Vectors-Shuttle, YAC, BAC, Transposons

Shuttle vectors

- The plasmid cloning vector that can exist and replicate in two different organisms is called shuttle vectors.
- It has two origins of replication, each of which is specific to a host.
- It is also called as **Bi-functional vectors**
- It can also exist in 2 different host.
- Ex: pHV14,pEB10,pHP3, etc. replicate both in bacillus subtilis & E.coli
- pJDB219 is another, can replicate E.coli and yeast

•Kado and tait (1893), and ideal shuttle vector have these characters:

- •It has origin of replication for 2 different hosts.
- •It must be small enough to carry large foreign DNA.
- •It must have suitable genetic markers.
- •It must be easily propagatable in vivo.
- •Cloned gene expression must be easily detected.
- •It must be non-pathogenic & shouldn't induce any stress in the host.

YAC

- Yeast artificial chromosomes(YAC) are derived cloning vectors used to clone large DNA
- fragments in yeast cells. They are linear in shape. Each YAC is made up of three important *regions, namely two telomeres, a centromere and an autonomously replicating sequence (ARS). The YAC having all these sequences is called a minichromosome. It be*haves like an additional chromosome in yeast cell. Eg.YAC2.
- The two telomeres, are located one on either end of the YAC. They are highly conserved and homologous to each other. Each telomere consists of multiple copies of palindromic sequence CCCAAA. Many one-nucleotide gaps occur in DNA strands at the telomere and are mostly confined to the terminal 100 bp region. The telomeres are essential for the stability of YAC in yeast cells. They are derivatives f of chromosomes of Tetrahymena, a protozoan.

The centromere (Cent) is derived from an yeast chromosome. It has three distinct regions- I, II and III. The regions I and III consist of 11 to 14 base pairs. They are separated from each other by the region II consisting of 82 to 89 base pairs rich in TA. Thus DNA of about 120 bp forms the kinetocore of YAC.
The centromere is important for the movement of YAC to daughter cells during cell division.
The ARS is a derivative of yeast chromosome. It has the ability to switch on continuous replication of YAC in which it occurs. It is highly unstable unless it is linked to a centromere.
For cloning purpose a selectable genetic marker such as ampicillin resistance gene (Amp') is inserted between the telomere and ARS. The foreign DNA is inserted into YAC in between the Centromere and telomere.

YAC advantages

- YAC behaves as an additional chromosome in yeast cells. So yeast genome is n disturbed.
- The re-isolation of YAC from transformed yeasts and manipulation are easy.
- Large DNA fragments can be inserted into YAC and introduced into yeast cells.
- YAC can use all enzymes of yeast for its replication, transcription and translation. So it does not need special cares.
- The selection of transformant yeasts is easy.

Uses

- YAC is used to form genomic libraries of prokaryotes and eukaryotes.
- It is used in human genomic project (HGP) to construct gene map of chromosomes in man.
- It is used to clone large DNA fragments for gene walking, a method for screening gene library.

BAC

- (BAC) The synthetic plasmid that behaves as an additional genetic material in bacteria, is called bacterial artificial chromosome (BAC). The BAC exists as a single copy in bacterial cell and is sharply inherited from parent to daughter cells during cell division. Mel Simon and coworkers first developed BAC in 1990 to clone large DNAs in E.coli. BACs are constructed from F plasmid (or F factor) of E.coli. They are us to clone DNAs larger than 100 Kb size. The upper limit of foreign DNA that can be accommodated in BAC is 300-350 Kb size.
- F-plasmid is a circular double-stranded DNA and is about 100 Kb in size. It has several genes, of which a stretch of DNA carrying oris, repE, parA, parB and parC is taken to construct the BAC called pBeloBAC11. A chloramphenicol resistance sequence (Chl') is linked with OriS end. T7 RNA polymerase promoter is isolated from T7 phage and attached to Parc gene. A promoter is isolated from SP6 phage and linked with Chl' gene. The T7 and SP6 promoters are joined by a MCS.
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The pBeloBAC11 consists of oris gene, oft repE gene, parA gene, par B gene, par C gene, ing T7 promoter, MCS, SP6 promoter and Chlr gene. The OriS is the origin of replication for BAC. The repE directs the initiation and orientation of replication of BAC. The genes ParA, Par B and ParC regulate the segregation of BAC into daughter cells during cell division. The gene Chl' is used as the selectable marker for BAC vector. T7 is a powerful promoter for RNA polymerase for transcription of MCS and foreign DNA cloned in it. The SP6 promoter helps for transcription of the other strand of foreign DNA. The MCS containing LacZ has Hind III site for gene cloning. Further, LacZ is designed in such a way that cloning inactivates Lac function for blue-white selection of recombinants.

Transposons

- Transposon is a DNA sequence, which moves from one genome to another. This phenomenon is called transposition. Transposition involves the duplication of the transposon. One copy is retained at the original site and the second copy is transferred.
- *Transposon is also named as transposable element. Babbara Mc Clintock named it as controlling element. It is also called mobile elements or jumping genes or selfish genes.*
- When a plasmid carrying a transposon is introduced into a bacterial cell containing a plasmid that lacks the transposon, in the progeny cells, both plasmids will contain transposons.
- Transposons occur both in prokaryotes and eukaryotes. They are found in E. coli, Drosophila, bacterial plasmids, maize, etc.
- Transposon can be passed from one organism to another only when it is part of a plasmid or virus or bacterial chromosome. It cannot replicate independently.
- *A transposon consists of terminal sequences at the ends and antibiotic resistant genes, transposase genes and insertion sequences in between the terminal sequences.*
- *. The terminal sequence of one end is the inverted sequence of the other end. So they are also called inverted sequences.*

Repeating DNA sequences located at either end of the transposon, enable it to insert into certain common but definite sites in bacterial, plasmid or viral DNA

Types of Transposons

Based on structural complexity three levels of transposons have been recognized

1. Simple transposons or IS elements
2. Complex transposons
3. *Composite transposons.*

Transposons

- *1. The IS Elements*
- **The IS elements are transposons. These are known as insertion sequences or in short as 'IS'. They are usually designated by IS followed by an identifying number**
- Is elements are normal constituents of bacterial chromosomes and plasmids. E.coli has 8 copies of IS1 and 5 copies of IS2.
- The genetic information contained in IS unit appears to be involved only in the insertion process.
- The IS element is a double stranded DNA. It is a short segment of DNA. It consists of 200 bp.
- *The IS element is made up of two terminal sequences and a middle transitional gene.*
- *The terminal sequence of one end is the inverted sequence of the other end. So they are also called inverted sequences.*
- This inverted repeat ranges from 18 to 40 bp long. These inverted sequences are called terminal sequences.
- *The terminal sequences seem to have a role in the mechanism of insertion of IS elements into DNA. These IS elements co-valently join to other genes to form transposons.*

2. Complex Transposons

They carry genes not involved in the transposition process, but carry gen antibiotic resistance. Such transposons are designated as “Tn” followed by an identifying number. For example, Tn3 consists of 4957 bp and has inverted ter peats of 38 pb each. The central region of Tn3 codes for three proteins viz, transp apA), TnpR (repressor), and beta-lactamase that inactivates amphicillin.

3.Composite transposons

They consist of gene containing a central regionflanked by 2 identical or nearly identical IS-like elements that have neither the same nor an inverted relative orientation. These arose by the association of two originally indepe**pendent IS elements. Eg. Tyl of** *yeast cells.*

Transposons

- Significance of Transposons
- Transposons help to transfer a gene of interest from one plasmid or from one bacterium to another.
- It helps to transfer DNA segment from one organism to another.
- It helps to restructure a genome.
- It helps in the construction of a recombinant DNA.
- It helps in the cloning of genes.
- They can cause deletion or inversion of regions of DNA lying between them
- In some cases, they have the ability to acquire and transpose other genomic DNA. This latter event could lead to the spread of antibiotic resistant genes to pathogenic organisms.
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