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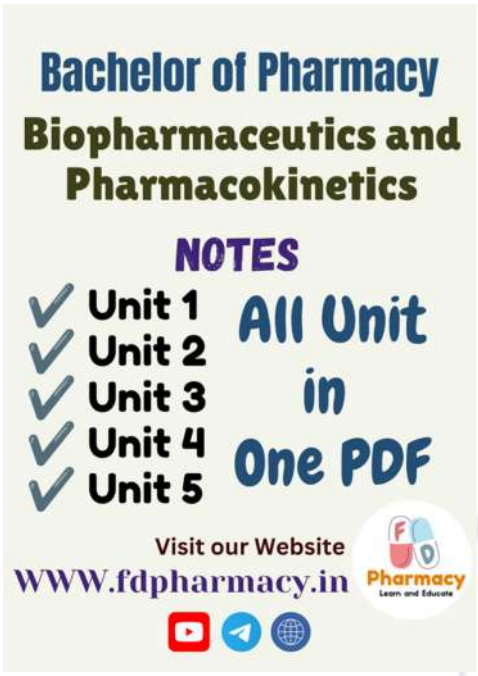
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PHARMACEUTICAL BIOTECHNOLOGY

UNIT 2

TOPIC :

- a) Study of cloning vectors, restriction endonucleases and DNA ligase.

Cloning Vectors

Cloning vectors are DNA molecules used to carry a foreign DNA fragment (gene of interest) into a host cell where it can replicate and produce multiple copies.

Simple meaning: A cloning vector acts as a DNA carrier for gene cloning.

Examples:

- Plasmids, Bacteriophages
- Cosmids, Artificial chromosomes

Need of Cloning Vectors

Cloning vectors are required to:

1. Insert a gene of interest into a host cell.
2. Produce multiple copies (clones) of DNA.
3. Store and maintain genetic material.
4. Help in genetic engineering research.
5. Assist in DNA sequencing and analysis.
6. Enable production of recombinant proteins.

Characteristics of Cloning Vectors

1. Origin of Replication (ori)

- Site from where DNA replication starts.
- Allows vector replication inside host cells.

2. Selectable Marker

- Helps identify transformed cells.
- Example: Antibiotic resistance genes.

3. Multiple Cloning Site (MCS)

- Contains several restriction enzyme sites.
- Helps insertion of foreign DNA.

4. Small Size

- Easy handling and transfer into host cells.

5. Stability

- Maintains vector integrity inside host cells.

6. Host Compatibility

- Vector should function properly within host organisms.

7. Unique Restriction Sites

- Allows controlled insertion of foreign DNA.

Types of Cloning Vectors

1. Plasmids

Plasmids are small, circular, double-stranded DNA molecules found mainly in bacteria.

Characteristics:

- Circular DNA structure
- Double-stranded DNA
- Self-replicating
- Small size (few kilobases)
- Carry useful genes like antibiotic resistance

Types of Plasmids

F (Fertility) Plasmid

- Helps bacterial conjugation.

R (Resistance) Plasmid

- Provides antibiotic resistance.

Col Plasmid

- Produces bacteriocins that kill other bacteria.

Degradative Plasmid

- Helps metabolism of unusual compounds.

Virulence Plasmid

- Increases pathogenicity.

pBR322 Vector

pBR322 is one of the most commonly used artificial plasmid vectors in genetic engineering.

Developed by:

- Bolivar
- Rodriguez

Name meaning:

- p = Plasmid
- BR = Bolivar and Rodriguez
- 322 = Identification number

Features of pBR322

- Circular double-stranded DNA
- Size = 4361 base pairs
- Contains origin of replication (ori)
- Contains ampicillin resistance gene (ampR)
- Contains tetracycline resistance gene (tetR)
- Contains rop gene

Restriction enzyme sites:

- EcoRI
- ClaI
- HindIII
- PstI
- BamHI
- SalI
- PvuI

Insertional Inactivation

Foreign DNA insertion can inactivate antibiotic resistance genes.

Examples:

- DNA inserted in ampR → Ampicillin resistance lost.
- DNA inserted in tetR → Tetracycline resistance lost.

Functions of pBR322

1. DNA cloning
2. Foreign DNA insertion
3. Gene transfer

2. Bacteriophage Vectors

Bacteriophages are viruses that infect bacteria.

Features:

- Carry foreign DNA into bacterial cells.
- Can carry 15–20 kb DNA fragments.
- Useful for gene cloning.

Example:

- Lambda (λ) phage

3. Cosmids

Cosmids are hybrid vectors formed by combining plasmid and bacteriophage properties.

Features:

- Circular DNA molecules
- Carry large DNA fragments
- Capacity: 35–45 kb
- Used in genomic library preparation

4. Artificial Chromosomes

- Artificial chromosomes are synthetic DNA molecules designed to behave like natural chromosomes.
- Used to carry very large DNA fragments.

Types of Artificial Chromosomes

YAC (Yeast Artificial Chromosome)

- Used in yeast cells
- Carries very large DNA fragments
- Capacity up to 1 Mb

BAC (Bacterial Artificial Chromosome)

- Used in bacteria like *E. coli*
- Capacity 100–300 kb
- Highly stable

Restriction Endonucleases

- Restriction Endonucleases are enzymes that cut DNA molecules at specific recognition sequences.
- They are called "Molecular Scissors" because they cleave DNA into smaller fragments.
- These enzymes are naturally found in bacteria and act as a defense mechanism against viral infection.

Meaning of Restriction Endonuclease

Restriction

- Restricts growth of invading viruses by cutting viral DNA.

Endonuclease

- Endo = Inside
- Nuclease = Enzyme that cuts nucleic acid

Thus, restriction endonucleases cut DNA internally at specific sites.

Types of Restriction Endonucleases

1. Type I Restriction Endonuclease

Characteristics:

- Cuts DNA at random sites away from recognition sequence.
- Complex enzyme system.
- Requires ATP.
- Not commonly used in genetic engineering.

2. Type II Restriction Endonuclease

Characteristics:

- Cuts DNA at specific recognition sequences.
- Highly precise.
- Most commonly used in recombinant DNA technology.
- Produces sticky ends or blunt ends.

Examples:

- EcoRI
- HindIII
- BamHI

Applications:

- Gene cloning
- DNA mapping

- Recombinant DNA technology

3. Type III Restriction Endonuclease

Characteristics:

- Cuts DNA near recognition sequence.
- Requires ATP.
- Less commonly used.

Recognition Sequence

- Restriction enzymes recognize specific DNA sequences called Recognition Sequences.
- Most recognition sequences are Palindromic Sequences.

Palindromic Sequence

- A DNA sequence that reads the same in opposite directions on complementary strands.

Example: EcoRI recognition site

5′ — GAATTC — 3′
3′ — CTTAAG — 5′
EcoRI cuts between:
G↓AATTC
CTTAA↑G
Produces sticky ends.

Examples of Restriction Endonucleases

Enzyme	Source Organism	Recognition Sequence
HindIII	*Haemophilus influenzae*	AAGCTT
EcoRI	*Escherichia coli* RY13	GAATTC
HpaI	*Haemophilus parainfluenzae*	GTTAAC
BamHI	*Bacillus amyloliquefaciens*	GGATCC

Applications of Restriction Endonucleases

1. Gene cloning
2. Recombinant DNA technology
3. DNA fingerprinting
4. Genetic engineering
5. Genome mapping
6. Biotechnology research

DNA Ligase

- DNA ligase is an enzyme that joins two DNA fragments together.
- It acts as a "Molecular Glue".
- DNA ligase forms phosphodiester bonds between DNA fragments.
- It joins:
    - 3'-OH end of one DNA strand
    - 5'-Phosphate end of another DNA strand

Functions of DNA Ligase

1. Joins DNA fragments.
2. Helps in DNA replication.
3. Repairs damaged DNA.
4. Essential in recombinant DNA technology.

Role in Cells

DNA Replication

- DNA ligase joins Okazaki fragments on the lagging strand.

DNA Repair

- Repairs breaks present in DNA molecules.

Types of DNA Ligase

1. Prokaryotic DNA Ligase

Characteristics:

- Found in bacteria (*E. coli*)
- Uses NAD^+ (Nicotinamide Adenine Dinucleotide) as energy source

2. Eukaryotic DNA Ligase

Characteristics:

- Present in plants, animals and humans
- Uses ATP as energy source

Types:

- DNA Ligase I
- DNA Ligase II
- DNA Ligase III
- DNA Ligase IV

3. T4 DNA Ligase

Characteristics:

- Obtained from bacteriophage T4
- Widely used in genetic engineering
- Efficiently joins sticky ends and blunt ends

Applications of DNA Ligase

1. Gene cloning
2. Recombinant DNA technology
3. DNA repair studies
4. Biotechnology research
5. Construction of recombinant DNA molecules
6. Genetic engineering experiments