

Genetic Engineering

Techniques of Gene Manipulation

Gene manipulation means changing the genetic material (DNA) of an organism in a controlled way to study genes, improve useful traits, or produce valuable products.

1. Isolation of DNA

- **The first step in gene manipulation is to extract and purify DNA from cells.**
 - Cells are broken open using chemicals or enzymes.
 - DNA is released from the cells.
 - Proteins and other unwanted materials are removed.
 - Pure DNA is collected for further use.

2. Cutting DNA with Restriction Enzymes

- **Restriction enzymes are special proteins that act like molecular scissors.**
 - They cut DNA at specific sequences called recognition sites.
 - Scientists use them to remove a desired gene from one organism.
 - The same enzyme can cut another DNA molecule, such as a plasmid, so the gene can be inserted into it.
- **Types of cuts produced:**
 - Sticky ends – have overhanging bases that join easily with matching DNA fragments.
 - Blunt ends – straight cuts without overhangs.

3. Joining DNA with DNA Ligase (DNA Ligation)

- After cutting DNA, the desired gene is joined to another DNA molecule.
 - DNA ligase acts like glue.
 - It joins DNA fragments by forming bonds between them.
- This forms recombinant DNA (DNA made by combining genes from different sources).

4. Gene Transfer

- After recombinant DNA is formed, it must be introduced into a **host cell**.
- This is called **gene transfer**.

Common Gene Transfer Methods

i. Transformation

- Recombinant DNA is introduced into bacteria.
- The host cell can copy the inserted gene and may produce its protein.

ii. Transfection

- DNA is introduced into animal cells.

iii. Electroporation

- Electric pulses create temporary pores in the cell membrane, allowing DNA to enter.

iv. Microinjection

- DNA is directly injected into a cell using a very fine needle.

v. Gene Gun Method

- DNA-coated particles are shot into cells, especially plant cells.

5. Gene Amplification

- Sometimes a gene needs to be copied many times.

This can be done by:

- Cloning – making many copies of a gene inside host cells.
- PCR (Polymerase Chain Reaction) – rapidly makes millions of copies of a DNA segment.
- Purpose: Amplification provides enough DNA for research or practical applications.

6. Gel Electrophoresis

- **Gel electrophoresis** is used to separate DNA fragments according to size.
 - DNA fragments move through a gel under electric current.
 - **Smaller fragments move faster** than larger ones.
 - It helps scientists analyze and isolate DNA fragments.

7. Blotting Techniques

- These techniques help detect specific DNA or RNA sequences.
 - **Southern Blotting** – used for **DNA** analysis.
 - **Northern Blotting** – used for **RNA** analysis.

Summary of Steps in Gene Manipulation

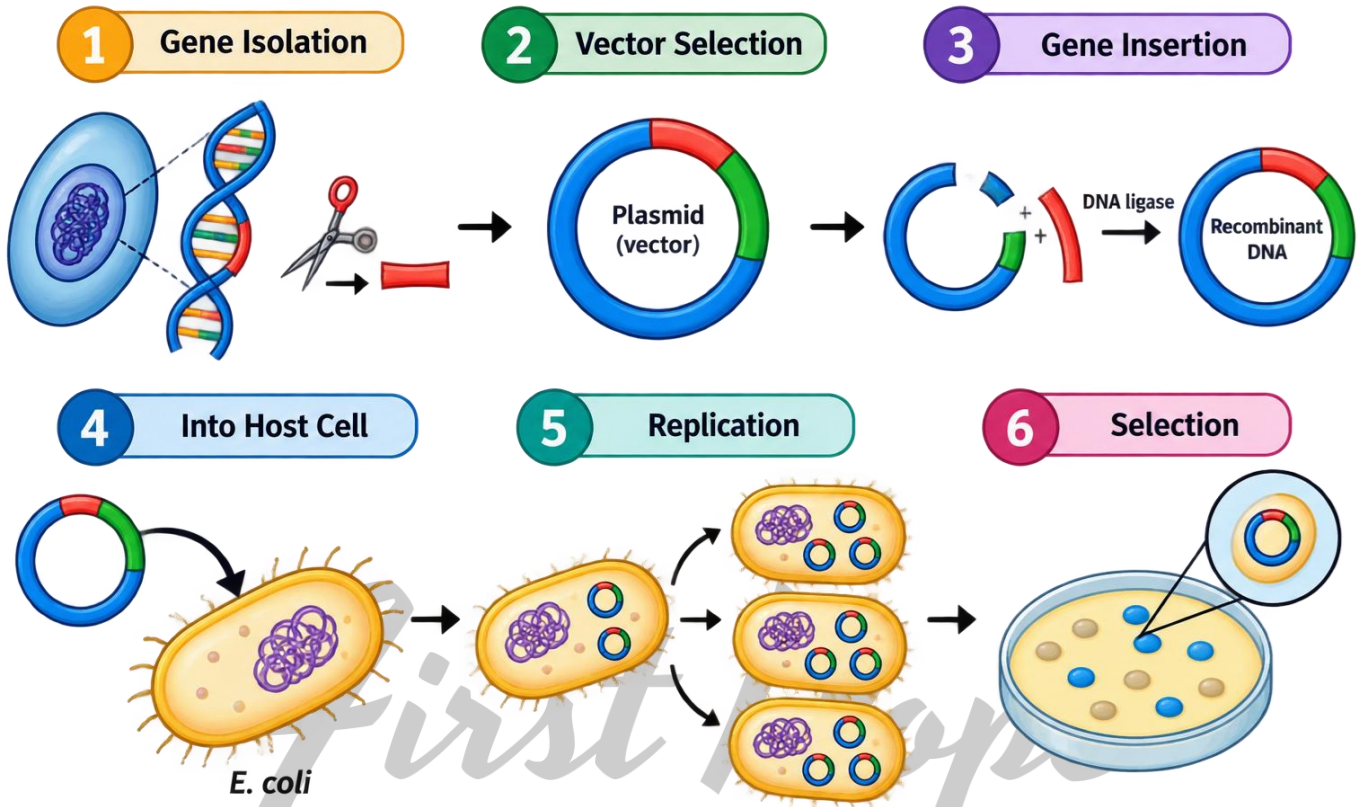
1. Isolation of DNA
2. Cutting DNA with restriction enzymes
3. Joining DNA using DNA ligase
4. Gene Transfer into host cells
5. Gene Amplification

6. Analysis by gel electrophoresis and blotting techniques

Cloning Strategies and Procedures

- **Gene cloning** means making many identical copies of a specific gene.

Steps in Gene Cloning



A. Isolation of Desired Gene

- First, the gene of interest is identified and isolated from the donor organism.
- Restriction enzymes are often used for this purpose.

B. Selection of Vector

- A vector is a DNA molecule used to carry the gene into a host cell.
- Plasmids are commonly used as vectors.

C. Insertion of Gene into Vector

- The desired gene and vector are cut with the same restriction enzyme.
- DNA ligase joins them together to form recombinant DNA.

D. Introduction into Host Cell

- The recombinant vector is introduced into a host cell, usually bacteria such as *E. coli*.
- This process is called transformation.

E. Replication of Recombinant DNA

- As the bacteria grow and divide, they copy the inserted gene.

- This produces many identical copies of the gene.

F. Selection and Identification

- Cells that contain the recombinant DNA are identified and separated from others.
- This ensures only useful clones are studied.

Cloning Strategies

1. Shotgun Cloning

- In this strategy, DNA is broken into many fragments.
- All fragments are inserted into vectors and cloned.
- Scientists then find the clone containing the desired gene.

2. Directed Cloning

- In this method, a specific gene is intentionally selected and cloned.
- It is more targeted than shotgun cloning.

3. Genomic Cloning

- Entire genomic DNA fragments are cloned.
- It includes coding and non-coding regions.

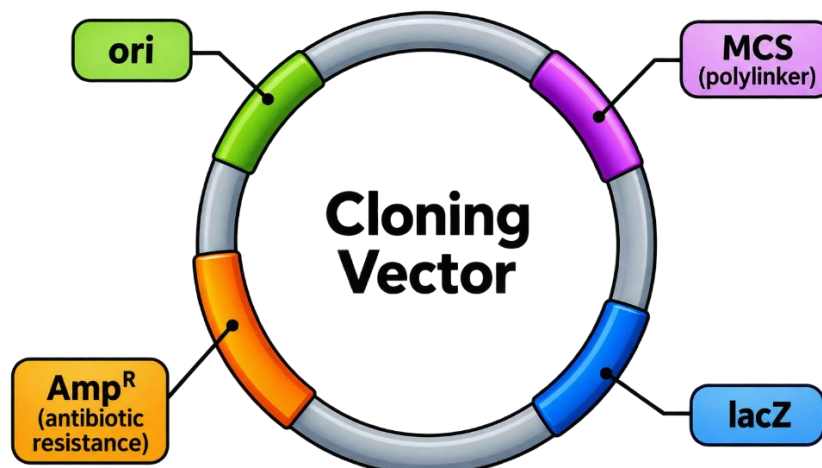
4. cDNA Cloning

- Complementary DNA made from mRNA is cloned.
- It contains only expressed genes without introns.

Cloning Vectors

- **Cloning vectors** are DNA molecules used to carry foreign DNA into a host cell for replication.

Characteristics of a Good Cloning Vector



1. Origin of Replication (Ori)

- This is the site where DNA replication starts.
- It allows the vector to make copies inside the host cell.

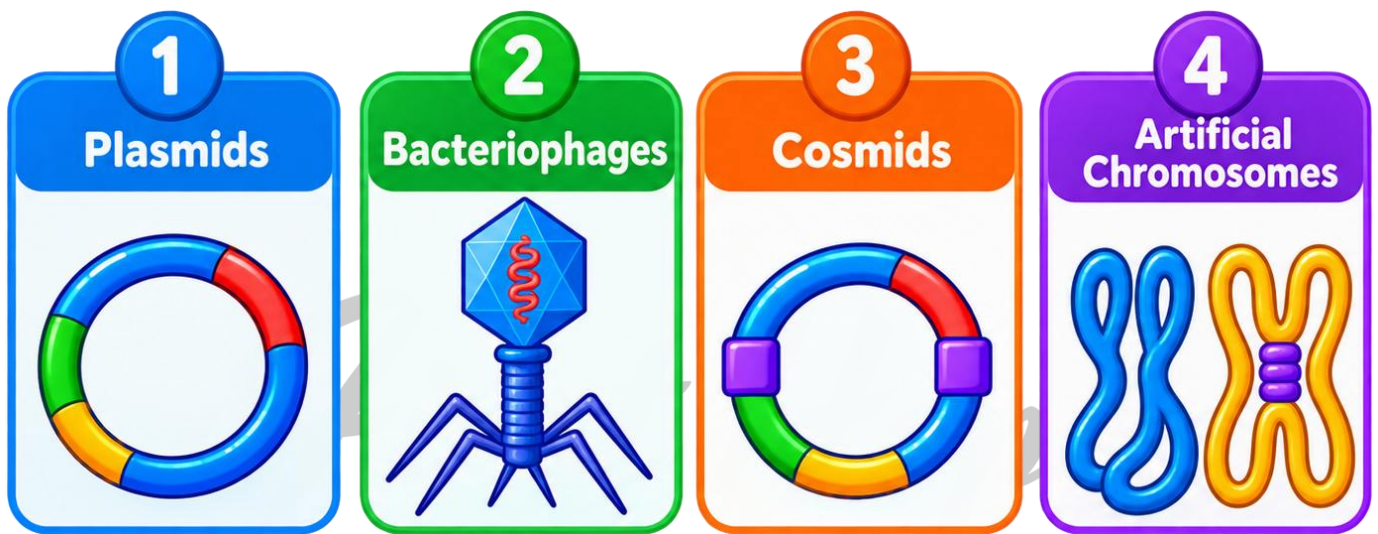
2. Selectable Marker

- These are genes used to identify cells carrying the vector.
- Antibiotic resistance genes are common markers.

3. Multiple Cloning Site (MCS)

- This region contains several restriction enzyme sites.
- It allows easy insertion of foreign DNA.

Types of Cloning Vectors



A. Plasmids

- Plasmids are small circular DNA molecules found in bacteria.
- They are commonly used for gene cloning.

B. Bacteriophages

- These are viruses that infect bacteria.
- They can carry larger DNA fragments than plasmids.

C. Cosmids

- Cosmids combine features of plasmids and bacteriophages.
- They are useful for cloning large DNA fragments.

D. Artificial Chromosomes

- Examples include BACs and YACs.
- They can carry very large DNA fragments.

Components of Cloning Vectors



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