BIOTECHNOLOGY

UNIT 2 NOTES

- CLONING VECTORS
- RESTRICTION ENDONUCLEASES
- DNA LIGASE
- rDNA TECHNOLOGY & ITS APPLICATIONS
- INTERFERON, HEPATITIS B, INSULIN
- PCR

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CLONING VECTORS

- Cloning vectors is DNA molecule used to carry a foriegn gene into a host cell & make multiple copies of it.
- It is simply a DNA carrier used for gene cloning (copying DNA).
- Example: Plasmids, Cosmids etc.

NEED OF CLONING VECTORS

- Cloning Vectors are used to:
- Insert a gene of interest into a host cell.
- Produce many copies (clones) of that gene.
- Store & maintain the gene for research.
- Help in DNA analysis & genetic studies.

CHARACTERISTICS OF CLONING VECTORS

- Origin of Replication: It allows the vector to replicate inside the host cell.
- Selectable Marker: It helps in identifying cells that contain the vector.
- Multiple Cloning site: It provides specific sites for insertion of foriegn DNA.
- Small size: Easy to handle & transfer into host cells.
- Stability: It ensures the vector remains stable inside the host cell.

- **Host Compatability:** It ensures proper functioning of the vector inside the host cell.
- **Unique restriction Site:** Allows precise & controlled insertion of DNA.

TYPES OF CLONING VECTORS

1. Plasmids
2. Bacteriophage
3. Cosmids
4. Artificial Chromosomes

1. PLASMIDS

- Plasmids are small, circular, double-standard DNA molecules found mainly in bacteria.
- They're separate from the main chromosomal DNA & replicate independently.

FEATURES OF PLASMIDS

- Circular DNA structure
- Double stranded DNA
- Self-replicating (have thier own origin of replication)
- Usually small in size (few kb)
- Provides advantages such as antibiotic resistance to bacteria.

Types Of Plasmids

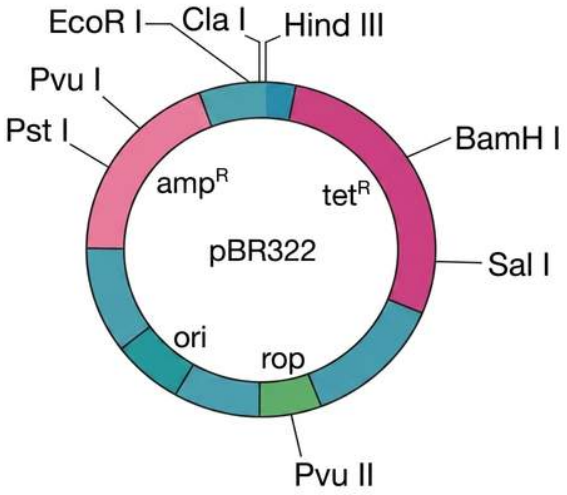
1. Fertility (F) plasmids: Helps in bacterial conjugation
2. Resistance (R) Plasmids: Provide antibiotic resistance
3. Col Plasmids: Produce bacteriocins (Toxins to kill other bacteria)
4. Degradative Plasmids: Helps in metabolism of unusual substance
5. Virulence Plasmids: Increase pathogenecity

pBR322

- pBR322 is the oldest & most commonly used Artificial Plasmid Vectors in genetic engineering.
- It is developed by Boliver & Rodriguez
- Name breakdown:
  - p: Plasmid
  - BR: Boliver & Rodrigez
  - 322: Identification Number

CHARACTERISTICS

- pBR322 is a circular double-stranded DNA
- It contains 4361 base pairs.
- It has important regions like:
  - amp^R (ampicillin resistance)
  - ori (origin of replication)
  - tet^R (tetracycline resistance)
  - rop gene
  - Multiple restriction sites



IMPORTANT REGIONS OF pBR322

① ori (origin of replication)

- It is the starting point of DNA replication.
- Helps plasmid to multiply inside bacteria.

② rop gene

- rop = repressor of primer.
- Helps in controlling plasmid copy number

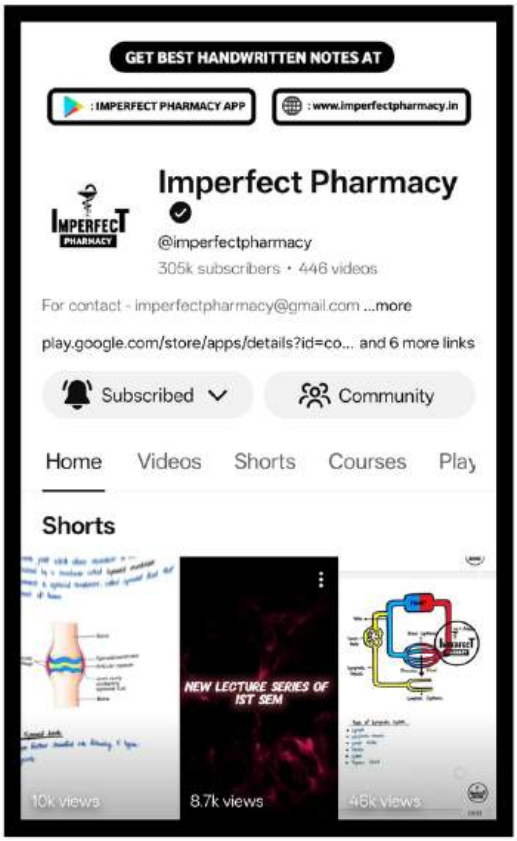
③ Antibiotic Resistance Gene

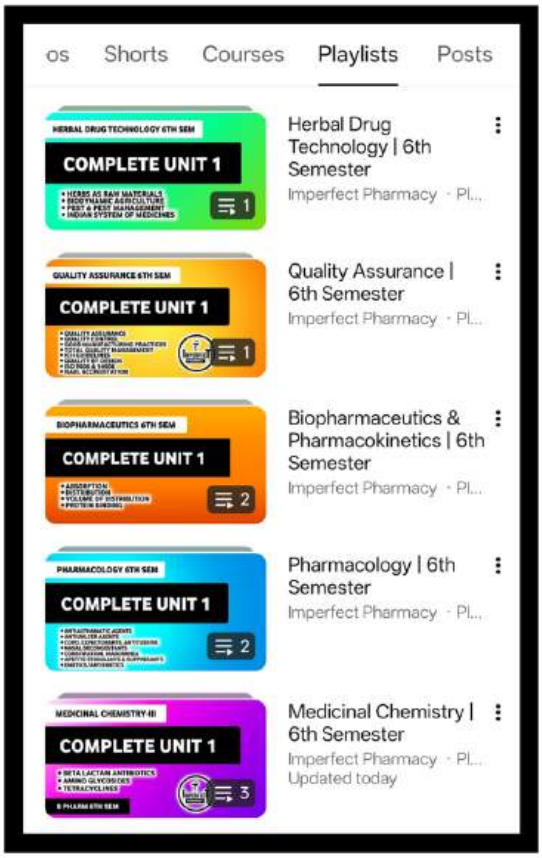
- amp^R (Ampicillin Resistance): Protects bacteria from ampicillin
- tet^R (Tetracycline Resistance): Provides resistance against tetracycline
- These are used as selectable markers.

④ Restriction Sites

- pBR322 contains 40+ unique restriction enzyme sites in which these are the most common ones:
  - EcoR I
  - Cla I
  - Hind III
  - Ba I
  - Sal I
  - Pst I
  - Pvu I
  - Pvu II

FOR LECTURE VIDEOS VISIT OUR CHANNEL





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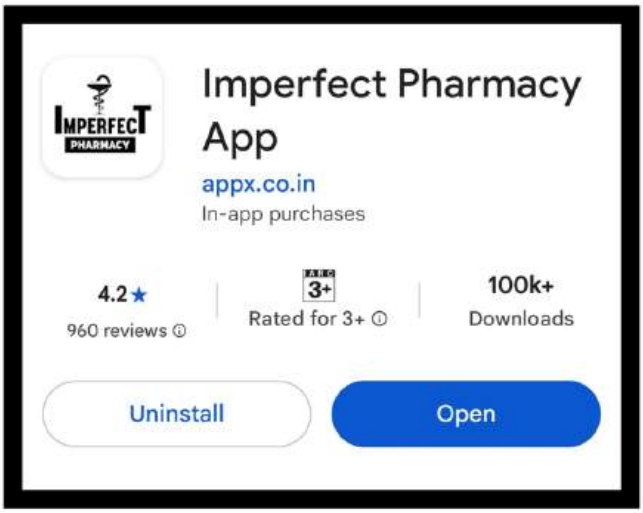


INSERTIONAL INACTIVATION

- When foriegn DNA is inserted:
- If inserted in amp^R: ampicillin resistance lost
- If inserted in tet^R: tetracycline resistance lost

FUNCTIONS OF pBR322

- It is used as cloning vector
- It helps in DNA insertion
- It is used in gene transfer
- It helps in selection of recombinant cells.

② BACTERIOPHAGE

- Bacteriophage are viruses that infect bacteria.
- They're used as vectors to carry foriegn DNA.
- They can carry 15-20kb DNA fragments.
- Common example: λ (lambda) phage

③ COSMIDS

- Cosmids are hybrid vectors made by combining features of plasmids + bacteriophage (λ phage).
- They're circular DNA (like plasmids)
- They can carry large DNA fragments (≈ 35-45kb)

④ ARTIFICIAL CHROMOSOMES

- Artificial Chromosomes are man-made DNA molecules that behave like real chromosomes are used to carry very large DNA fragments in cloning.

TYPES OF ARTIFICIAL CHROMOSOMES

ⓐ YAC (Yiest Artificial Chromosomes)

- Used in yeast cells
- Can carry very large DNA fragments (up to 1mb)

ⓑ BAC (Bacterial Artificial Chromosomes)

- Used in bacteria (E.coli)
- Capacity: 100-300 kb

© HAC (Human Artificial Chromosomes)

- Used in Human cells
- Very large DNA (more tha 1mb) capacity

Restriction Endonucleases

- Restriction Endonucleases are enzymes that cut DNA at specific sequences.
- They act like Molecular Scissors.
- They're found in Bacteria.
- Restriction: They protect bacteria from viruses by cutting viral DNA, thus 'restrict' viral growth.
- Endonucleases: Endo = Inside
  Nucleases = Enzyme that cuts Nucliec Acids

Types Of Restriction Endonucleases

They're mainly of 3 types:

① Type I
② Type II
③ Type III

① Type I

- They cut DNA at random sites (far from recognition site)
- They are complex enzymes & not used in genetic engineering.

② Type II

- They cut DNA at specific sites
- They're simple, precise & widely used in Gene cloning.
- Example: EcoR I, Hind III, BamH I

③ Type III

- They cut DNA near the recognition site.
- They're less commonly used.

Recognition Sequence

- Each enzyme recognizes a specific DNA sequence, usually Palindromic Sequence
- Palindromic sequence: Same when read in both directions.
- Example: EcoR I recognizes:

5′ — GIAATTC — 3′

3′ — CTTAAG I — 5′

Example Of Some Re Enzymes & Cleavage Sites

Restriction Endonucleases	Sources	Cleavage Sites
Hind III	Haemophilus influenza - d	5′-AAGICTT -3′ 3′-TTCGIAA - 5′
Eco RI	Escherichia coli RY 13	5′— GIAATTC—3′ 3′—CTTAAG I—5′
Hpa I	Haemophillus parainfluenza	5′— GITTAAC—3′ 3′—CAATTG I—5′
Bam HI	Bacillus Amiloliquefaciens H	5′— GIGATCC—3′ 3′— CCTAGG I—5′

DNA LIGASE

- DNA ligase is an enzyme that joins two DNA fragments together.
- It acts like a "molecular glue".
- It forms phosphodiester bonds b/w DNA fragments.
- Joins 3'-OH end of one strand with 5'-phosphate end of another
- DNA ligase is naturally present in cells & used in DNA replication (Joins Okazaki fragments) & DNA repair.

TYPES OF DNA LIGASE

① PROKARYOTIC DNA LIGASE

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

② EUKARYOTIC DNA LIGASE

- Found in higher organisms (humans, animals, plants)
- Uses ATP as energy source
- They're further subdevided as:
  - DNA ligase I
  - DNA ligase II
  - DNA ligase III
  - DNA ligase IV

③ T4 DNA ligase

- It is obtained from bacteriophage T4.
- It is widely used in genetic engineering.

APPLICATIONS

- Gene cloning
- Recombinant DNA technology
- DNA repair studies
- Biotechnology & research

rDNA Technology

- Recombinant DNA (rDNA) technology is a technique in Molecular Biology in which DNA from different sources is combined artificially to form a new DNA molecule.
- This new DNA is called recombinant DNA (rDNA)
- The phenomenon of rDNA formation is known as Genetic Engineering.
- Example: Human insulin production using bacteria

Principle Of rDNA Technology

- The principle of rDNA technology is based on cutting & joining DNA from different sources to form a new DNA molecule, which is then introduced into a host cell to produce desired proteins.

Components Of rDNA Technology

① **Donor DNA:**
- It is the DNA that contains the gene of interest.
- Example: Human Insulin Gene

② **Vector:**
- It is the carrier DNA that transfers gene into host cell.
- Common vectors: Plasmids, Bacteriophages

③ **Host Cell:**
- It is the organism, where rDNA is inserted.
- Example: Bacteria (E.Coli, Yeast)

④ **RESTRICTION ENDONUCLEASES:**
- It is the enzyme which cuts DNA at specific sites.
- They're also known as 'Molecular Scissors'

⑤ **DNA LIGASE:**
- It is the enzyme that joins DNA fragments.
- It is also known as 'Molecular Glue'.

STEPS INVOLVED IN rDNA TECHNOLOGY

It involves following steps:

- Isolation of Desired Gene
- Cutting of DNA
- Selection of Vector
- Ligation
- Introduction into Host cell
- Cloning & Expression

① ISOLATION OF DESIRED GENE

- First the gene of interest (useful gene) is identified & isolated from the donor organism.
- Example: Insulin gene from human cells
- The main purpose is to obtain the specific DNA fragment that codes for a desired protein.

② Cutting Of DNA

- Special enzymes called restriction endonucleases act like molecular scissors.
- They cut DNA at specific sequences.

③ Selection Of Vector

- A vector is a DNA molecule used to carry the desired gene into a host cell.
- The most common vectors are Plasmids, Bacteriophages & Cosmid.

④ Ligation

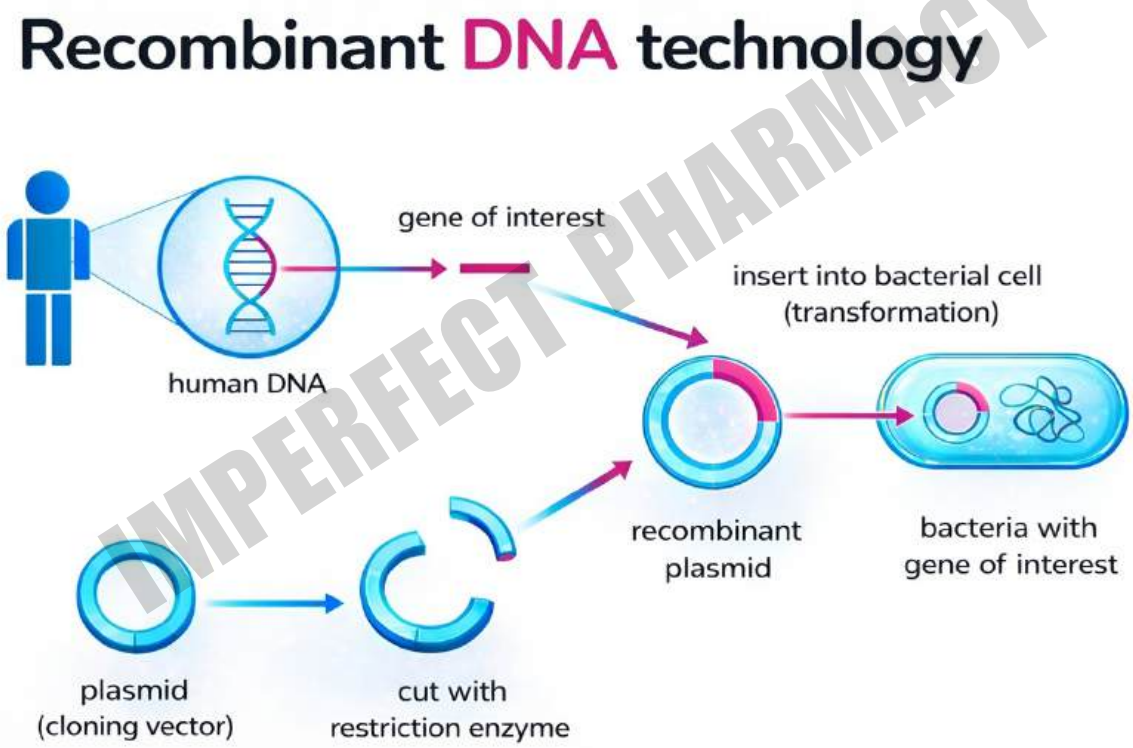
- The desired gene is joined with the vector using an enzyme called DNA ligase.
- This forms rDNA.
- Desired gene + Vector DNA = Recombinant DNA

⑤ Introduction Into Host Cell

- The rDNA is introduced into a suitable host cell such as:
  - Bacteria (E.coli)
  - Yeast
  - Animal Cells

⑥ Cloning & Expression of Gene

- Inside the host:
- The host cell multiplies.
- The inserted gene replicates
- The gene produces the desired protein (expression).
- Example: Bacteria produce large amounts of insulin.



Application Of rDNA Technology

① Production Of Hormones

- rDNA technology is used to produce human hormones like insulin & growth hormone using bacteria.

② Production Of Vaccines

- Vaccines are made by inserting antigen genes into microorganisms.
- These vaccines are safe because they don't contain the whole pathogen but still trigger immunity.

③ Gene Therapy

- It is used to treat genetic diseases by replacing defective with normal genes.

④ Production Of GM Crops

- Genes are inserted into plants to improve qualities like pest resistance & high yield.

⑤ Production Of Enzymes

- Microorganisms are genetically modified to produce useful enzymes.

⑥ DNA FINGERPRINTING

- rDNA technology helps in identifying indivisuals based on their unique DNA pattern.
- It is used in crime investigation & paternity testing.

⑦ PRODUCTION OF MONOCLONAL ANTIBODIES

- Specific antibodies are produced using genetic engineering techniques.
- These are used in disease diagnosis & treatment, especially in cancer.

INTERFERONS

- Inteferons are antiviral proteins (mainly glycoproteins) produced by body cells in respose to viral infection.
- It act as a first line defense against viral attack.
- They help protect cells by inhibiting virus replication & activating the immune system.

TYPES OF INTERFERONS

① Interferon - α
② Interferon - β
③ Interferon - γ

① INTERFERON - α

- They're produced by WBCs
- They've strong antiviral activity.

② INTERFERON - β

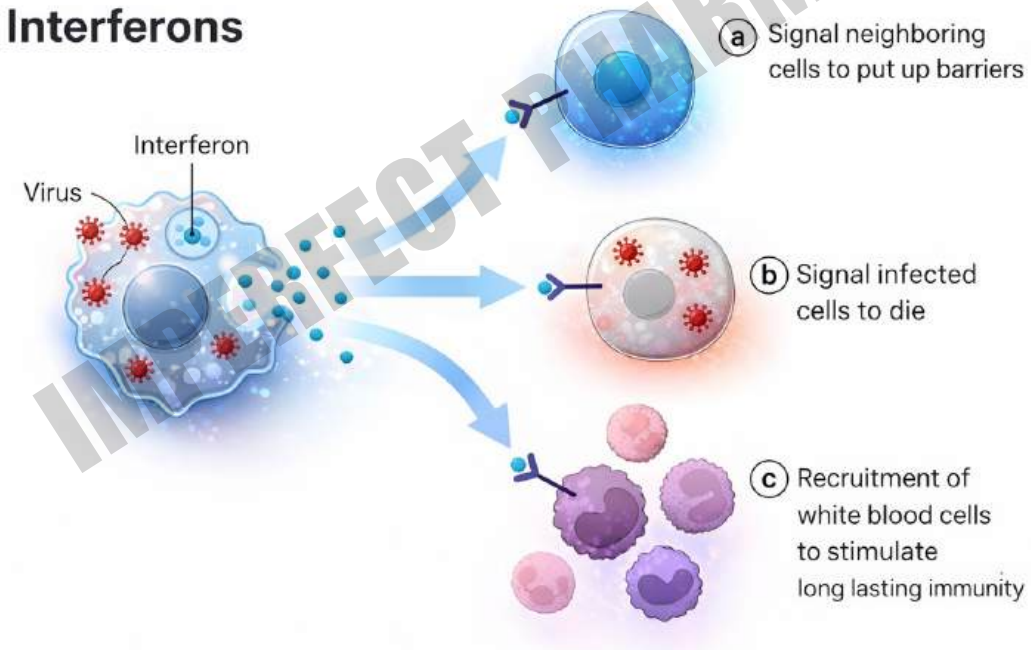
- They're produced by fibroblasts.
- They play an important role in viral defense & immune regulation.

③ INTERFERON - γ

- They're produced by T- lymphocytes
- They activates immune cells.

Mechanism Of Action

- Virus attack on cell
- Cell releases interferons
- Interferons binds to nearby cells
- Activates gene inside nearby cells
- Produce Antiviral proteins
- These proteins:
  - Stop viral replication
  - Destroy Viral RNA
  - Activate immune cells



Production Of Interferon By rDNA Technology

- Interferons are antiviral proteins produced by human cells.
- Using recombinant DNA (rDNA) technology, interferon genes are inserted into microorganisms like (E. coli or Yeast) to produce large amounts of interferons.

Steps Involved

- Isolation & Preparation of Desired Gene
- Preparation of Vector
- Formation of
- Introduction into Host cell
- Cloning & Expression

① Isolation & Preparation Of Desired Gene

- mRNA from interferon producing cells is isolated.
  mRNA is converted cDNA using reverse transcriptase enzyme.
- This cDNA represents the interferon gene.

② Preparation Of Vector

- A bacterial plasmid (vector) is selected.
- Plasmid is cut using restriction endonuclease enzymes.

③ Formation Of rDNA

- The interferon cDNA is inserted into plasmid using DNA ligase.
- rDNA is prepared.

④ Introduction Into Host Cell

- Recombinant plasmid is introduced into competent E.coli cells.

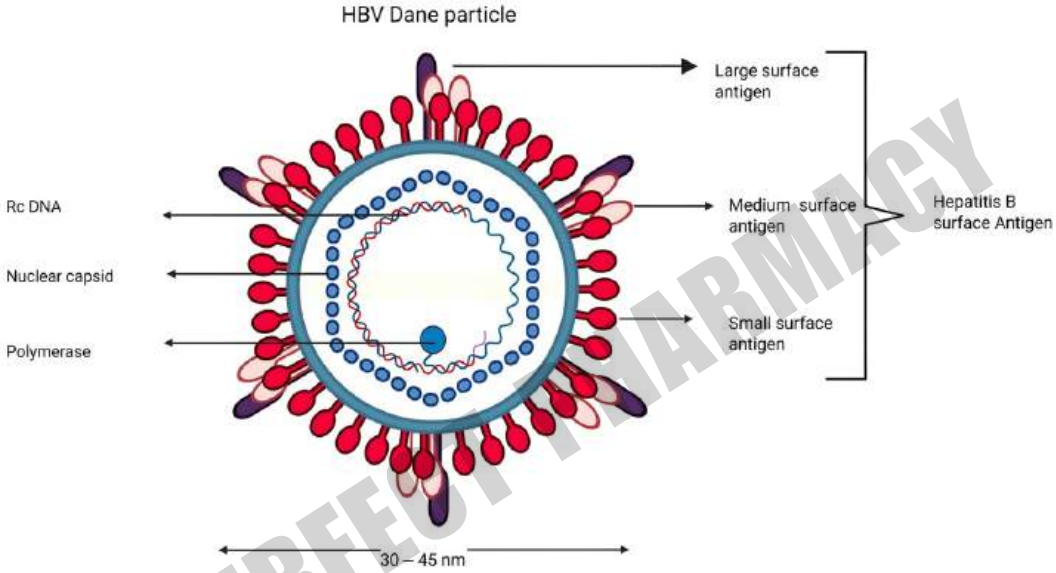
⑤ Cloning & Expression

- Bacteria are grown in culture (fermenter).
- They multiply & express the interferon protein.

Note: In present time mostly Yeast host (Saccharomyces cerevisiae) are preferred over bacterial host for production of interferons.

Hepatitis B Virus

- Hepatitis B Virus (HBV) is a DNA Virus that infects the liver.
- It causes Hepatitis B, which can be acute or chronic.
- It is spherical shaped (Dane particle)
- It contains HBsAg as surface antigen.



Production Of Hepatitis B Vaccine

- The Hepatitis B Vaccine is a recombinant vaccine produce by inserting the HBsAg gene of the Hepatitis B virus into a host cell using rDNA technology, leading to production of antigen without using the live virus.

Steps Involved

- Gene isolation
- Insertion into Vector
- Transformation
- Expression
- Fermentation
- Purification & Vaccine Preparation

① Gene Isolation

- Gene coding for HBsAg (surface antigen) is isolated from HBV DNA.

② Insertion Into Vector

- The gene is inserted into plasmid vector using restriction enzymes & ligase.

③ Transformation

- Recombinant plasmid is introduced into yeast cells (Saccharomyces cerevisiae)

④ Expression

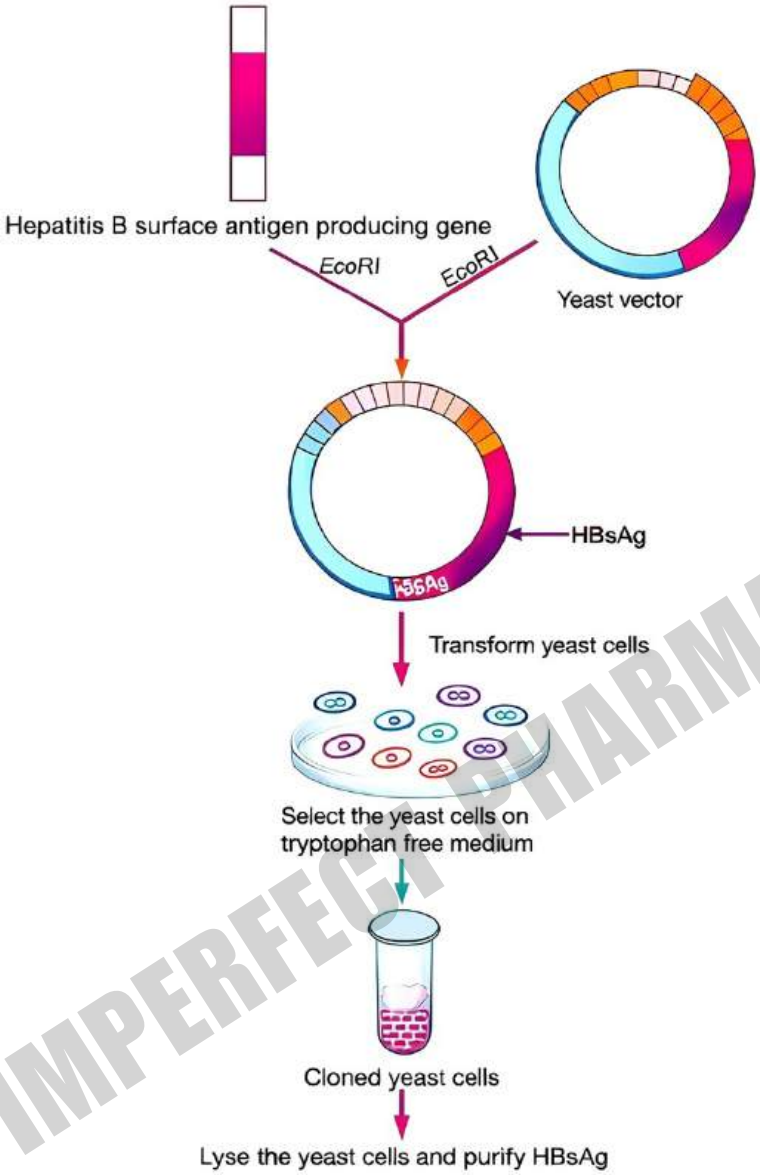
- Yeast cell produce HBsAg Protein.

⑤ Fermentation

- Yeast are grown at large scale in bioreactors for mass production.

⑥ Purification

- HBsAg is extracted & purified.
- Purified HBsAg combined with other ingredients (e.g. aluminium salts) & bottled.
- Now it's ready for vaccination in humans.



ADVANTAGES OF rDNA VACCINE

- Safe (no live Virus)
- Highly effective
- No risk of infection
- Can be produced in large amounts.

INSULIN

- Insulin is a peptide hormon produced by β- cells of pancreas (Islets of Langerhans).
- It plays a crucial role in regulating blood glucose levels.
- It is composed of 51 amino acids arranged in two chains (A & B) connected by disulfide bonds.
- It is secreted in response to increased blood glucose levels.
- It helps in glucose uptake by cells, especially muscle & adipose tissue.

FUNCTIONS

- Lowers blood glucose levels
- Promotes glycogenesis (glucose → glycogen)
- Inhibits gluconeogenesis
- Promotes fat storage (lipogenesis)

PRODUCTION OF INSULIN

- Recombinant DNA (rDNA) technology allows production of human insulin using microorganism like E. coli or Yeast avoiding animal sources.
- Genetech and Eli Lily developed the first recombinant human insulin (Humwilin) in 1978 & brought it to market in 1982
- Insulin consists of two polypeptide chains (A & B).
- In earlier days both chains are produced separately in bacteria & later combined to form active insulin.

STEPS INVOLVED

- Isolation of A & B chain Genes
- Preparation of Recombinant Plasmids
- Formation of Fusion Proteins
- Transformation into E. coli
- Expression of A & B
- Isolation & Cleavage of Fusion Proteins
- Combination of A & B Chains (Disulfide Bond Formation)
- Formation of Active Insulin

① ISOLATION OF GENES

- Human insulin gene is identified.
- Separate DNA sequences are isolated for:
  - A chain (21 amino acids)
  - B chain (30 amino acids)

② Preparation Of Recombinant Plasmids

- Two different plasmids are prepared:
  - One containing A chain gene
  - One containing B chain gene
- Plasmids are cut using restriction enzymes (e.g., EcoRI)

③ Formation Of Fusion Protiens

- Each gene is inserted into plasmid along with β-galactosidase gene
- This forms fusion proteins to protect small insulin chains from degradation inside bacteria.

④ Transformation Into E. Coli

- Recombinant plasmids are introduced into E. Coli cells
- Two separate bacterial cultures are prepared:
  - One produces A chain
  - One produces B chain

⑤ Expression Of Chains

- Bacteria synthesizes:
  - A chain fusion protein
  - B chain fusion protein

⑥ Isolation & Cleavage

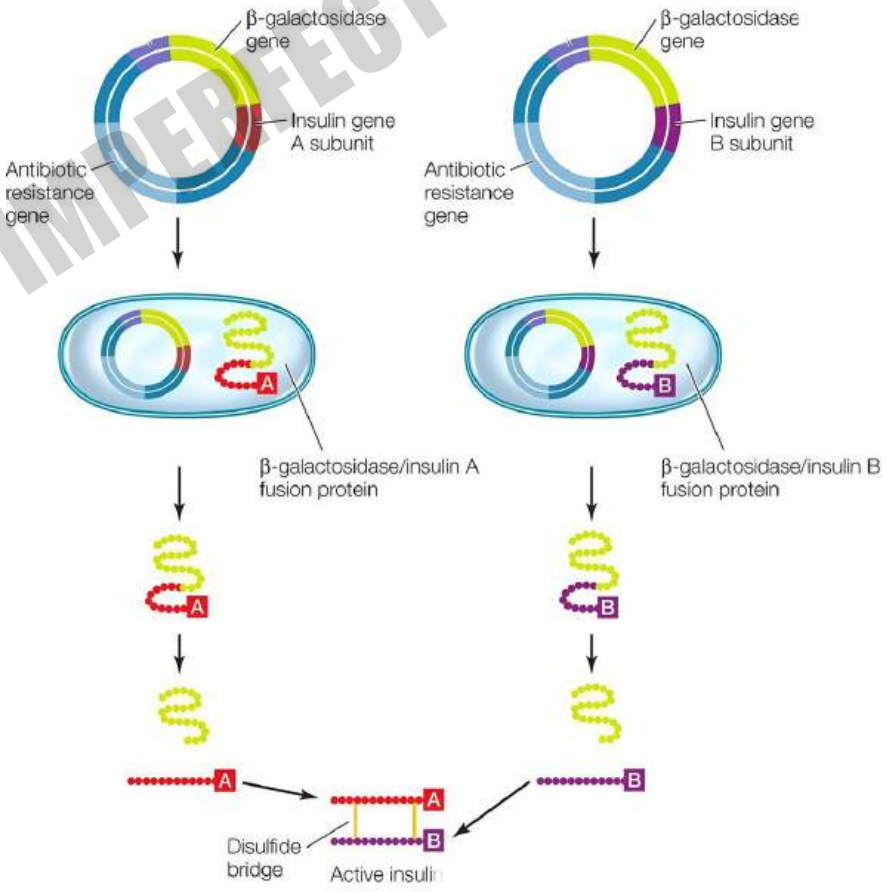
- Fusion proteins are extracted
- They're treated with chemicals to separates A & B chains from fusion proteins.

⑦ Combination Of A & B Chains

- Purified A & B chains are mixed under controlled conditions.
- Disulfide bonds (S—S bonds) are formed.

⑧ Formation Of Active Insulin

- Correct folding leads to formation of biologically active insulin.



POLYMERASE CHAIN REACTION

- Polymerase Chain Reaction (PCR) is a laboratory technique used to make millions of copies of a specific DNA segment in a short time.
- It is developed by Kary Mullis (1983) & recieved nobel Prize in Chemistry in 1993.

PRINCIPLE

- PCR is based on DNA replication mechanism, but performed artificially.
- DNA strands separate on heating.
- Primers binds to target sequence
- DNA polymerase synthesizes new strand

COMPONENTS OF PCR

① TEMPLATE DNA

- It is the DNA which you want to copy

② PRIMERS

- They're short DNA sequences that mark the starting point.

③ DNA POLYMERASE

- They're special heat stable enzyme (commonly taq polymerase).

④ dNTPs (Deoxynucleotide Triphosphates)

- They're building blocks of DNA (A, T, G, C)

⑤ Buffer Solution

- It maintains proper pH & conditions.

Steps Of PCR

PCR Consists of 3 main steps:

① Denaturation
② Annealing
③ Synthesis

① Denaturation

- The reaction mixture is heated to 94°-98°C
- At this temperature, the double-stranded DNA separates into two single strands due to breaking of hydrogen bonds.
- This step prepares the DNA to act as a template.

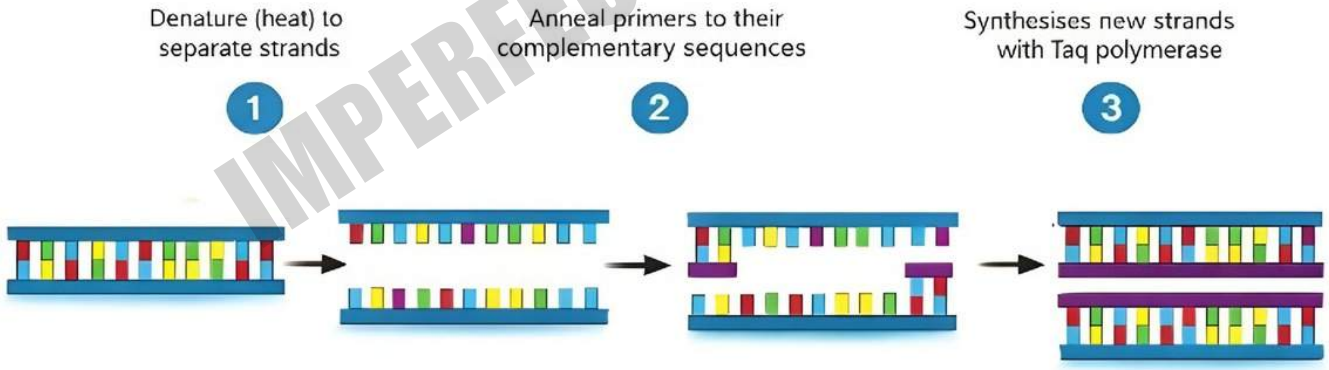
② Annealing

- The temperature is reduced to 50-65°C.
- Primers bind (anneal) to their complementary sequences on the single-stranded DNA.

③ SYNTHESIS (EXTENSION / ELONGATION)

- The temperature is raised to 72°C.
- DNA Polymerase synthesizes a new DNA strand by adding nucleotides in the 5′ → 3′ direction.
- As a result, a new complementary DNA strand is formed.

NOTE: The steps of Denaturation → Annealing → Extension are repeated for 25-35 cycles, leading to exponential amplification of the target DNA.



Advantages Of PCR

- Very fast (few hours)
- Highly sensitive (detects even small DNA amount)
- Specific (due to primers)
- Requires small sample.

Applications Of PCR

(1) Medical Applications

- Used for diagnosis of infectious diseases (e.g. COVID-19, TB, HIV)
- Detection of genetic disorders

(2) Forensic Science

- Used in DNA fingerpointing
- Helps in crime investigation
- Used for paternity testing

(3) Agriculture

- Detection of genetically modified organisms (GMOs)
- Identification of plant pathogens

(4) Environmental & Microbiology

- Detection of micro-organism in water/soil
- Monitoring environmental pollution.

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