VECTOR,GENE CLONING AND GENOMIC LIBRARY

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Vector-The Cloning Vehicles

- Defination:

  Vector are the DNA molecules which can carry a foreign DNA fragment to be cloned.
- They are self replicating in an appropriate host cell.
- The most important vectors are
- Plasmids
- Bacteriophages
- Cosmids

Characteristics of an ideal vector

- An ideal vector should be small in size,with a single restriction endonuclease site,an origion of replication and 1-2 genetic markers.
- Naturally occurring plasmids rarely possess all this characterstic.

1)Plasmids

- Plasmids are extrachromosal,double standard,circular,self-replicating DNA molecules.
- High copy number(10-100 per cell).
- Low copy number(1-4 per cell).

Types of plasmids

- Conjugative Plasmids:

  Conjugative plasmide are the large show stringent control of DNA replication and are present in low numbers.

- Non-conjugative plasmids:

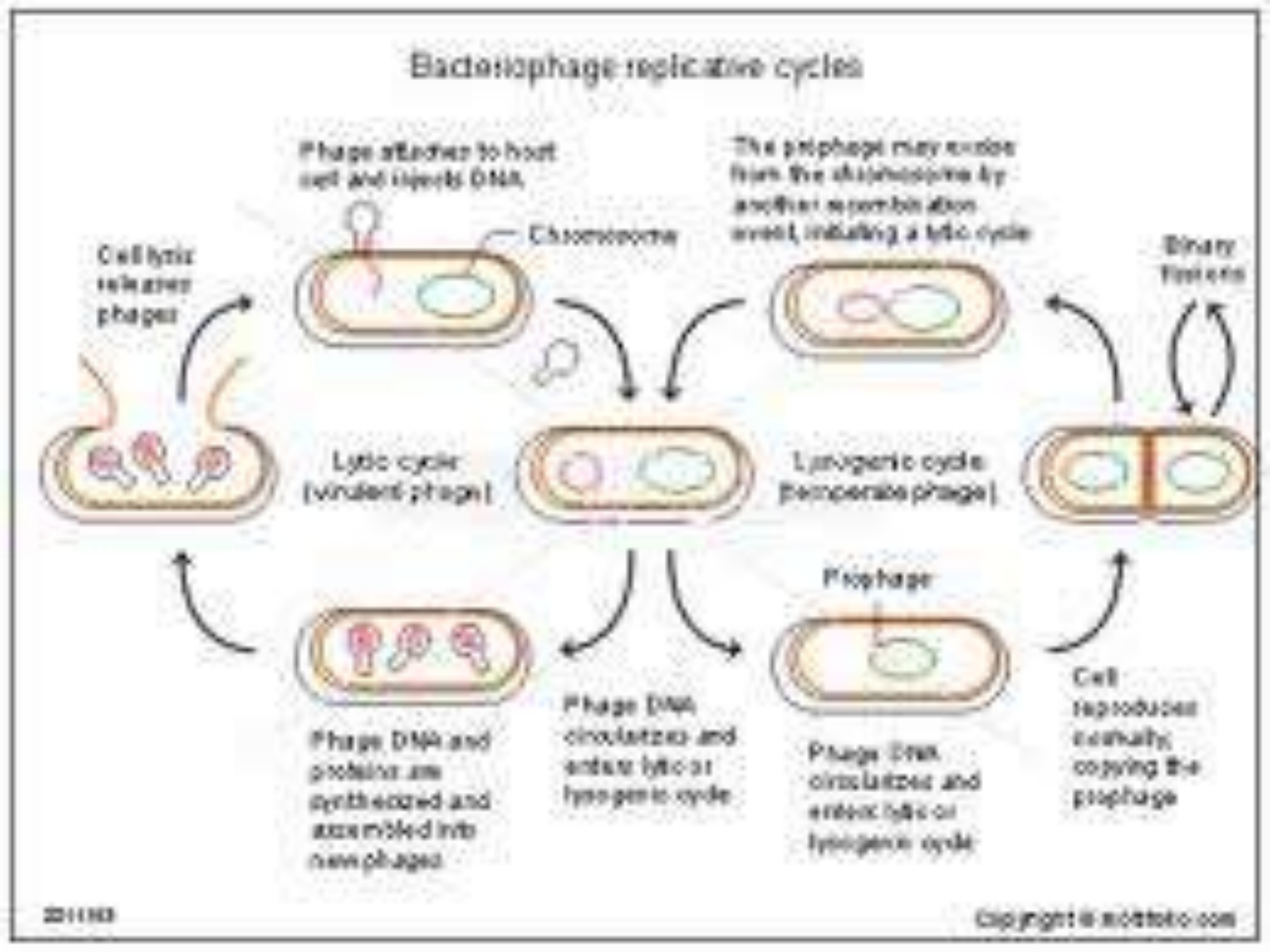
  Non-conjugative plasmids are small show relax control of DNA replication and are present in high numbers.

Another classification is based on copy numbers.

- Stringent plasmids: Presents in limited number.
- Relax plasmids:It occur in large number in each cell.
- F-plasmids:it posses the genes for their own trasfer from one cell to another cell.
- R-plasmids:it carry genes resistance to antibiotics.

2)Bacteriophages

- Bacteriophages or simply phages are the viruses that replicate within bacteria.
- Phase vector can accept short fragment of foreign DNA in their genomes.
- The advantage with phages is that they can take up larger DNA segments than plasmids.
- Bacteriophage fuctions as a vector.
- Phase lamda consists of a head and a tail.
- Its shape is comparable to miniature hypodermic syringe.
- Phage lamda injects its DNA in to the cell inside E-coli.


Bacteriophage replicative cycles
Phage attaches to host cell and injects DNA
Chromosome
The prophage may excise from the chromosome by another recombination event, initiating a lytic cycle
Cell lytic releases phages
Binary fission
Lytic cycle (virulent phage)
Lysogenic cycle (temperate phage)
Prophage
Phage DNA and proteins are synthesized and assembled into new phages
Phage DNA circularizes and enters lytic or lysogenic cycle
Phage DNA circularizes and enters lytic or lysogenic cycle
Cell reproduces normally, copying the prophage
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The phage DNA has two cycle

- Lytic cycle
- Lysogenic cycle

- Lytic cycle: The circular DNA Replicate and its also directs the synthesis of many proteins necessary for the head,tail etc of the phase.

- The circular DNA is then claved and packed in to the head of phage.

- About hundreds phage particles are produce within 20 min after the entry of phage into E.coli.

- The host cell is then subjected lysis and the phage are released.

- phage will reproduced when in infects bacterial cell.

Lysogenic Cycle

- In this phage DNA becomes intergated in to the E.coli chromosomes and replicates along with the host genome.
- No phage particles are sythesized in this pathways.

3)Cosmids

- Cosmides can be constructed by adding fragment of phage lamda DNA including cossite,to plasmids.
- A foreign DNA can be inserted into cosmids DNA.
- The recombinant DNA so formed can be packed as phages and injected in to E.coli.
- Once inside the host cell cosmids behave just like plasmids and replicated.
- The advantage with cosmids is that they can carry larger fragments of foreign DNA compared to plasmids.

Gene cloning starategies:

- A clone refers to a group of organisms,cells, molecules or other objects,arising from a single individual.
- Gene cloning starategies following aspects
- Generation of desird DNA fragments.
- Insertion of these fragments into a cloning vector .
- Introduction of the vector into the host cell.
- Selection or screening of the recipient cell for the recombinant DNA molecule.

Cloning from genomic DNA or mRNA:

The use of mRNA in cloning is preferred for the following reasons:

- mRNA representes the actual genetic information being expressed.
- Selection and isolatation mRNA is easy.
- as introns are removed during processing, mRNA reflects the coding sequence of the gene.
- The syenthesis of recombinant protein is easy with mRNAcloning.

Gene libraries

- The collection of DNA fragments from a particular species represent gene libraries.
- The creation or construction of gene libraries is accomplished by isolating the complete genome.
- Which is cut into fragments and clone in suitable vectors.
- In prokaryotic organism the stuctural genes coding for proteins are continuous.
- In eukaryotes organism the coding regions of structural genes are separated by non-coding regious.

Creating A gene library

- The DNA from the source organism is digested by restrication endonuclease to results in fragment .
- All possible DNA fragments of variable size can be produced.
- The desired fragments can be isolated & cloned.

Other vectors for creating genomic libraries

- These includs cosmids,bacterial, artificial chromosomes(BACS) & yeast artificial chromosomes.

PCR as an alternative to genomic library construction

- PCR with primers can be used to isolate target DNA directly from the genome.
- Long PCR:
- This is achived by using a combination of two DNA polymerase enzyme besides lowering the reaction tempreture.
- Long PCR has been applied for the stuctured analysis of human gene & genomes of HIV.
- PCR can be employed for amplifying selected DNA fragments from genomic libraries.

REFERENCE:

- Dr. U. SATYANARAYANA,Textbook of "Biotechnology"UPPALA AUTHOR PUBLISHER INTERLINKS, Page no:82,89,120.

THANK YOU