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. The vector therefore contains features that allow for the convenient insertion or removal of a DNA fragment to or from vector. Numerous cloning vectors are in current use, and the choice between them often depends on the size of the DNA segment that needs to be cloned and on the intended application for the cloned gene.

Cloning vectors most common types:

- Plasmid:

Many types of plasmids are found in nature, in bacteria and some yeasts. They are circular DNA molecules, relatively small when compared to the host cell chromosome, although plasmids are generally dispensable (i.e. not essential for cell growth and division), they often confer traits (such as antibiotic resistance). One of the earliest commonly used cloning vectors is the pBR322 plasmid. Other cloning vectors include the pUC series of plasmids. Plasmids utilized in genetic recombination technologies and cloning share some common features including origin of replication (Ori), selectable marker site such as Ampicillin which is resistant antibiotic that helps cell growth and restriction enzyme site or multiple cloning site (MCS) (fig. 1).

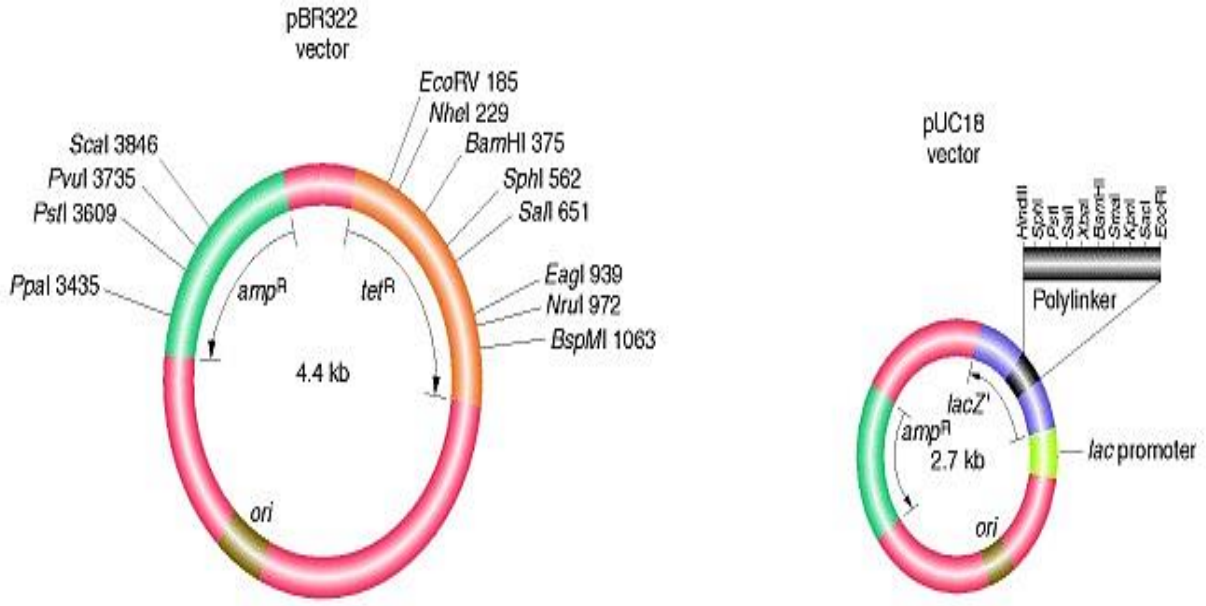

Figure1: Two plasmids designed as vectors for DNA cloning, showing general structure and restriction sites. Important regions indicated are the genes for ampicillin and tetracycline resistance (Apr and Tcr) and the origin of replication (ori).

- Viral vectors.

Viral vectors, in which the gene or genes of interest are incorporated into the genome of a virus, offer many advantages for cloning and the subsequent applications of cloned genes. Because viruses infect cells with high efficiency, the cloned gene can be introduced into cells at a significantly higher frequency than by simple transformation. The bacteriophages used for cloning are the phage λ and M13 phage.

Phage lambda (λ):

The genome of phage is 48.5 kb in length and encodes some 46 genes. The utility of phage as a cloning vector depends on the fact that not all the genome is essential for the phage to function. Thus, there is scope for the introduction of exogenous DNA. In λ cloning vectors, sequences of the bacteriophage genome that are dispensable for virus replication have been removed and replaced with unique restriction sites for insertion of cloned DNA. DNA inserts can be as large as about 15 kb and still yield a recombinant genome that can be packaged into phage particles. To isolate genomic clones of human DNA, for example, random fragments of human DNA with an average size of about 15 kb are ligated to λ vector arms. These recombinant DNA molecules can then be efficiently packaged into phage particles by mixing DNA with λ proteins (called packaging extracts) in vitro. The phage particles are then used to infect cultures of E. coli. Since each recombinant phage forms a single plaque, recombinants carrying unique inserts of human DNA can be isolated. In addition, recombinant phages containing particular genes of interest can be identified by nucleic acid hybridization or other screening methods, as discussed in the next section (fig 2).

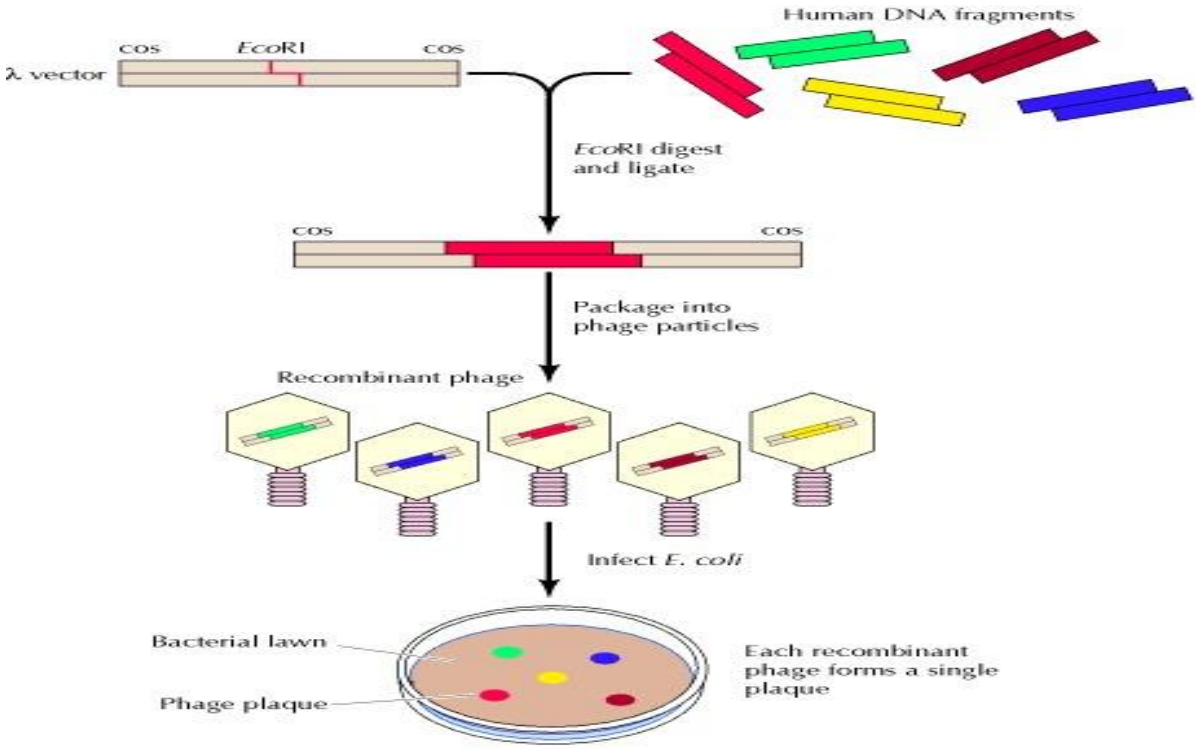

Figure 2: Cloning in bacteriophage λ vectors, the vector contains a restriction site (e.g., an EcoRI site) for insertion of cloned DNA. In addition, cos sites (cohesive ends), which are required for packaging DNA into phage particles, are present on both ends of the vector DNA. Insert DNA (e.g., human DNA) is ligated to the vector, and the recombinant molecules are packaged into phage particles by being mixed with phage proteins. The recombinant phages are then used to infect E. coli. Each recombinant phage, which carries a unique insert of cloned DNA, forms a single plaque in the infected bacterial culture. Progeny phage carrying unique DNA inserts can then be isolated from individual plaques and grown in large quantity.

- Cosmid vectors

Cosmids are conventional vectors that contain a small region of bacteriophage λ DNA containing the cohesive end site (cos). Cosmid vectors accommodate inserts of approximately 45 kb. These vectors contain bacteriophage λ sequences that allow efficient packaging of the cloned DNA into phage particles. In addition, cosmids contain origins of replication and the genes for antibiotic resistance that are characteristic of plasmids, so they are able to replicate as plasmids in bacterial cells. Cosmids are most commonly used to generate large insert libraries (fig 3).

- Bacterial Artificial Chromosomes (BACs)

BACs are Cloning vectors that can accommodate large pieces of DNA up to 1 million base pairs. Because of their stability, ability to accept large DNA inserts, and ease of handling, BACs are now the preferred vector for building DNA libraries of complex organisms including those representing the human and mouse genomes. A DNA fragment is cloned into BAC vectors in a similar fashion to cloning into general cloning vectors; DNA is ligated to a linearized vector and then introduced into an E. coli cloning strain by electroporation. BAC vectors were originally created from part of an unusual plasmid present in some bacteria called the F' plasmid.

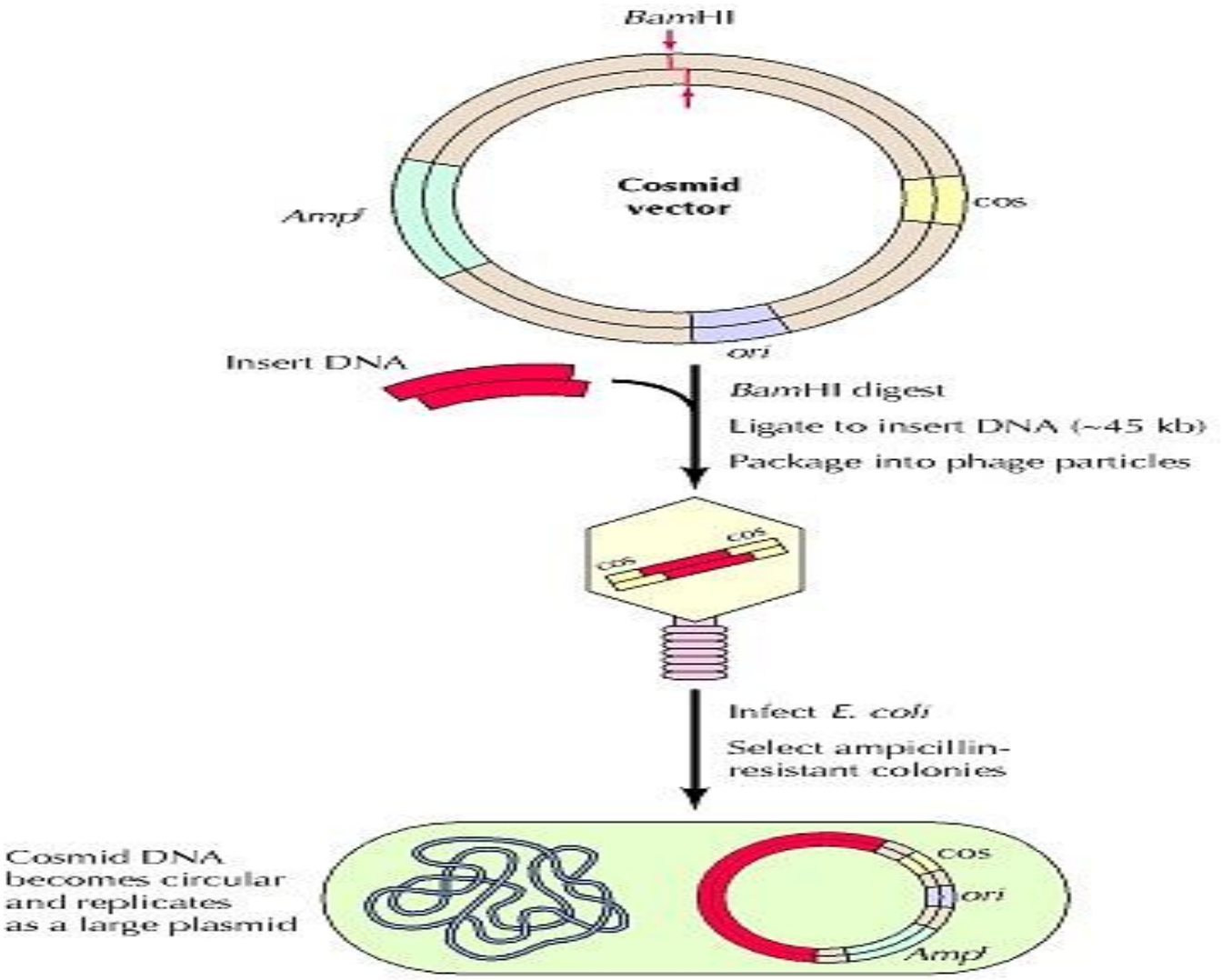

Figure 3: Cloning in cosmid vectors, A cosmid is a plasmid containing cos sites, which allow DNA to be packaged into λ phage particles. Large fragments of insert DNA (approximately 45 kb) are ligated to a cloning site (e.g., BamHI) to yield molecules of approximately the size of the λ genome (48.5 kb), which is appropriate for incorporation into phage particles. The recombinant molecules are made linear (so that a cos site is present at each end), packaged into phage, and used to infect E. coli. The cosmid DNA becomes circular again within the infected bacteria, yielding molecules that replicate as plasmids and confer ampicillin resistance to infected cells.

F’ helps bacteria give its genome to another bacterium but this only happens rarely when bacteria are under a lot of stress Also, F’ has origins of replication and bacteria have a way to control how F’ is copied. In 1992, Hiroaki Shizuya took the parts of F’ that were important and turned it into a vector (fig 4).

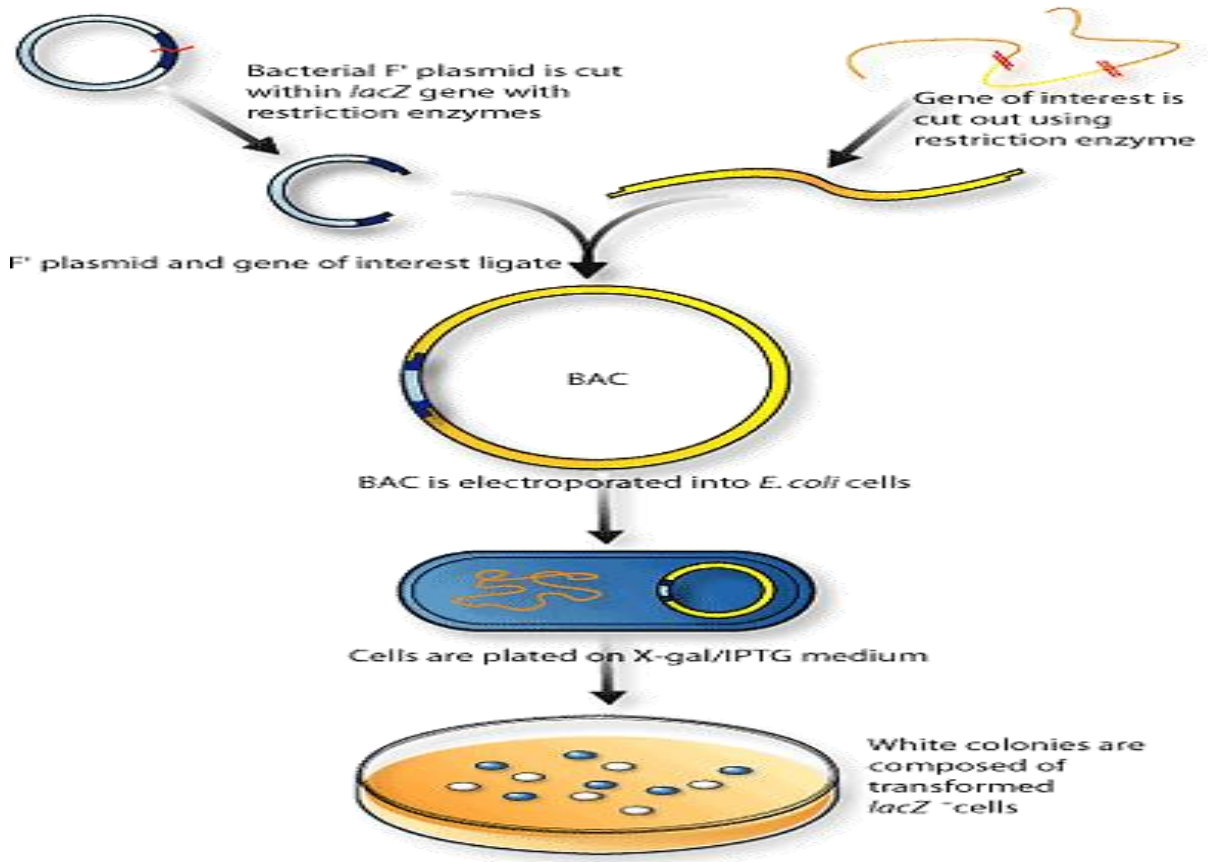

Figure 4: Transforming a bacterium using a BAC vector.

- Yeast artificial chromosomes (YACs)

Yeast artificial chromosomes (YACs) have become essential research tools as they enable large fragments of DNA to be cloned. Artificial chromosomes in the budding yeast Saccharomyces cerevisiae were developed following the isolation of the sequence elements for origins of replication, telomeres, and a centromere s. In 1983, these elements were combined together on the same linear vector and introduced into yeast cells, creating the first artificial chromosomes (fig 5).

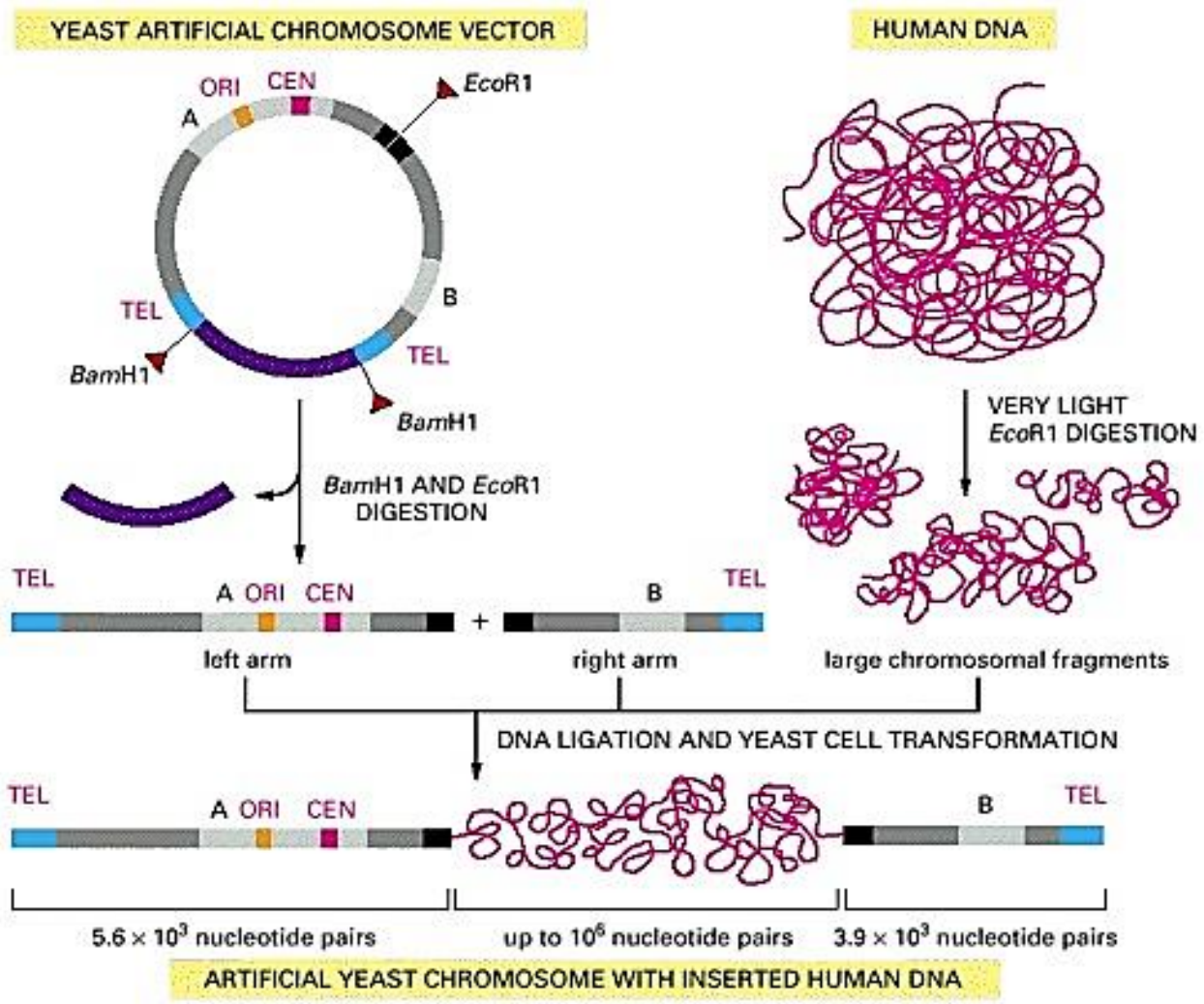

Figure 5: A YAC vector allows the cloning of very large DNA molecules. TEL, CEN, and ORI are the telomere, centromere, and origin of replication sequences, respectively, for the yeast Saccharomyces cerevisiae. BamH1 and EcoR1 are sites where the corresponding restriction nucleases cut the DNA double helix. The sequences denoted A and B encode enzymes that serve as selectable markers to allow the easy isolation of yeast cells that have taken up the artificial chromosome.