

Notes

Cloning Vectors : We know that plasmids and bacteriophages have the 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. Some plasmids may have only one or two copies per cell whereas others may have 15-100 copies per cell. Their numbers can go even higher. If we are able to link an alien piece of DNA with bacteriophage or plasmid DNA, we can multiply its numbers equal to the copy number of the plasmid or bacteriophage. Vectors used at present, are engineered in such a way that they help easy linking of foreign DNA and selection of recombinants from non-recombinants.

- ❖ **Origin of replication (ori)** : This is a 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 also responsible for controlling the copy number of the linked DNA. So, if one wants to recover many copies of the target DNA it should be cloned in a vector whose origin support high copy number.
- ❖ **Selectable marker** : In addition to 'ori', the vector requires a selectable marker, which helps in identifying and eliminating nontransformants and selectively permitting the growth of the transformants. Transformation is a procedure through which a piece of DNA is introduced in a host bacterium (you will study the process in subsequent section). Normally, the genes encoding resistance to antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for E. coli. The normal E. coli cells do not carry resistance against any of these antibiotics.
- ❖ **Cloning sites**: In order to link the alien DNA, the vector needs to have very few, preferably single, recognition sites for the commonly used restriction enzymes. Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning (Figure 11.4). The ligation of alien DNA is carried out at a restriction site present in one of the two antibiotic resistance genes. For example, you can ligate a foreign DNA at the BamH I site of tetracycline resistance gene in the vector pBR322.