be recovered from the host cell, purified, and analyzed in various ways.

Two types of vectors are most commonly used: E. coli plasmid vectors and bacteriophage λ vectors. Plasmid vectors replicate along with their host cells, while λ vectors replicate as lytic viruses, killing the host cell and packaging the DNA into virions.

Properties of Vector: All commonly used cloning vectors plays an important role in molecular cloning and to accomplish this task they must possess some specific features which make them the carrier DNA molecule.

Four important features of all cloning vectors are:-

Origin of replication (ori):- It is necessary for their replication in the host cell. Frequently most of the cloning is performed by using E. coli because of ease and convenience. The ColE1 origin of replication is found in many plasmids necessary for their propagation and maintenance in E. coli. Some vectors also include elements that allow them to be maintained in another organism in addition to E. coli.

Cloning site: -All cloning vectors contain unique restriction sites for manipulation either by insertion or deletion of specific DNA sequence within the vector. This may be a multiple cloning site (MCS), which contains many unique restriction sites.

Selectable marker: - Vector contains selectable marker genes which distinguish between the transformed and non-transformed cells. Different types of selectable markers are carried by the vector for e.g. antibiotic resistance marker which includes resistance to ampicillin, kanamycin or penicillin etc., auxotrophic selection marker and more.

There recovery from the host cells is quite easy and there are many possible choices of vector are available depending on the purpose of cloning.

Types of Vectors:

Numerous cloning vectors are in current use, and the choice between them often depends on the size of the DNA fragment that needs to be cloned, copy number, cloning method and on the intended application for the cloned gene. Large insert may not be stably maintained in a general cloning vector, especially for those with a high copy number, therefore cloning large fragments may require more specialized cloning vectors.

There are many types of cloning vectors, but the most commonly used ones are:-

1)Plasmids

2)Bacteriophage such as phage λ

3) Cosmids

4) Bacterial and yeast artificial chromosomes (BACs and YACs).

The choice of vector depends on insert size and application. Vectors like plasmids, phages and cosmids can insert DNA up to10, 20, and 45 kbs respectively. The size of mammalian genes is larger than 100 kbps in size so originally there were limitations in cloning complete gene sequences. To overcome this problem new generation vectors are engineered which have properties of host cell chromosomes. These include artificial chromosome vectors like bacterial artificial chromosomes (BACs) and yeast artificial chromosomes (YACs).

Let us discuss each vector in detail Plasmids Bacterial plasmids are small circular DNA molecules that replicate their DNA independently of the bacterial chromosomes. These extra-chromosomal DNAs, which occur naturally in bacteria, yeast, and some higher eukaryotic cells, exist in a parasitic or symbiotic relationship with their host cell. Range of plasmids varies in size from a few thousand base pairs to more than 100 kilo base pairs (kbs). Different types of plasmids have been found in bacteria. However, the plasmids with properties like drug resistance genes are routinely used as vectors. This drug resistance phenotype helps to distinguish between the transformed and non-transformed cells by plasmids. The major advantage of using plasmids as a vector is there large copy number per cell and the efficient means of amplifying cloned DNA.

There are different types of genes present on plasmid that provides benefit to the host cell. For example, some bacterial plasmids encode enzymes that inactivate antibiotics and allows host cell to survive by providing drug-resistance. This additional presence of plasmids with drug resistance gene has become a major problem in the treatment of a number of common bacterial pathogens. Other then drug-resistance genes many of these plasmids also contain genes responsible for forming macromolecular tube or pilus. The major function of this tube is to transfer the copy of plasmid to other host cell of the same or related bacterial species. The genes responsible for such type of transfer are called “transfer genes”.

Plasmid DNA as a vector

Plasmids are naturally occurring extra-chromosomal double-stranded circular DNA molecules that carry an origin of replication and replicate autonomously within bacterial cells. The plasmids most commonly used in recombinant DNA technology replicates in E. coli. Engineered plasmid vector contains an origin of replication, multiple cloning site and

selectable marker to optimize their use as vectors in DNA cloning. To simplify working with plasmids, their length is reduced; many plasmid vectors are only ≈3kb in length.

Plasmid DNA Replication The replication origin (ORI) is a specific DNA sequence of 50 – 100 base pairs that must be present in a plasmid to replicate. Host-cell enzymes bind to ORI, initiating replication of the circular plasmid. Once DNA replication is initiated at ORI, it continues around the circular plasmid regardless of its nucleotide sequence. Thus any DNA sequence inserted into such a plasmid is replicated along with the rest of the plasmid DNA; this property is the basis of molecular DNA cloning.

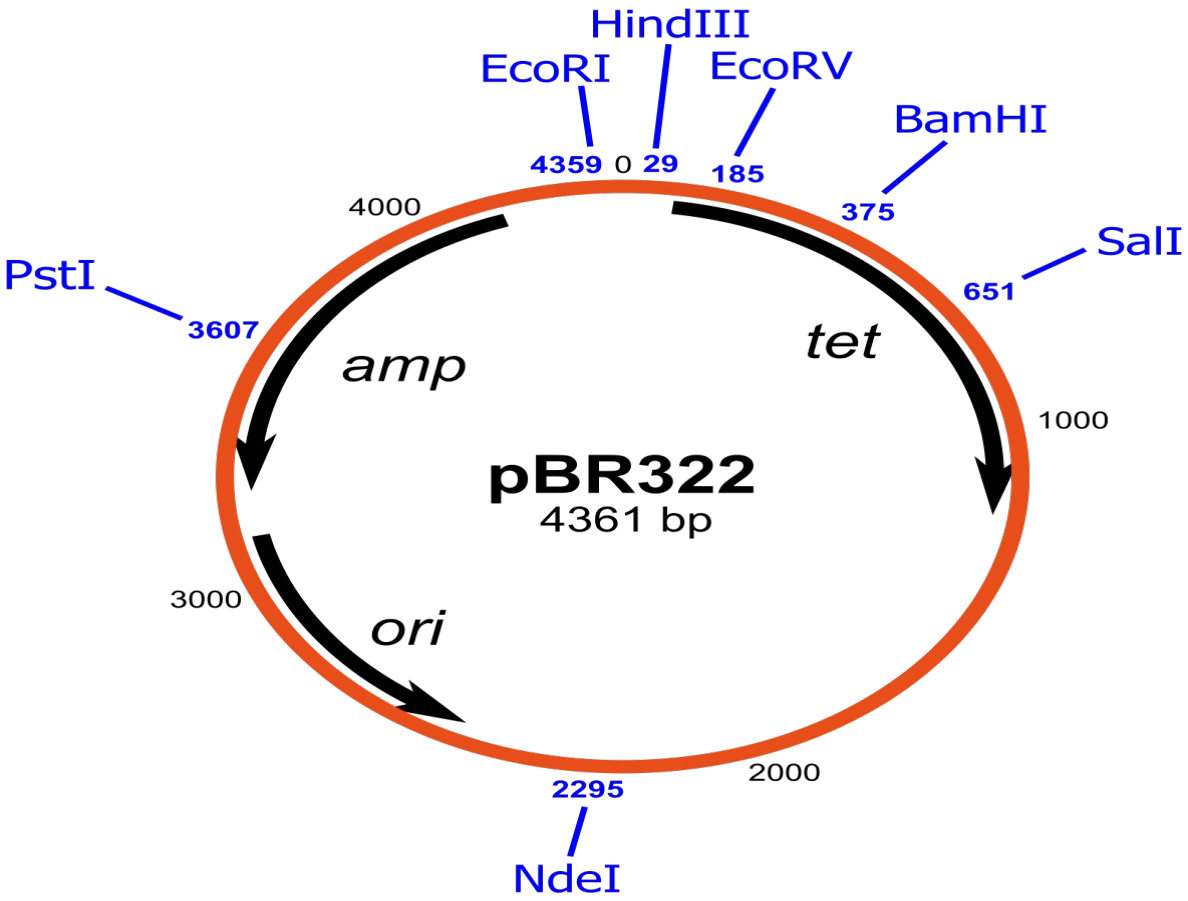
Selection of Transformed Cells Once the vector inserted with DNA fragment of interest transformed to the host cell then the next crucial step is to select the transformed and non transformed cells. To distinguish between them plasmid vector must contain a selectable gene, most commonly a drug-resistance gene encoding an enzyme that inactivates a specific antibiotic. For example the transformed vector carry gene(β-lactamase) for ampicillin-resistance which inactivates the antibiotic ampicillin. Those cells which contain plasmid are able to survive in presence of antibiotic and can be easily selected from the large number of cells that do not contain plasmid, by growing them in an ampicillin-containing medium.

Examples of Plasmid vectors:-

Two plasmid vectors that have been extensively used in genetics are pBR 322 and pUC. Basically these vectors are derived from natural plasmids, and later both have been genetically modified for convenient use as recombinant DNA vectors.

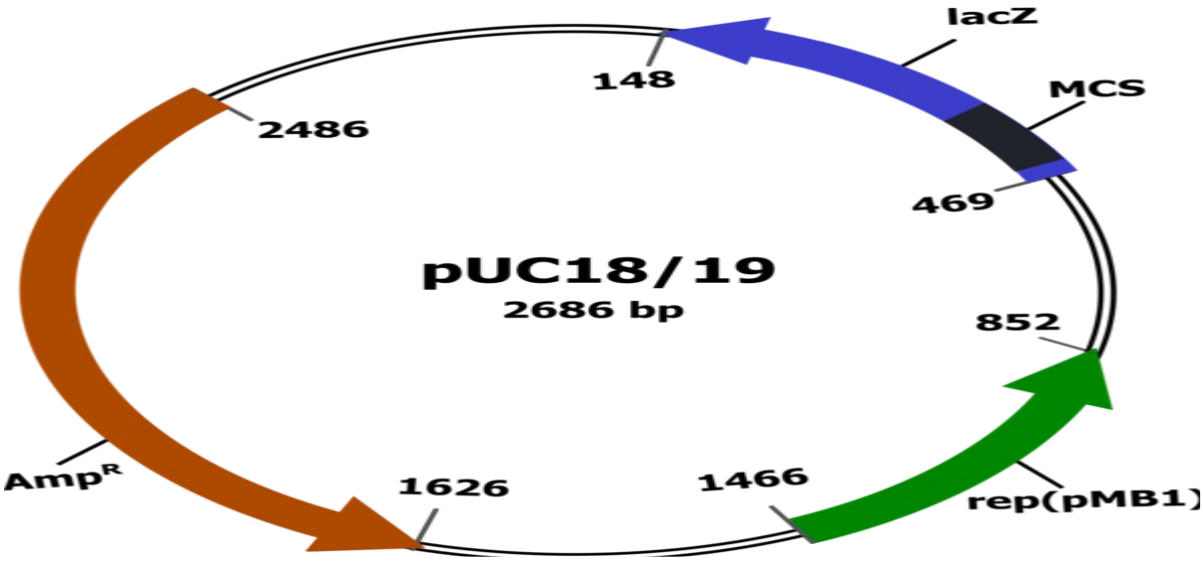
pBR322:

The plasmid vector pBR322, constructed in 1974 by Bolivar and Rodriguez, was one of the first genetically engineered plasmids to be used in recombinant DNA technology. Its structure is simple and contains two antibiotic resistance genes(tetRand ampR). One is for tetracycline resistance and another for ampicillin. Both genes contain unique restriction target sites that are useful in cloning. The molecule is a doublestranded circle and 4,361 base pairs in length. The main drawback of this early vector is the low copy number that means they replicate only to one or two copies per cell.



pUC18

The pUC plasmid is a more advanced vector, and is a derivative of pBR322. It is a “high copy number” plasmid (> 500 copies per bacterial cell).It contains genes for resistance to ampicillin (ampR), and for the enzyme beta-galactosidase (lacZ). The major difference between pBR322 and pUC 18 is the presence of this E.coli β- galactosidase gene into which a multiple cloning site is inserted which contain unique restriction sites useful for inserting donor fragments. Digestion with any one of the endonuclease will make a single cut that linearize the circular plasmid DNA, and allow it to recombine with foreign DNA that has been cut with the same endonuclease. The insert size for these plasmids should less than 20 Kbps; if it is larger then there are chances of losing the cloned DNA fragments.



Viral Vectors

In viral vectors the gene of interest is inserted into the virus genome. The use of viral vector offer many advantages for cloning and the subsequent applications of cloned genes. As compared to simple transformation the viral vectors introduced cloned gene into the cell with higher efficiency. There are also special vectors designed for production of high levels of proteins encoded by the cloned genes. Viral vectors are also the vehicles of choice for gene-therapy strategies.

Examples of viral vectors Phage lambda It is a bacterial virus or also known as bacteriophage that infects the E.coli cells. It consists of head, a tail and tail fibers. The head contain the phage linear DNA genome.

There are several reasons of Phage λ to be used as cloning vector.

 The maximum size of insert or DNA fragment of interest can be of 50 Kbps and λ phage head will selectively pack the donor DNA.

 By using the tools of genetic engineering like restriction enzymes and ligase, the new chimeras of phage molecules are prepared and can be either introduced into E. coli directly by transformation or packaged into phage heads in vitro.

 Another important feature of a phage vector is that recombinant molecules are automatically packaged into infective phage particles, which can be conveniently stored and handled experimentally.

Single-stranded phages

This is another category of bacteriophage which contains single stranded DNA which is also known as infective strand. When they infect the bacteria this ssDNA converts into the ds DNA i.e. the replicative form. This double-stranded replicative form further can be isolated and used for cloning. Phage M13 is the one most widely used for this purpose.

Cosmids

Cosmids vectors are hybrids of λ phages and plasmids, and their DNA can replicate in the cell like that of a plasmid or be packaged like that of a phage.

They carry λ cos site which helps in packaging into a phage head. They infect bacteria as do phages but do not lyses the host cells. Most of the cosmids vectors are about 5 Kbps and around 33 to48 kb of DNA can cloned in these vectors.

YAC and BAC Another category of vectors includes Yeast artificial chromosomes (YACs) and Bacterial artificial chromosome (BAC). These vectors have become essential

research tools as they enable large fragments of DNA to be cloned. Insert of up to 3,000 kbs may be carried by yeast artificial chromosome.YAC vectors contain all the elements needed to maintain a eukaryotic chromosome in the yeast nucleus: a yeast origin of replication, two selectable markers, and specialized sequences derived from the telomeres and centromeres, regions of the chromosome needed for stability and proper segregation of the chromosomes at cell divisions. 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 used to sequence the genome of organisms in genome projects, for example the Human Genome Project.

Expression vectors

For identification and expression of specific cloned gene in the form of protein requires special types of vectors known as expression vectors. These vectors include features of bacterial transcription and translation therefore able to express the gene in bacteria. The cloned gene is inserted next to appropriate bacterial transcription and translation start signals.

Major Application of Vectors

Each type of vectors has their significant use in the area of genetic engineering. Plasmid: Subcloning and downstream manipulation, cDNA cloning and expression assays.

Phage: Genomic DNA cloning, cDNA cloning, and expression libraries.

Cosmids: Genomic library construction.

BACs (Bacterial artificial chromosomes): Analysis of large genomes.

YACs (Yeast artificial chromosomes): Analysis of large genomes, YAC transgenic mice.

CONCLUSION:

Recombinant DNA, the term generally reserved for DNA molecules produced by joining segments derived from different biological sources. The recombinant DNA molecule is placed in a host cell, either prokaryotic or eukaryotic. The host cell then replicates (producing a clone), and the vector with its foreign piece of DNA also replicates. The foreign DNA thus becomes amplified in number, and following its amplification can be purified for further use. Cloning vectors are carrier DNA molecules with various specific features. There are many possible choices of vector depending on the purpose of cloning. The largest variety of cloning vectors has been developed for use in the bacterial host E. coli. The choice of vector depends upon the insert size and application. Development of vectors accelerated the field of recombinant DNA technology and opens the pathways for cloning of desired genes for various purposes.