

Cloning vector

Definition

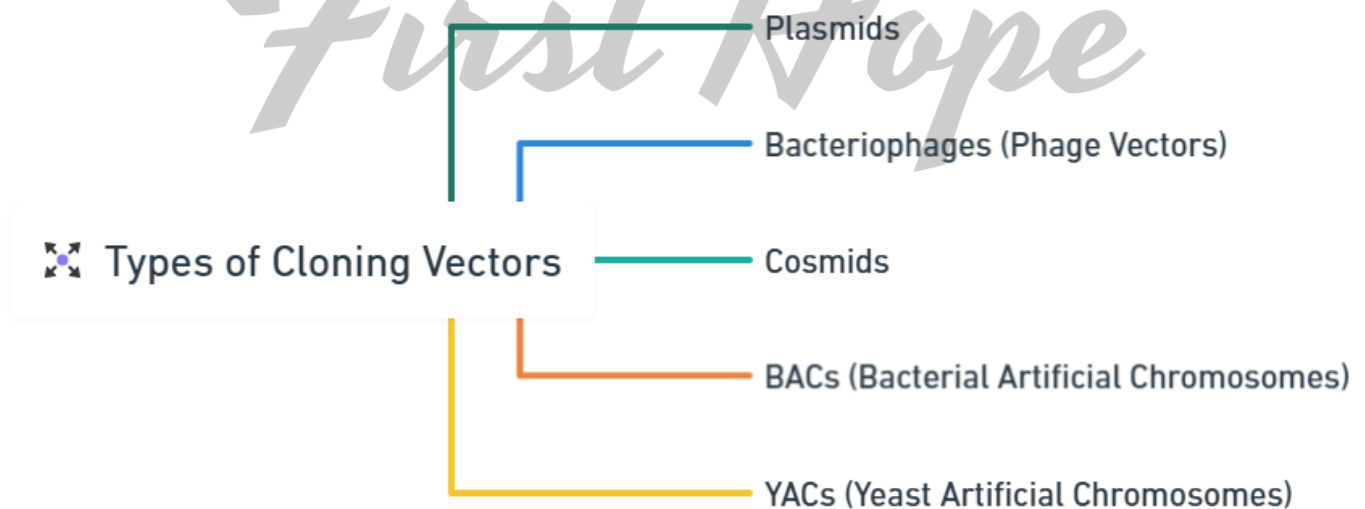
- A **cloning vector** is a small, self-replicating DNA molecule that is used to transfer foreign genetic material into a host cell.
- It acts as a carrier for the DNA fragment of interest, allowing it to be replicated and expressed in the host.

Characteristics of an Ideal Cloning Vector

A good cloning vector must have the following features:

1. **Origin of Replication (ori)** → Enables the vector to replicate independently in the host.
2. **Selectable Marker** → A gene (e.g., antibiotic resistance) that helps identify cells containing the vector.
3. **Multiple Cloning Site (MCS)** → Contains recognition sites for multiple restriction enzymes to facilitate gene insertion.
4. **Small Size** → Smaller vectors are easier to manipulate.
5. **High Copy Number** → Ensures large-scale production of recombinant DNA.

Types of Cloning Vectors



1. **Plasmids** → Small, circular DNA molecules found in bacteria (e.g., pBR322, pUC19).
2. **Bacteriophages (Phage Vectors)** → Viruses that infect bacteria (e.g., λ phage).
3. **Cosmids** → Hybrid of plasmids and bacteriophage λ DNA, can carry larger DNA fragments.
4. **BACs (Bacterial Artificial Chromosomes)** → Used for cloning very large DNA fragments (100-300 kb).
5. **YACs (Yeast Artificial Chromosomes)** → Used for cloning extremely large DNA fragments (up to 1000 kb).

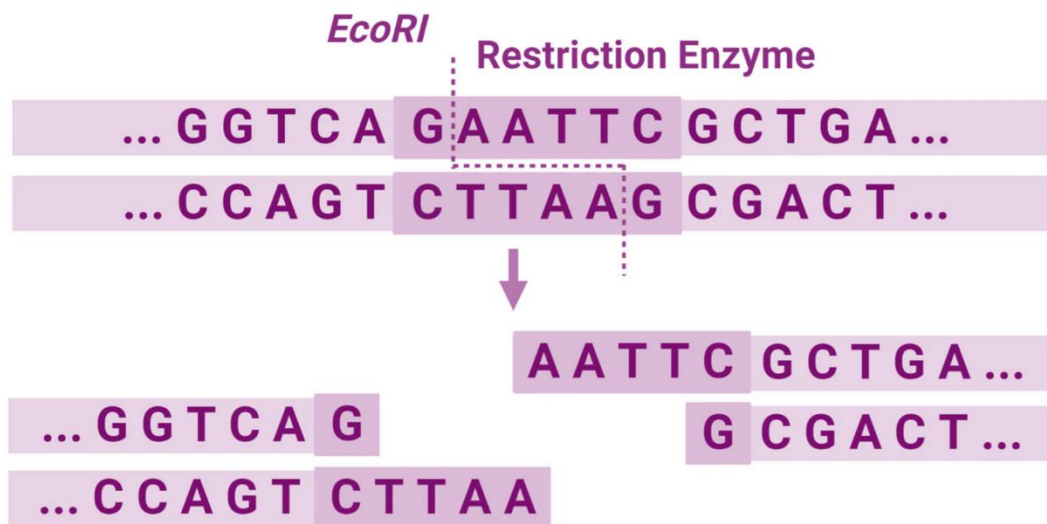
Examples of Cloning Vectors

- **pBR322** → One of the first widely used plasmid vectors, contains ampicillin and tetracycline resistance genes.
- **pUC19** → A high-copy-number plasmid with a lacZ marker for blue-white screening.
- **λ Phage** → Can carry larger DNA fragments than plasmids.

Restriction Endonucleases (Restriction Enzymes)

Definition

- Restriction endonucleases are enzymes that cut DNA at specific sequences, acting as molecular scissors.
- These enzymes are naturally found in bacteria, where they protect against foreign DNA (e.g., viral DNA) by cutting it.



Types of Restriction Enzymes

Type I

- Cut DNA at random locations, often hundreds of base pairs away from their recognition site.
- Less useful for precise genetic engineering.

Type II

- The most commonly used type in laboratories.
- Cut **within** or at short distances from their recognition sites, producing predictable and reproducible fragments.
- Recognition sites are often **palindromic** (the same sequence read 5'→3' on both strands).

Type III

- Cut DNA at sites a short distance away (about 20–30 base pairs) from the recognition site.
- Less frequently used in routine cloning compared to Type II.

Common Restriction Enzymes and Their Recognition Sites

- **EcoRI** → Recognizes 5'-GAATTC-3' and produces sticky ends.
- **HindIII** → Recognizes 5'-AAGCTT-3' and produces sticky ends.
- **HaeIII** → Recognizes 5'-GGCC-3' and produces blunt ends.

Types of DNA Ends After Cutting

Sticky Ends (Cohesive Ends)

- The enzyme cuts the two DNA strands at offset positions, leaving overhanging single-stranded regions.
- These overhangs can base-pair (anneal) with complementary overhangs cut by the same enzyme.

Blunt Ends

- The enzyme cuts both DNA strands at the same position, leaving no single-stranded overhang.
- While this creates more stable DNA ends, blunt-end ligations can be less efficient compared to sticky-end ligations.
 - **Sticky Ends** → Single-stranded overhangs that help in DNA ligation.
 - **Blunt Ends** → No overhangs, making ligation less efficient.

DNA ligase

- DNA ligase is an enzyme essential for DNA replication and repair.
- It catalyzes the formation of phosphodiester bonds between adjacent nucleotides, sealing breaks in the sugar-phosphate backbone of DNA.

Mechanism of Action

- Catalyzes the formation of phosphodiester bonds between 3'-OH and 5'-phosphate ends of DNA strands.
- Requires ATP or NAD⁺ as an energy source.

Specificity

- Highly specific for joining complementary DNA strands at the break site.
- Can distinguish between single-stranded and double-stranded DNA ends.

Applications

- Used in molecular biology and genetic engineering techniques:
 - **DNA cloning** – joins compatible DNA fragments to form recombinant DNA.



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