Vectors in Genetic Engineering

Cloning Vector:

By cloning, one can produce unlimited amounts of any particular fragment of DNA. In principle, the DNA isolated and cut pieces are introduced into a suitable host cell, usually a bacterium such as Escherichia coli, where it is replicated, as the cell grows and divides.

However, replication will only occur if the DNA contains a sequence which is recognized by the cell as an origin of replication. Since such sequences are infrequent, this will rarely be so, and therefore, the DNA to be cloned, has to be attached to a carrier, or vector DNA which does contain an origin of replication.

Criteria of an Ideal Vector:

Vectors are those DNA molecules that can carry a foreign DNA fragment when inserted into it. A vector must possess certain minimum qualifications to be an efficient agent for the transfer, maintenance and amplification of the passenger DNA.

1. The vector should be small and easy to isolate.

2. They must have one or more origins of replication so that they will stably maintain themselves within host cell.

3. Vector should have one or more unique restriction sites into which the recombinant DNA can be inserted.

4. They should have a selectable marker (antibiotic resistance gene) which allows recognition of transformants.

5. Vector DNA can be introduced into a cell.

6. The vector should not be toxic to host cell.

Types of Vector:

Based on the nature and sources, the vectors are grouped into bacterial plasmids, bacteriophages, cosmids and phagemids (Fig. 22.3).

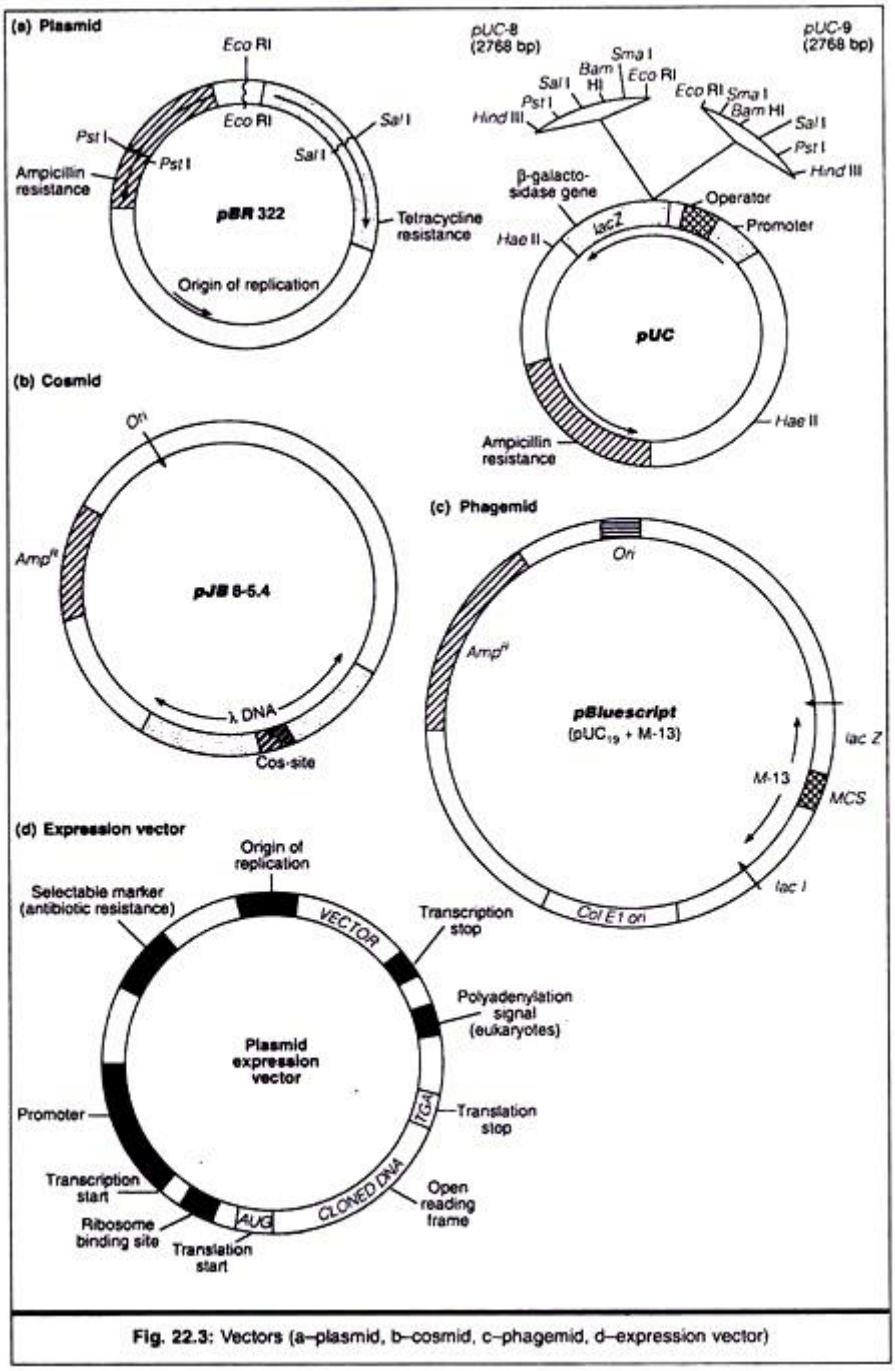

Fig. 22.3: Vectors (a–plasmid, b–cosmid, c–phagemid, d–expression vector)

(a) Plasmid:

Plasmids are the extra-chromosomal, self-replicating, and double stranded closed and circular DNA molecules present in the bacterial cell. A number of properties are specified by plasmids such as antibiotic and heavy metal resistance, nitrogen fixation, pollutant degradation, bacteriocin and toxin production, colicin factors, etc.

Plasmids have following advantages as cloning vehicle (Cohen et a. 1973):

1. It can be readily isolated from the cells.

2. It possesses a single restriction site for one or more restriction enzymes.

3. Insertion of foreign DNA does not alter the replication properties.

4. It can be reintroduced into cell.

5. Selective marker is present.

6. Transformants can be selected easily by using selective medium.

7. Multiple copy numbers are present in a cell.

Some plasmid vectors are pBR 322, pBR 327, pUC vectors, yeast plasmid vector and Ti, Ri plasmids. Ti and Ri Plasmids are widely used in plant system for genetic transformation.

Among higher plants, Ti plasmid of Agrobacterium tumefaciens or Ri plasmid of A. rhizogenes are the best known vectors. T-DNA, from Ti or Ri plasmid of Agrobacterium, is considered to be very potential for foreign gene transfer in cloning experiments with higher plants.

pBR 322 and pUC Vectors:

pBR322 is a derived plasmid from a naturally occurring plasmid col El, composed of 4362 bp DNA and its replication may be more faster. The plasmid has a point of origin of replication (ori), two selectable marker genes conferring resistance to antibiotics, e.g., ampicillin (amp^r), tetracycline (tet^r) and unique recognition sites for 20 restriction endonucleases.

Tetracycline resistance gene has a cloning site and insertion of foreign segment of DNA will inactivate the tet^r gene. The recombinant plasmid will allow the cells to grow only in presence of ampicillin but will not protect them against tetracycline .

Another plasmid used in gene cloning is pUC vector available in pairs with reverse orders of restriction sites relative to lac^z promoter. This is a synthesized plasmid possessing ampicillin resistance gene (amp^r), origin of replication from pBR322(on) and lac^z J gene from E. coli. pUC 8 and pUC 9 make one such pair.

(b) Bacteriophage:

The bacteriophage has linear DNA molecule, a single break will generate two fragments, foreign DNA can be inserted to generate chimeric phage particle. But as the capacity of phage

head is limited, some segments of phage DNA, not having essential genes, may be removed. This technique has been followed in λ (Lambda) phage vectors to clone large foreign particle.

Plasmid can clone up to 20 to 25 kb long fragments of eukaryotic genome. The examples of different Lambda phage vectors are λ gt 10, λ gt 11, EMBL 3, etc. M-13 is a filamentous bacteriophage of E. coli whose single stranded circular DNA has been modified variously to give rise M-13 series of cloning vectors.

(c) Cosmid:

Cosmids are plasmid particles, into which certain specific DNA sequences, namely those for cos sites are inserted which enable the DNA to get packed in X particle. Like plasmids, the cosmids perpetuate in bacteria without lytic development. The cosmids have high efficiency to produce a complete genomic library

(d) Phagemid:

These are prepared artificially by combining features of phages with plasmids. One commonly used phagemid is pBluescript IIKs derived from pUC-19.

(e) Plant and Animal Viruses:

A number of plant and animal viruses have also been used as vectors both for introducing foreign genes into cells and for gene amplification. Cauliflower Mosaic Viruses (CaMV), Tobacco Mosaic Viruses (TMV) and Gemini Virus are three groups of viruses that have been used as vectors for cloning of DNA segments in plant system. SV 40 (Simian Virus 40), human adenoviruses and retroviruses are potential as vectors for gene transfer into animal cells.

(f) Artificial Chromosomes:

Yeast Artificial Chromosome (YAC) or Bacterial Artificial Chromosome (BAC) vectors allow cloning of several hundred kb pairs which may represent the whole chromosome. It can be cloned in yeast or bacteria by ligating them to vector sequences that allow their propagation as linear artificial chromosome.

(g) Transposons:

Transposable elements like Ac-Ds or Mu-1 of Maize, P-element of Drosophila may also be used for cloning vector and transfer of gene among eukaryotes.

Expression Vector:

A vector that has been constructed in such a way that inserted DNA molecule is put under appropriate promoter and terminator sequences for high level expression through efficient transcription and translation. Example: Use of promoters ('nos' from T-DNA) or expression cassettes (pRT plasmids) (Fig. 22.3d).

Shuttle Vector:

There are plasmids capable of propagating and transferring genes between two organisms (e.g., E. coli and A. tumefciciens). It has unique origins of replication for each cell type as well as different selectable markers. It can, therefore, be used to shuttle gene from prokaryotes to eukaryotes. Example: pBin19.