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B. Pharm
Semester-V

Subject Name: Pharmaceutical Biotechnology
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Chapter-2
RECOMBINANT DNA TECHNOLOGY

Study of cloning vector:

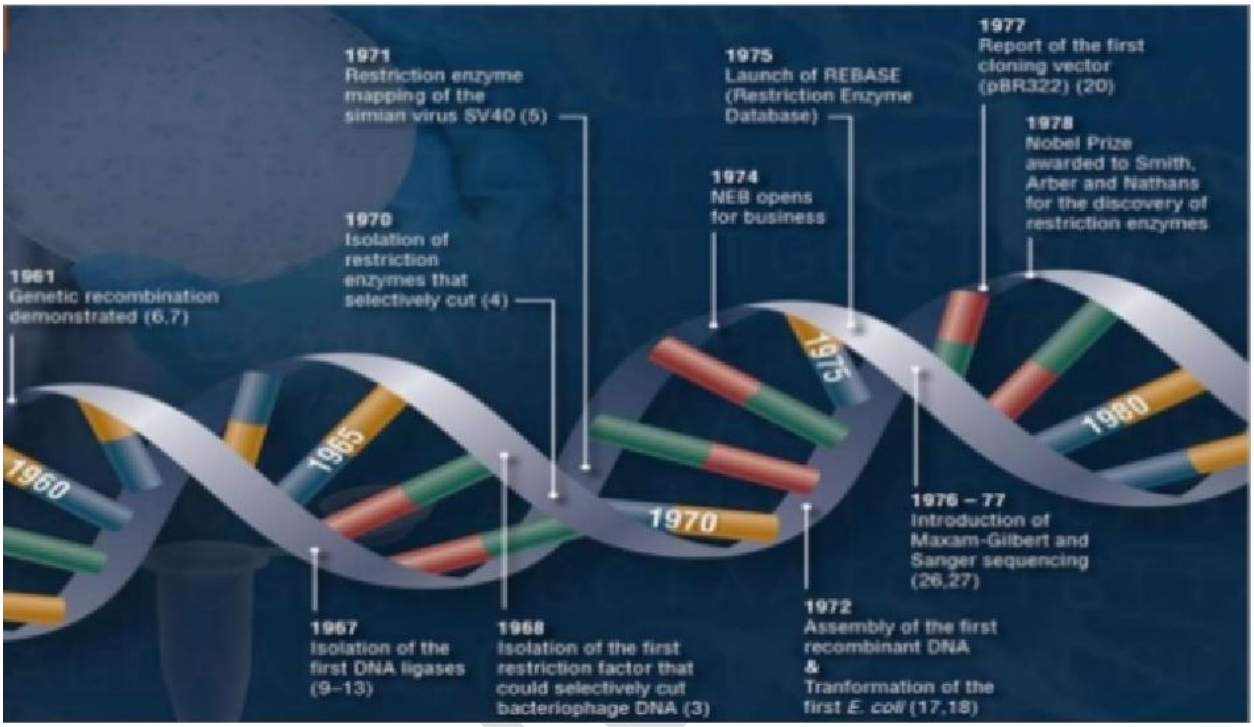
- The molecular analysis of DNA has been made possible by the cloning of DNA. The two molecules that are required for cloning are the DNA to be cloned and a cloning vector.
- A cloning vector is a small piece of DNA taken from a virus, a plasmid or the cell of higher organism, that can be stably maintained in an organism and into which a foreign DNA fragment can be inserted for cloning purpose.
- Most vectors are genetically engineered.
- The cloning vector is chosen according to the size and type of DNA to be cloned.
- The vector therefore contains features that allow for the convenient insertion or removal of DNA fragment in or out of the vector.
- After a DNA fragment has been cloned into a cloning vector, it may be further sub cloned into another vector designed for more specific use.

Why Cloning Vector?

- Cloning vector is used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and expressed.
- It is used to amplify single molecules of DNA into many copes.
- Cloning vectors are DNA molecules that are used to "transport" cloned sequences between biological hosts and the test tube.
- Without cloning vector, molecular gene cloning is totally impossible.

History:

- Scientists (herbert boyer, keiichi itakura and arthur riggs) recognized a general cloning vector with unique restriction sites for cloning in foreign DNA and the expression of antibiotic resistance genes for selection of transformed bacteria.
- In 1977 they described the first vector designed for cloning purpose pBR322 – a plasmid.
- This vector was small – 4kb in size and had two antibiotic resistance genes for selection.



Features of a Cloning Vector:

- Origin of replication :
- This makes autonomous replication in vector.
- Ori is a specific sequence of nucleotide from where replication starts.
- When foreign DNA is linked to the sequence along with vector replication, foreign DNA also start replicating within host cell.

Cloning Site:

- Cloning site is a place where the vector DNA can be digested and desired DNA can be inserted by the same restriction enzyme.
- It is a point of entry for genetic engineering work.
- Recently recombinant plasmids contain a multiple cloning site (MCS) which have many restriction sites.

Selectable Marker:

- It is a gene that resistance to particular antibiotics or selective agents that would normally kill the host cell.
- A cloning vector contains a selectable marker, which confer on the host cell an ability to survive and proliferate in a selective growth medium containing the particular antibiotics.

Marker Gene or Reporter Gene:

- Reporter gene used in cloning vector to Facilitate the screening of successful clones by using features of these gene that allows successful clone to be easily identified.
- Such feature present in cloning vectors is used in blue-white selection.

Additional Properties of Vectors:

- It should be small, short.
- Compatible with host cell.
- Incompatible with other vector.
- Should become high in copy number.
- It should able to express itself utilizing the host machinery.
- It should be able to move under two systems.

Type of cloning vector:

- **Plasmid**
- **Bacteriophage**
- **Cosmid**
- **Bacterial Artificial Chromosome (BAC)**
- **Yeast Artificial Chromosome (YAC)**
- **Human Artificial Chromosome (HAC)**
- **Retroviral Vector**

Plasmid:

- Plasmid is an autonomously replicating circular double stranded extra chromosomal DNA which is physically separated from a chromosomal DNA and can replicate independently.
- Most commonly found in bacteria, sometimes they are present in eukaryotic organisms.
- The size of the plasmid varies from 1 to 200 kb.
- Most general plasmids may be used to clone DNA insert of up to 10 kb in size.

- Many plasmids have high copy number and high copy number is useful as it produces greater yield of recombinant plasmid.
- However low copy number plasmid may be used in certain circumstances for e.g. when the protein from the cloned gene is toxic to the cells.
- E.g 1) F plasmid 2) pUc18 3) pBR322

Why plasmid are good cloning vectors:

- Small size
- Circular
- Replication independent host cell.
- Several copies may be present.
- Have antibiotic resistance.

Disadvantage Using Plasmid:

- Cannot accept large fragments.
- Size range from 0-10 kb.

Bacteriophage:

- The bacteriophage used for cloning is the phage λ and M13 phage.
- There is an upper limit on the amount of DNA that can be packed into a phage
- (maximum of 53kb)
- There is also lower limit for vector DNA so that cannot packed properly

λ phage:

- λ phages are viruses that can infect bacteria. The major advantage of the λ phage vector is its high transformation efficiency, about 1000 times more efficient than the plasmid vector.
- The assembly process of the λ virion. The extreme ends of the λ DNA are known as COS sites.
- Each is single stranded, 12 nucleotides long.
- One end of λ DNA May base- pair with the other ends of a different λ DNA, forming concatemers.
- The two ends of a λ DNA may also bind together, forming a circular DNA. In the host cell because ligase may seal the join of the COS site.
- The filled head is then attached to the tail, forming a complete λ virion.

Cosmid:

- It was first constructed by collins and hohn in 1978. it is combination of essential elements of the plasmid and λ system.
- It is short circular DNA molecule containing COS site and origin of replication, selectable marker and several unique restriction enzymes site.
- Cosmid vector is a combination of the plasmid vector and the COS site which allows the target DNA to be inserted into the λ head.
- Size of cosmid vector 45 kb and λ vector 25kb to carry.

ADVANTAGES:

- Cosmid pick up relatively larger DNA than plasmids do.
- They are used in establishment of gene libraries of lower and higher organism.

DISADVANTAGES:

- The packing enzyme fails to pack recombinant Cosmids into the phage head if COS site is missing.

Yeast Artificial Chromosomes:

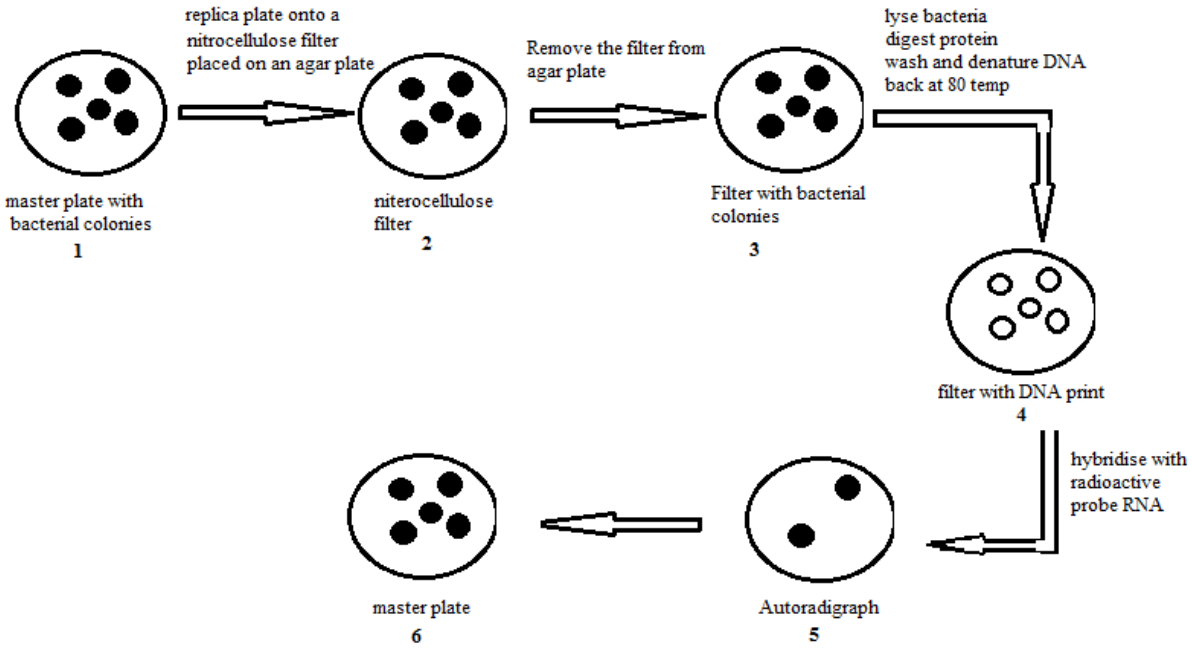
- The YAC vector is capable of carrying a large DNA fragment size up to 2MB, but its transformation efficiency is very low.
- The final types of yeast cloning vector to consider is the YAC it is a totally new approach to gene cloning.
- YAC are valuable because they can carry as much as 500kb to 1mb of foreign DNA.
- YAC are designed to replicate as a plasmid in bacteria when there is no foreign DNA inserted into the vector.
- Once the fragment of DNA is inserted YAC are transferred into YEAST CELL. Where they replicate as eukaryotic chromosomes.

Bacterial Artificial Chromosome (BAC):

- BAC vector are similar to standard E.coli plasmid vector.
- Contain the origin and genes encoding. The ori binding protein required for plasmid replication.
- Derived from a naturally occurring large plasmid the F' plasmid.
- Low copy number (1-2 copies)
- BAC insert size is 150-350kb.

- BAC is preferred for different kind of genetic studies of infectious diseases because they accommodate much larger sequences without the risk of rearrangement.
- And BAC is more stable than other types of cloning vectors.

Colony Hybridization by Replica Plate Method:



- The replica plate method is used in genetic engineering for identifying transformed bacterial cells.
- Only some of the cells transformed with a specific DNA may actually have foreign DNA.
- Thus, it is essential to screen those cells having foreign DNA by the technique of colony hybridization.
- The principle of colony hybridization involves in-situ hybridization of transformed bacterial cells with a radioactive probe sequence.
- Due to the specificity of probe a single colony can be rapidly identified amongst thousands of colonies.

Hybrid Released Translation (HRT) Method:

Double stranded cDNA

↓ Denaturation

Single stranded cDNA

↓ mRNA of desired protein

cDNA - RNA hybrid

↓

Single stranded DNA (complementary to mRNA of target gene)

↓

Double stranded DNA (coding for target gene)

↓ As probe

Used for isolation of rDNA gene.

Immunochemical Detection:

- Immunochemical detection relies on the ability of the desired gene to produce its protein; however in this method the presence of protein is detected by a specific antibody. There are many gene cloning procedures in which eukaryotic genes are identified in prokaryotic hosts by antibody recognition.
- While immunochemical detection involves challenging the fixed lyste with a primary antibody and then exposing to a labeled secondary antibody.
- In this method primary antibody bound to a polyvinyl sheet, on which the lysed, antigen – producing colonies or plaques are replica plated.

- The polyvinyl sheet is then exposed to labeled secondary antibody, which although is specific for the same antigen as the primary, but binds to different epitope.
- Colonies in which the target genes are produced are identified by autoradiography or by a colour change.

Expression of Cloned Gene within the Host:

- To express a cloned gene in a transformed cell means that it has to transcript and translate. Sometimes functional proteins are produced by post- translation modifications.
- Protein synthesis is based on the encoded information in the sequence of the DNA strand bases. Structural gene directs the cells machinery for sequential liner arrangements of amino acid units to form a particular protein.
- In prokaryotic transcript would not carry RBS; therefore eukaryotic genes should be supplied at minimum a prokaryotic promoter and RBS for expression.
- Many the eukaryotic genes having coding the same, but control signals are different. Many eukaryotic genes having coding the same, but control signals are different.