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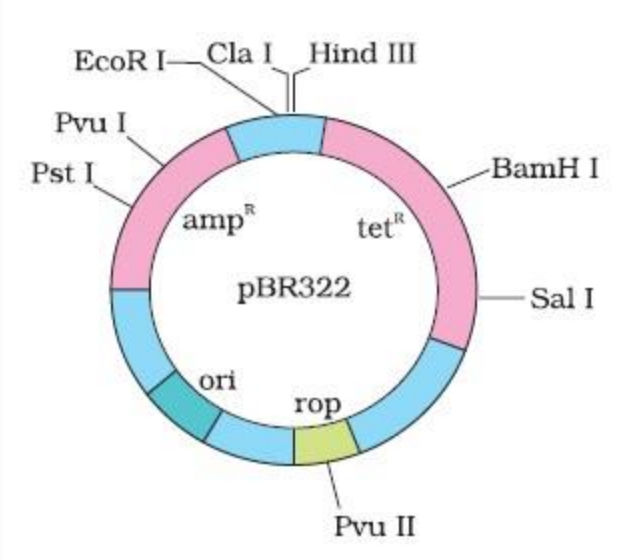
Cloning Vector

Plasmids and Bacteriophages is commonly used vector for cloning. They have ability to replicate within bacterial cells independent of the control of chromosomal DNA. Bacteriophages because of their high number per cell, have very high copy numbers of their genome within the bacterial cells.

Following features are required to facilitate cloning into a vector-

a. **Origin of replication (ori)** – the sequence from where replication starts and any piece of DNA when linked to this sequence can be made to replicate within the host cells.This sequence is responsible for controlling the copy number of the linked DNA.
b. **Selectable marker**-help in the identifying and eliminating non transformants and selectively permitting the growth of the transformants. **Transformation** is a procedure through which a piece of DNA is introduced in a host bacterium. Generally,the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for E. coli.
c. **Cloning sites**– to link the foreign DNA, the vector need to have single recognition sites for the commonly used restriction enzymes as presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning. The ligation of foreign DNA is carried out at a restriction site present in one of the two **antibiotic resistance genes**.



E. coli cloning vector pBR322 showing restriction sites (Hind III, EcoR I, BamH I, Sal I, Pvu II, Pst I, Cla I), ori and antibiotic resistance genes (ampR and tetR). rop codes for the proteins involved in the replication of the plasmid.

Insertional inactivation:

The most efficient method of screening for the presence of recombinant plasmids is based on the principle that the cloned DNA fragment disrupts the coding sequence of a gene. This is termed as Insertional Inactiviation.

For example, the powerful method of screening for the presence of recombinant plasmids is referred to as Blue-White selection. This method is based upon the insertional inactivation of the lac Z gene present on the vector. The lac Z gene encodes the enzyme beta-galactosidase, which can cleave a chromogenic substrate into a blue coloured product. If this lac Z gene is inactivated by insertion of a target DNA fragment into it, the development of the blue colour will be prevented and it gives white coloured colonies. By this way, we can differentiate recombinant (white colour) and non-recombinant (blue colour) colonies.

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