Lecture No.6

Recombinant DNA Technology

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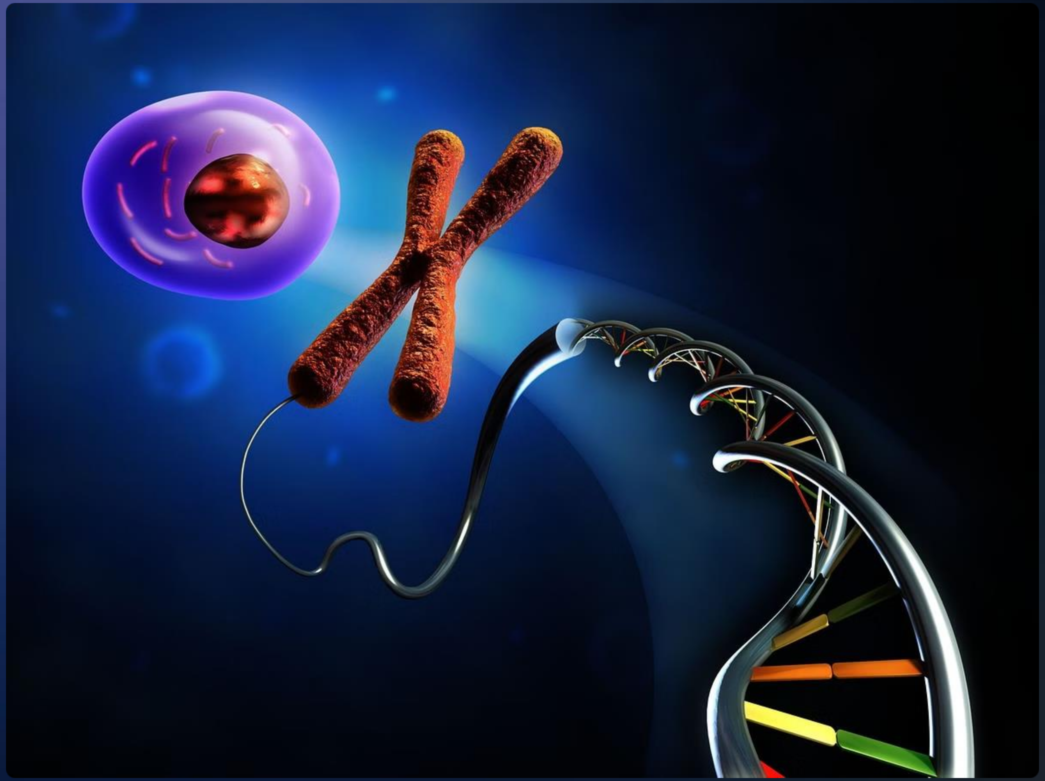
Recombinant DNA Technology

Recombinant DNA technology is a technique that enables the combination of DNA from two or more sources to create a new DNA sequence. The resulting recombinant DNA can then be inserted into an organism, enabling it to express a new gene or trait. This technology has transformed many fields, allowing us to:

- Produce proteins like insulin and hormones.
- Develop genetically modified crops with improved traits.
- Explore gene therapy options to treat genetic disorders.



What is DNA?

Genetic Code

DNA stands for deoxyribonucleic acid and carries the genetic instructions that are used to build and maintain an organism.

Nucleotides

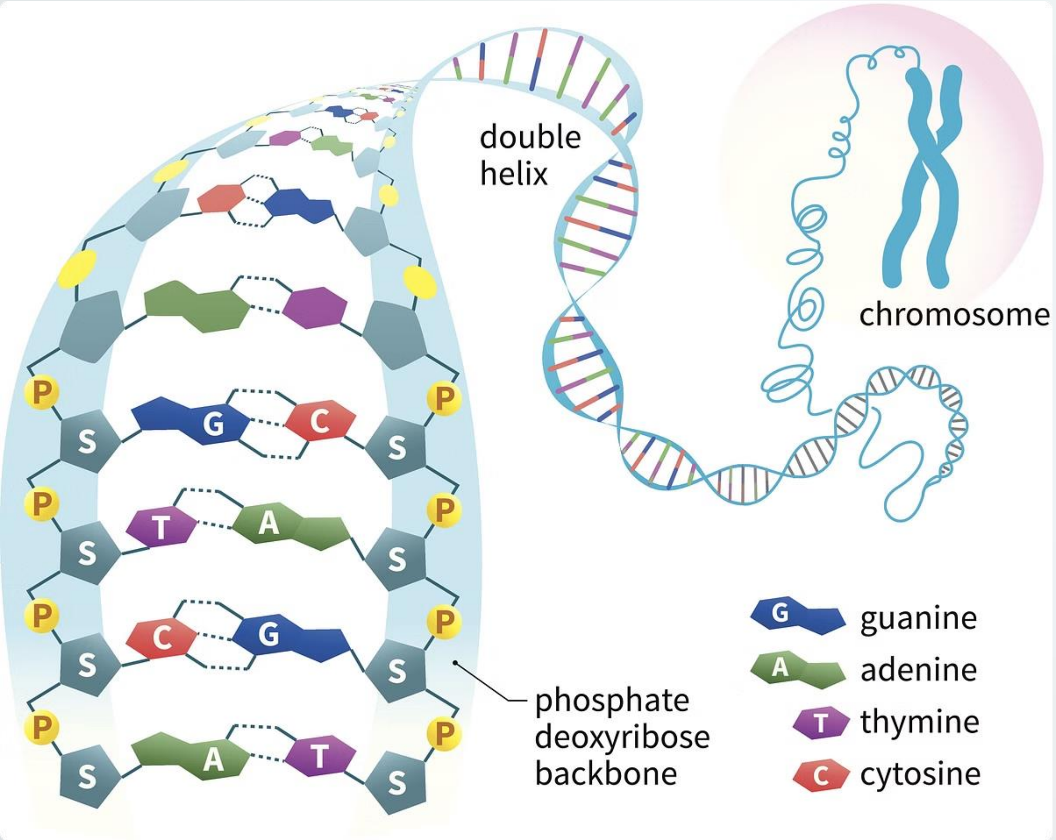
DNA is a molecule composed of two long chains of nucleotides that are twisted together into a double helix.

Four Bases

Each nucleotide contains a sugar, a phosphate group, and one of four nitrogenous bases: adenine (A), guanine (G), cytosine (C), and thymine (T).

Base Pairing

The order of these bases determines the genetic code, which is read by cells to create proteins and other molecules.

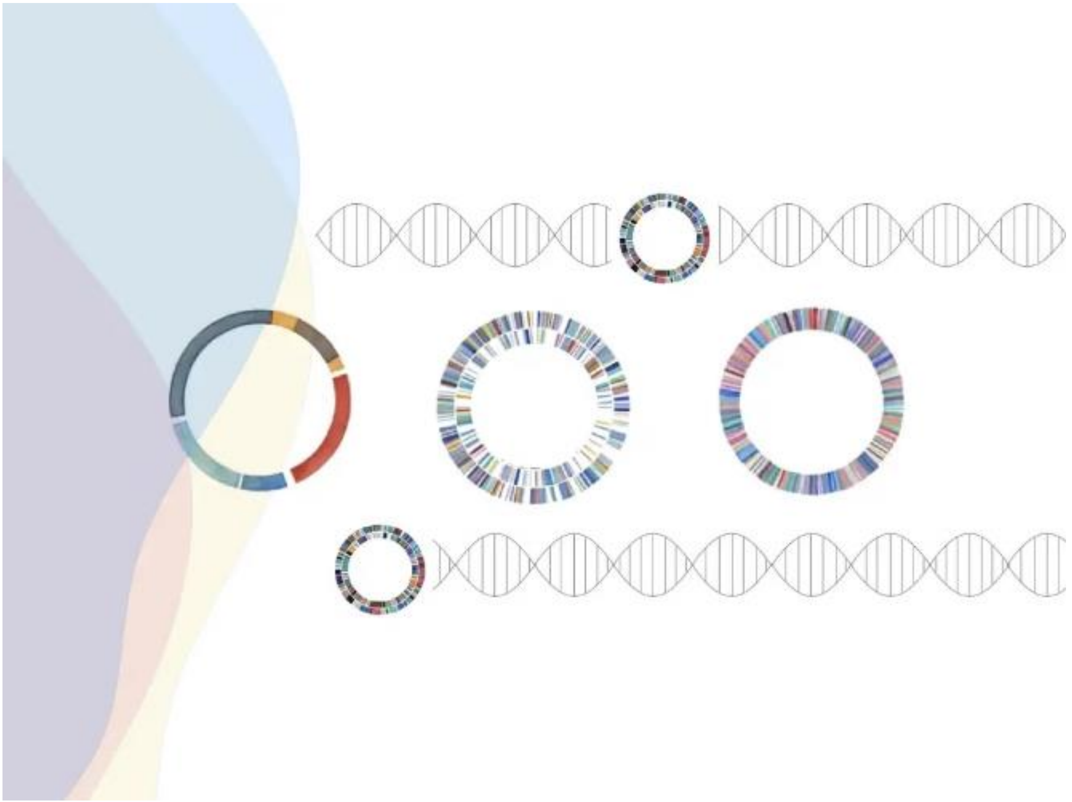


Steps Involved in Recombinant DNA Technology

Recombinant DNA technology typically involves several steps, each crucial for ensuring the successful insertion and expression of the new gene.

Step 1: Isolation of the Desired Gene

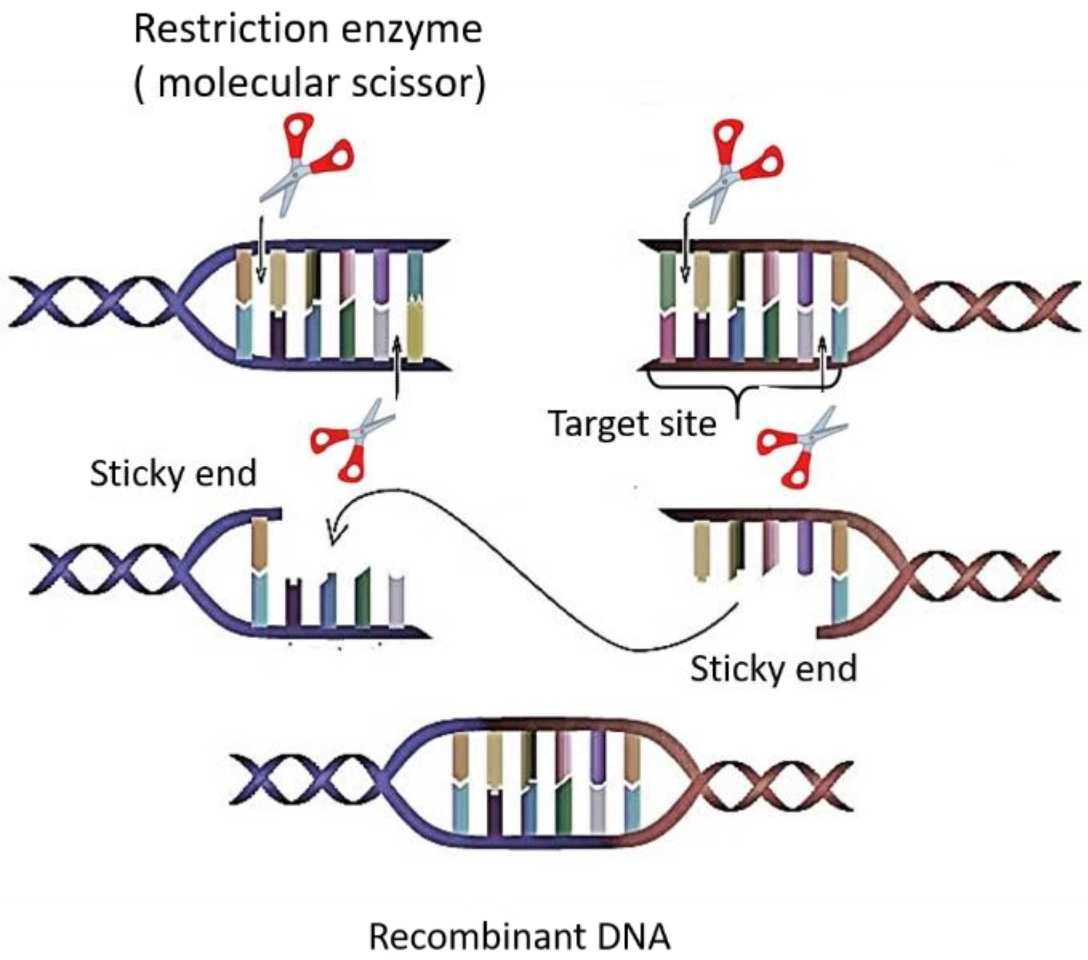
- The first step involves identifying and isolating the gene of interest. This gene could be for a specific protein or trait, such as human insulin. DNA is extracted from the donor organism, and the desired gene is isolated using specific enzymes.



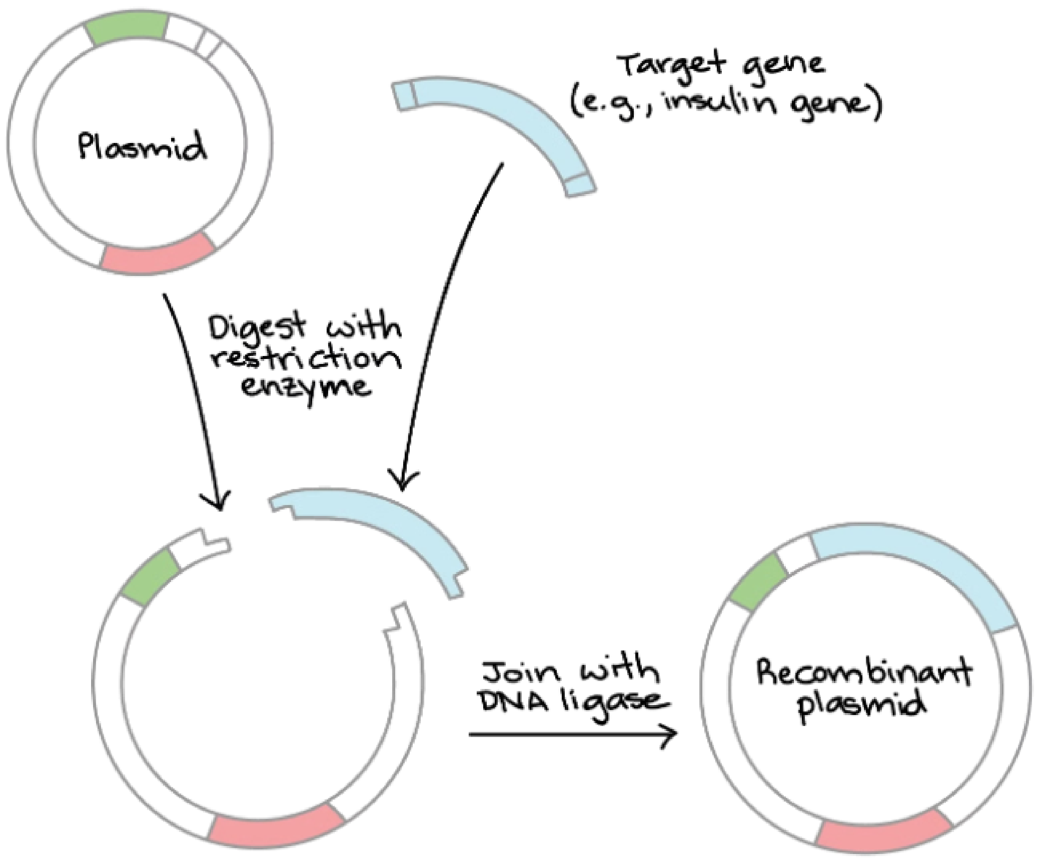
Step 2: Cutting the DNA with Restriction Enzymes

Restriction enzymes act like molecular scissors, cutting DNA at specific sequences called restriction sites. These enzymes are crucial for generating fragments of DNA that can be combined with other DNA sequences.

There are hundreds of restriction enzymes, each recognizing a unique sequence of 4-8 base pairs. This specificity allows scientists to precisely cut and paste DNA fragments, which is essential for gene cloning.



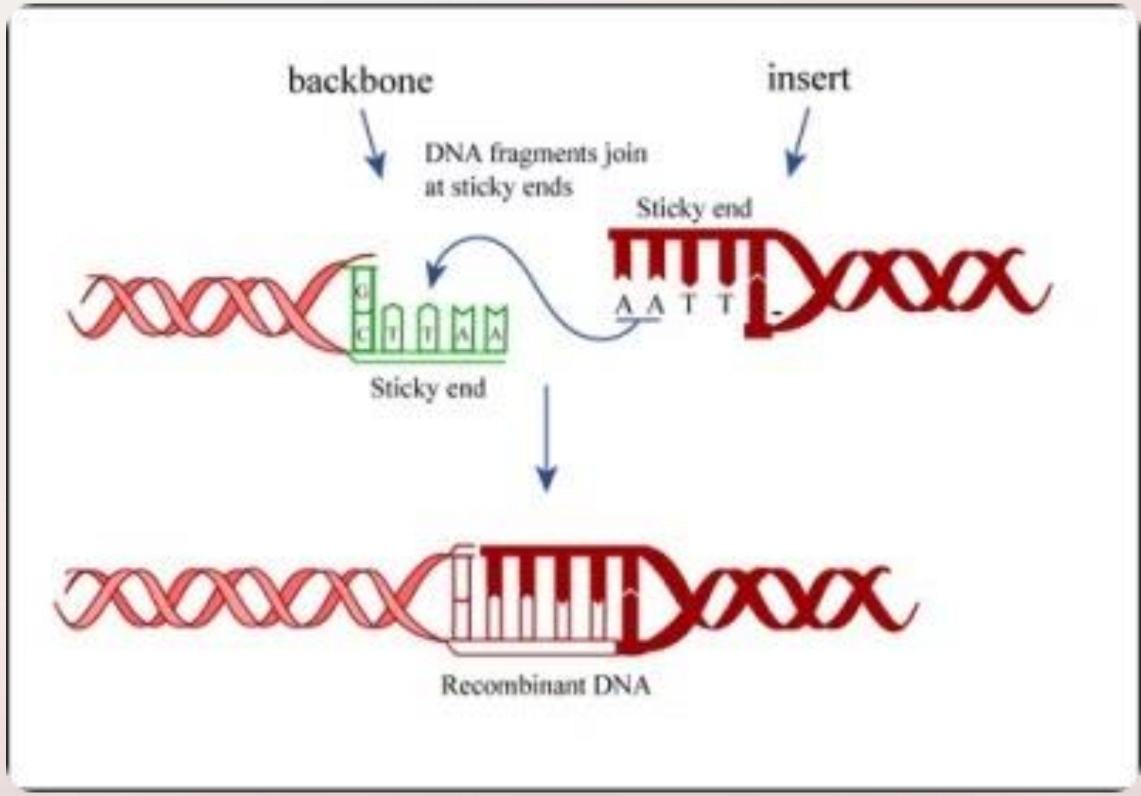
Step 3: Insertion of the Gene into a Vector



- A vector is a DNA molecule that carries the gene of interest into a host cell. Common vectors include plasmids (circular DNA found in bacteria) and viruses. The vector is also cut with restriction enzymes to match the ends of the target gene, enabling them to connect.

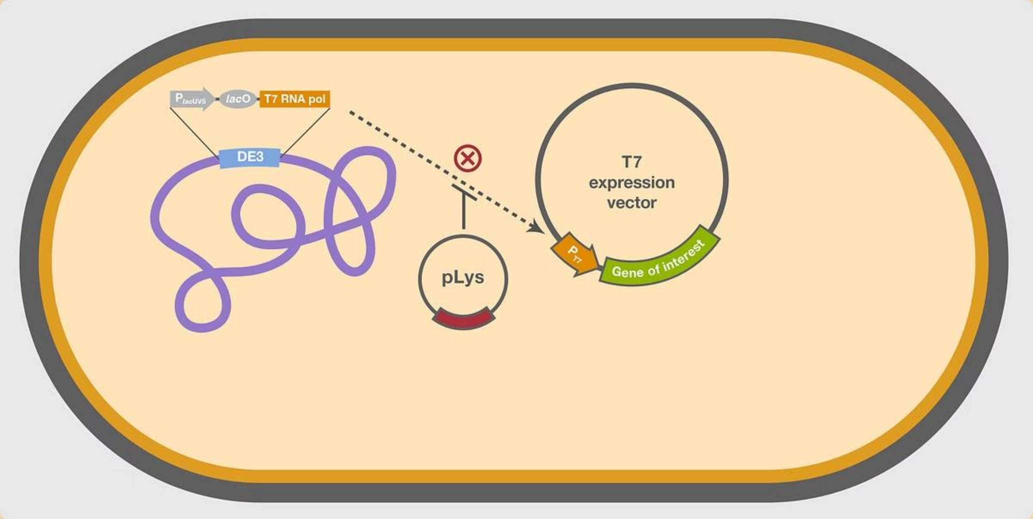
Step 4: Ligation

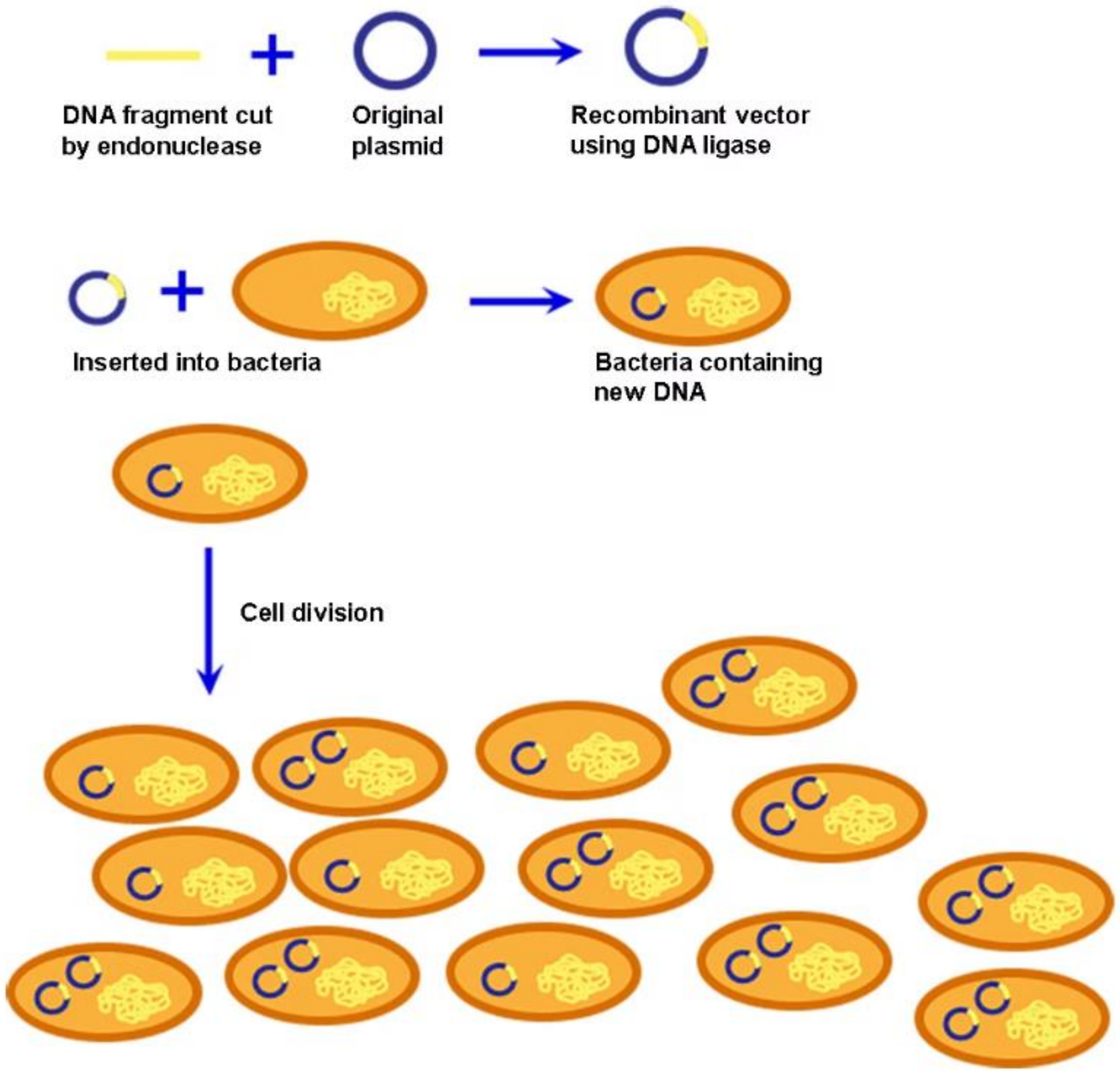
- DNA ligase, an enzyme, is used to join the target gene with the vector, creating recombinant DNA. This process is called ligation, where the gene and vector DNA are "glued" together.



Step 5: Insertion into the Host Cell

- The recombinant DNA is introduced into a host cell, often bacteria, through a process called transformation. The host cell takes up the recombinant DNA and incorporates it into its own DNA, allowing it to express the new gene.

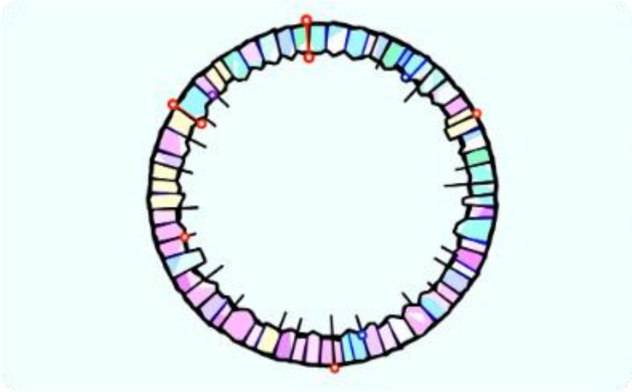




Step 6: Cloning and Selection of Transformed Cells

- The host cells are grown in culture to replicate and form colonies. Only those cells that have successfully incorporated the recombinant DNA are selected and further cultured. Techniques like antibiotic selection help isolate the transformed cells.

Cloning Vectors

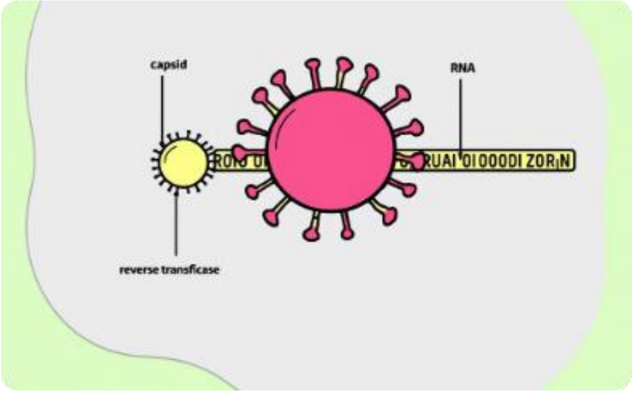


Plasmids

Circular DNA molecules that replicate independently of the host's chromosome, often used to carry foreign genes into bacteria.

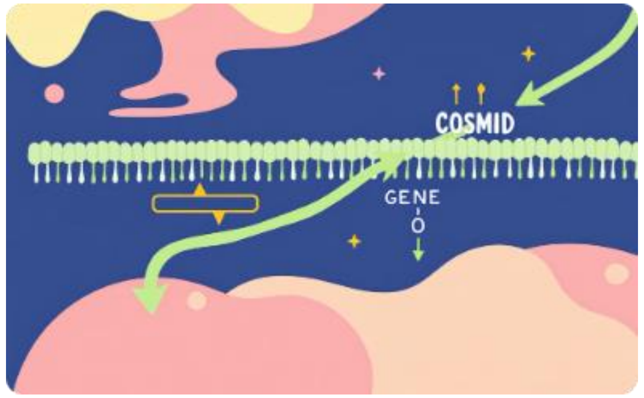
Bacteriophages

Viruses that infect bacteria, can be engineered to deliver genes into bacterial cells.



Retroviruses

RNA viruses that integrate their genetic material into the host's genome, can be used to deliver therapeutic genes into cells.



Cosmids

Hybrid vectors combining features of plasmids and bacteriophages, enabling the cloning of larger DNA fragments.

Step 7: Expression of the Gene

- The gene of interest is expressed in the host cells, allowing for the production of the desired protein or trait. These proteins can then be harvested, purified, and used for various applications.

Restriction Enzymes

Specific Cleavage

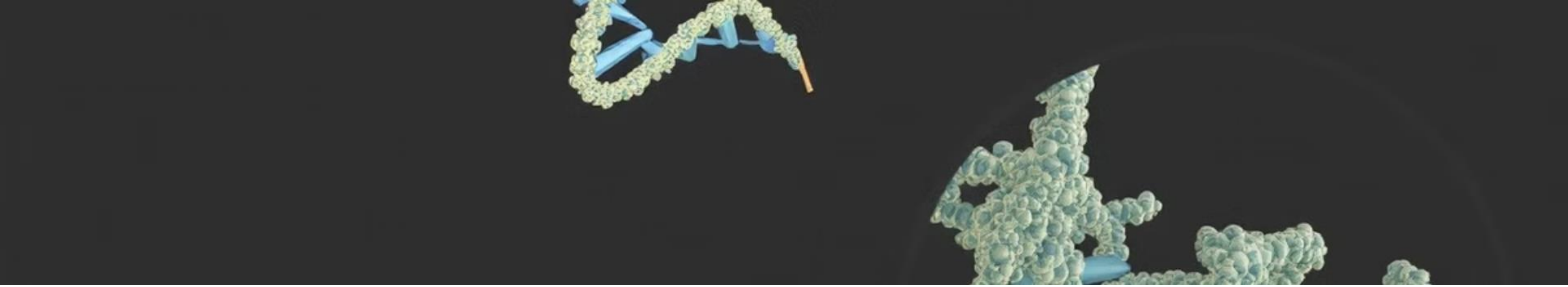
Restriction enzymes cut DNA at specific sequences, known as recognition sites. They act like molecular scissors, breaking the phosphodiester bonds in DNA.

Sticky Ends

Many restriction enzymes produce "sticky ends," short single-stranded overhangs that can base-pair with complementary sequences, facilitating the joining of DNA fragments.

Types of Enzymes

- Type I
- Type II
- Type III
- Type IV



DNA Ligase

Function

DNA ligase is an enzyme that joins two strands of DNA together.

This enzyme is essential for DNA replication, repair, and recombination.

Mechanism

DNA ligase works by forming a phosphodiester bond between the 5' phosphate end of one DNA fragment and the 3' hydroxyl end of another.

Types

There are two main types of DNA ligase, DNA ligase I and DNA ligase IV.

They differ in their substrate specificity, cofactor requirements, and cellular function.

3. Medical Applications of Recombinant DNA Technology

Recombinant DNA technology has numerous applications in medicine, with transformative impacts on healthcare and treatments.

- **Production of Therapeutic Proteins**
  - One of the most prominent applications is the production of human insulin for diabetes management. Recombinant DNA technology enables the production of synthetic insulin identical to human insulin, providing an effective treatment for diabetic patients.
- **Vaccine Development**
  - Recombinant DNA technology allows for the development of safer vaccines, like the hepatitis B vaccine. By inserting the gene for a viral protein into a vector, scientists can produce vaccines that stimulate immunity without introducing the live virus.

- **Treatment of Hemophilia**
  - Recombinant clotting factors produced using recombinant DNA technology are used to treat hemophilia patients, reducing their dependence on blood transfusions and minimizing the risk of blood-borne infections.

- **Gene Therapy**
  - Recombinant DNA technology is used in gene therapy to correct defective genes in patients with genetic disorders. By inserting a healthy version of a gene into the cells of patients with genetic diseases, it's possible to alleviate or potentially cure disorders like cystic fibrosis and muscular dystrophy.

Gene Therapy

Treating Genetic Disorders

Gene therapy aims to correct defective genes responsible for inherited diseases.

It uses vectors to deliver therapeutic genes into target cells.

Types of Gene Therapy

Somatic gene therapy targets specific cells in the body without affecting offspring.

Germline gene therapy modifies genes in eggs or sperm, potentially altering future generations.

Approaches

Gene replacement therapy aims to replace faulty genes with functional copies.

Gene silencing therapy targets specific genes to inhibit their expression.

Thanks