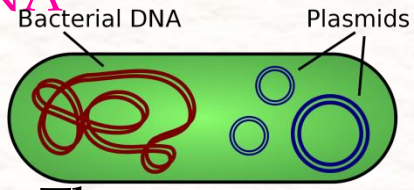
Plasmids, Cosmids, Phasmids

Introduction

- The DNA that carries the desired gene to the host cell is called "**gene cloning vector**". It is also known as Cloning vectors, Cloning vehicles Or carries DNA

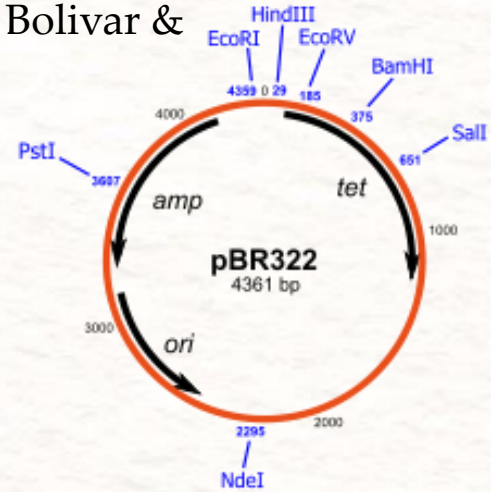


Plasmids:

- They are circular, double stranded DNA usually present in prokaryotic cells. They can carry a foreign DNA of 5-15kbp size to bacteria. Eg: PBR^{322} . Some plasmids carry gene to plant cells. Eg: Ti plasmid.
- It is small, extrachromosomal DNA present in bacterial cells. They are inherited sharply without the influence of chromosomal DNA. They replicate independently due to the presence of an origin of replication.
- Size is 1kbp-200kbp and have limited number of genes.
- Most bacteria contain more than one copy of each plamid. The number of copies of a plasmid present in a cell is called copy number. It can be increased by treating the bacterial culture with Chloramphenicol (an inhibitor of protein synthesis)

- The eukaryotic expect yeasts do not have plasmids. The yeast contains YEP- Yeast episomal plamid, YIP- Yeast intergrating plamid & ARS- Automatically replicating sequence in the cells.
- .Some plasmids are isolated from bacteria and directly used for gene cloning. They are called Natural plamid. Eg RP4 plasmid of Pseudomonas , CoL E1 of E.coli and YEP, YIP of yeast
- Most of Natural Plasmid cannot be used
- because they are large in size
- have no genetic markers
- no unique site
- confer Pathogenicity to the host

- Plasmid vectors created from wild plasmids are called artificial/ constructed/based / derived vectors. During construction unwanted portions are removed from the wild type and desired sequences are inserted. Eg. PBR 322 RSE 1010, psc 101 puB Constructed plasmids are of much use in gene transfer.
- **PBR 322 :-** artificial plasmid
- gene cloning Vector for E.coli
- constructed from 2 vectors & Psc 101 COL E1 and transposons P- plasmid. **BR** -name of workers F. Bolivar & Rodriguez **322** -specific number.
- circular, double stranded plasmid DNA.
- 4363 bp 528 restriction sites for 66 Rest enzymes. Among 20 RE cut it at unique restriction sites
- selectable gene marker [TetR & AmpR]
- Regulation & expression of a gene inserted in this good



- PBR 322 is used as a base plasmid. for invitro construction of derived plasmid vectors such as Puc 8 & puc 9 & Puc 10 etc. and cosmids.
- Eg. Somatostatin gene of man is introduced into, E. coli through PBR 322.

Cosmids:

- a type constructed plasmids containing complementary single stranded sites (cos-sites) Of λ DNA.
- They carry DNA segments of 25-45 kbp size to bacteria . Eg PHV 79.
- It is a derived vector
- It is linear inside the phage Cosmid
- It is circular and behaves like a plasmid inside the host cell.

Cosmid has an origin of replication a selectable markers and gene cloning sites of the Plasmid DNA

- Cosmids was first Constructed by Collins and Hohn in 1978. Eg . Col El Cosmid, PHC79, PJB8, PWE cosmid.

Advantage:

•It pick up larger DNA fragments than plasmid

• So they are used to establish gene Libraries of lower & higher Organisms.

• Gene cloning through cosmids helps in the study of non-sense sequences in the genome of Organisms

•Cosmids help to clone large genes and gene clusters in bacteria.

Phagemid or Phasmids:

A Phagemid a hybrid Vector that has λorigin of replication from the plasmid and the λ Phage DNA. It is constructed by inserting a linearized Plasmid DNA into cleaved λ DNA. This å known as lifting the plasmid λ DNA serves as a site for homologous recombination with chromosomal DNA of E.coli. It helps in vivo multiplication of phage particles that have recombinant phagemids. The plasmid portion i responsible for the independent existence of phage mid as plasmid in E.coli. It may be released free in E. coll. Eg: λZAP.

Bacteriophage vectors

- Bacteria which attacks virus is called bacteriophage used as a vector. In Lambdaphage vector , gene is transferred from one bacteria to other.
- Most of the bacteriophage don't have host cells.
- It has half protein and half DNA .
- It has 20 faces head- 55nm wide.
- Molecular weight 37,500.
- Tail is 180nm length and has bending capacity.
- In tail 35 rings are seen.
- No coat around the tail
- 20-25-kb length DNA fragments can be transferred.
- Used in c DNA library for easy and fast cloning vectors.