



The following are the features that are required to facilitate cloning into a vector.

- (i) **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.
- (ii) **Selectable marker** : In addition to 'ori', the vector requires a selectable marker, which helps in 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 (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.

- (iii) **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. The recombinant plasmids will lose tetracycline resistance due to insertion of foreign DNA but can still be selected out from non-recombinant ones by plating the transformants on tetracycline containing medium. The transformants growing on ampicillin containing medium are then transferred on a medium containing tetracycline. The recombinants will grow in ampicillin containing medium but not on that containing tetracycline. But, non-recombinants will grow on the medium containing both the antibiotics. In this case, one antibiotic resistance gene helps in selecting the transformants, whereas the other antibiotic resistance

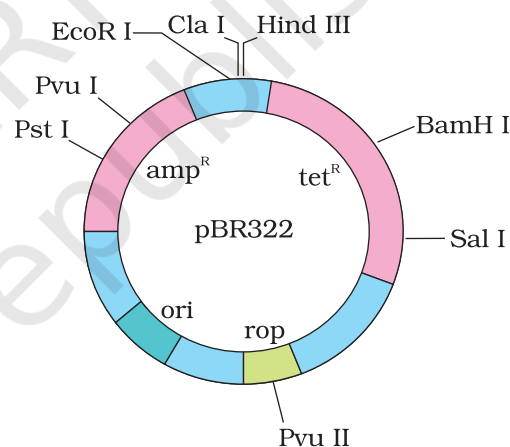


Figure 11.4 *E. coli* cloning vector pBR322 showing restriction sites (*Hind* III, *Eco*R I, *Bam*H I, *Sal* I, *Pvu* II, *Pst* I, *Cla* I), *ori* and antibiotic resistance genes (*amp*^R and *tet*^R). *rop* codes for the proteins involved in the replication of the plasmid.