

## **Cloning Vectors**

Vectors are the vehicles to transfer DNA between organisms. Vectors are capable of self replication in the host cell, so that the inserted DNA fragment will also replicate along with the vector and clones of DNA can be produced. Most cloning vectors are obtained from naturally occurring extra chromosomal elements. Commonly used vectors are plasmid, phage, cosmic, phagemid, Tie plasmid, artificial chromosomes etc.

Properties of an ideal vector.

1. A vector should have a replication origin which is recognized by host cells replication machinery.
2. A vector should contain selectable marker like resistance towards antibiotics.
3. A vector should have several unique restriction sites for cloning DNA fragment.
4. A vector should be small enough to be isolated without undergoing degradation during purification.

### **PLASMID**

Plasmid is a circular double stranded Extra-chromosomal self replicating DNA found in bacteria.

A typical plasmid vector contains polylinker which can recognize several different restriction enzyme, ampicillin resistant gene (amp) for selectine amplification and replication origin (ORI)

The insertion of DNA fragment in to the cloning vector is carried out by treating the vehicle and the foreign DNA with a restriction enzyme that create the same overhang. Then ligating the fragments together. A plasmid can clone up to 15 Kb of DNA.

### **Bacteriophage**

Virus infecting bacteria are called batriophage. Bacteriophage are capable of cloning large pieces of DNA. Most commonly used bacteriophages are Lamda phage and M13.

## **Lamda phage**

Lamda phage is the commonly used bacteriophage vector. Lamda phage have high transformation efficiency. The DNA to be cloned is first inserted in to the Lamda DNA, by replacing a non essential region. Then by an invitro assembly system, the Lamda virion carrying the recombinant DNA can be formed. Then these virions are allowed to infect bacterial cell. Inside the bacterial cell, the recombinant viral DNA is replicated. Multiplication of virus takes place by cycles of cell lysis and infection of surrounding cells.

## **M13**

The DNA of in M13 is single stranded and circular. Used for obtaining single strand copies of cloned DNA. M13 infect cells by adsorbing to entering through F pili. Thus These phages only infect F+ cells. These phages do not lyse the host cell; instead the progeny viruses are extruded through the layers of the cell membrane. Infected cells will continue to grow and extrude thousands of progeny virus particle.

## **COSMID**

Combination of plasmid vector and the cos site of Lamda phage. Plasmid part helps in selection due to the presence of marker gene. Cos sequence allows them to be packaged in a phage coat & to be transduced to a recipient by the Lamda injection machinery. No genes for viral proteins . so viral particles are not formed in the host . Host cell lysis absent. Cosmod have high transformation efficiency. It can carry up to 45 kb of foreign DNA

## **Phagemid or phasmid**

Phagemid or phasmid is hybrid of the filamentous phage M13 and plasmid. Phasmid contain the origin of replication and selectable marker from plasmid. Phasmid contain an intergenic sequence from filamentous phage, necessary for initiation and termination of viral DNA synthesis and for morphogenesis of phage particle. Phagemid replicate as a plasmid but when cells harboring these plasmids are infected with a suitable filamentous phage (helper phage), phagemid genome behaves like that of a phage.

## **Ti plasmid**

Ti plasmid is a circular plasmid of the soil bacterium *Agrobacterium tumefaciens* and used to transduce its genetic material to plants. This bacterium infects tissue wounds in plants and induces plant cell to proliferate, resulting in a cancerous tissue mass or crown gall near the infection site. A special region on the plasmid, the T DNA containing approximately 8 genes that encode disease characteristic is incorporated into the plant's genome. The T DNA which integrates into the plant's chromosome can be used to transfer foreign genes into plants.

## **Artificial chromosome**

An artificially synthesized chromosome, having a centromere, thus transmissible during cell division after introduction into a cell is called artificial chromosome.

Eg. Yeast artificial chromosome (YAC)

Bacterial artificial chromosome (BAC)

YAC vector is capable of carrying a large DNA fragment (up to 2mb).