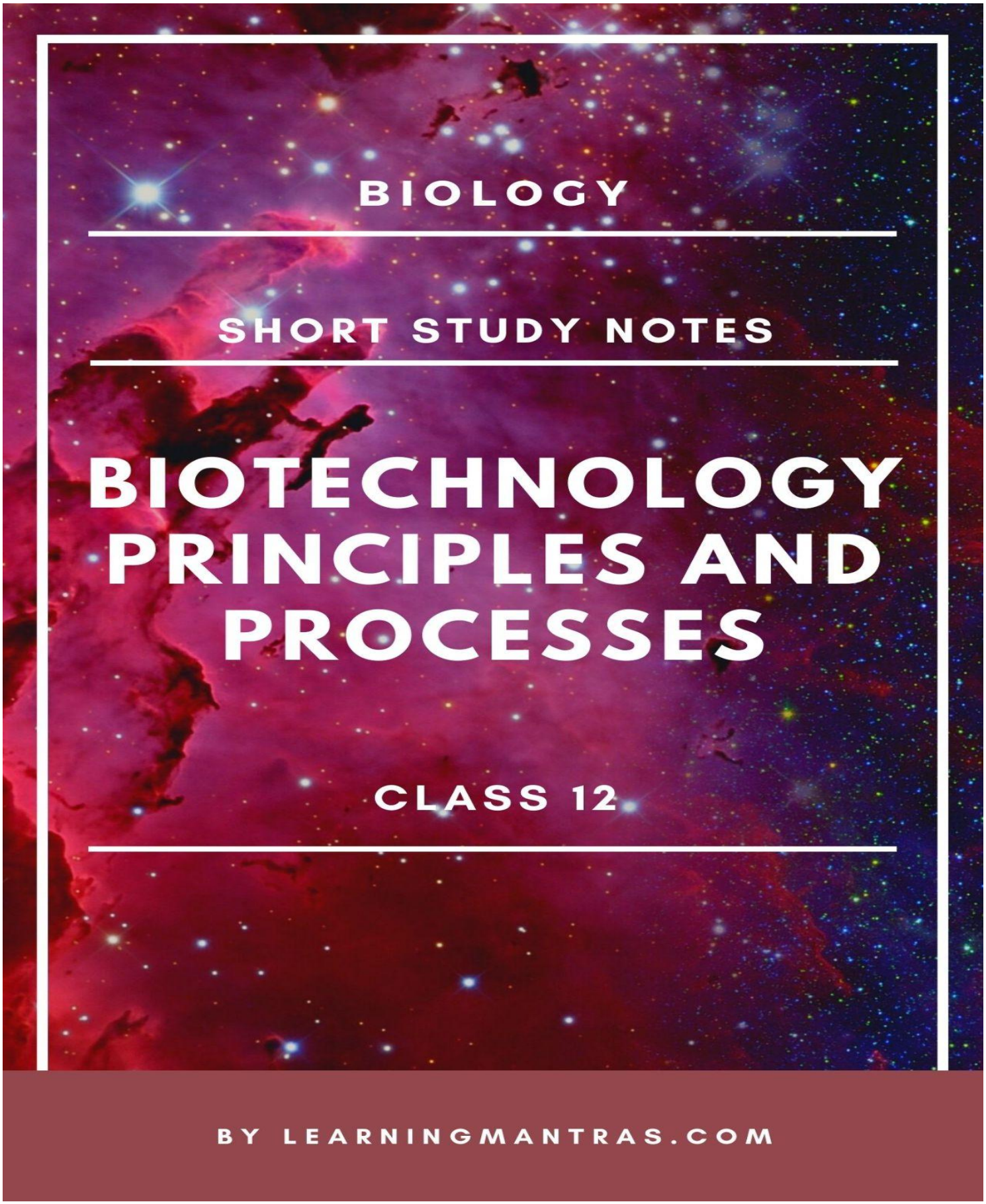

BIOLOGY
SHORT STUDY NOTES
BIOTECHNOLOGY PRINCIPLES AND PROCESSES
CLASS 12
BY LEARNINGMANTRAS.COM

Biotechnology Principles and Processes

Biotechnology is defined as the broad area of biology which uses both the technology and the application of living organisms and their components to develop, modify and produce useful products for human welfare.

- Biotechnology is a combined term of Biology and Technology.
- Biotechnology can be defined as the use of microorganisms, plants or animal cells or their components to produce products and processes useful to humans.
- According to European Federation Technology (EFT) biotechnology is the integration of natural science and organisms, cell, parts thereof and Molecular analogues for products and services.
- The term 'Biotechnology' was coined by Karl Ereky in 1919.

Traditional biotechnology: This technology uses bacteria and other microbes in the daily usage for preparation of dairy products. e.g. Curd, Ghee & Cheese. This biotechnology also extends to preparation of alcoholic beverages like beer, wine etc.

Modern biotechnology: It is based on rDNA technology. It manipulates genetic information in organism. e.g. human gene producing insulin has been transferred and expressed in bacteria

Principles of Biotechnology

According to modern Biotechnology, the main principles of Biotechnology are:

Genetic Engineering

It is the direct manipulation of the genome (DNA and RNA) of an organism. It involves the transfer of new genes to improve the function or trait into host organisms and thus changes the phenotype of the host organism.

The techniques of genetic engineering mainly include:

- DNA fragments are isolated from the donor organism.
- It is inserted into the vector DNA.
- It is transferred into an appropriate host.
- Cloning of the recombinant DNA in the host organism.

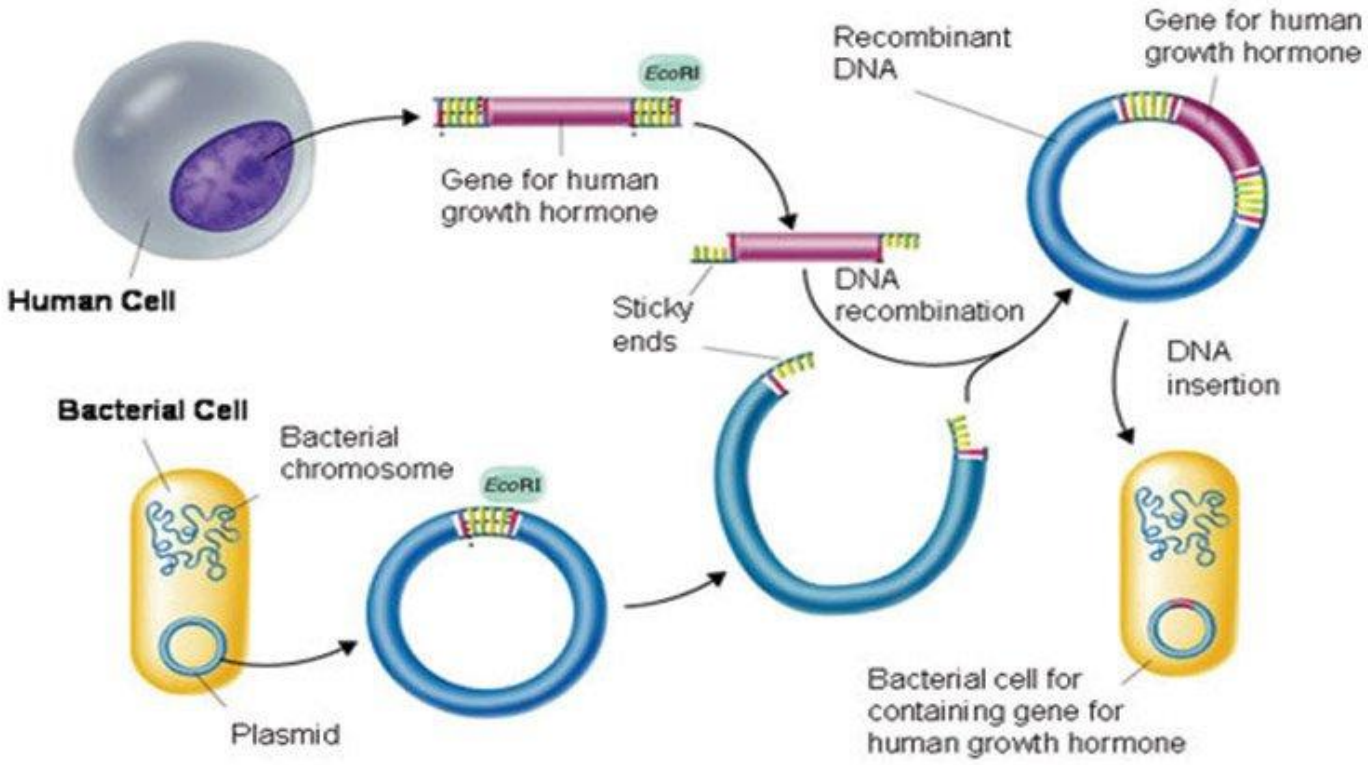
Bioprocess engineering (Chemical engineering)

It is the maintenance of sterile conditions to enable growth of only desired microbes for manufacture of biotechnological products like antibiotics, vaccines, enzymes etc.

Bioprocess engineering is the multiplication of cells in the bioreactors. A large amount of culture is obtained in the process which produces a higher yield of the required protein. The products that are obtained are subjected to a series of processes. The products are purified by downstream processing and subjected to quality check before undergoing further trials. This process is used to manufacture antibiotics, vaccines and other therapeutic drugs.

Recombinant DNA Technology

Recombinant DNA technology is also known as Genetic Engineering. It is the process of joining together two DNA molecules from two different organisms. This is known as the recombinant DNA.



The steps involved in the processes of Recombinant DNA technology are:

- Isolation of DNA.
- Cutting of DNA using restriction endonucleases.
- Isolation of a desired Gene fragment.
- Amplification of desired Gene.
- Ligation of the desired DNA fragment into the vector.
- Transfer of the recombinant DNA into the host.
- Culture of the transformed cells in a nutrient medium.
- Extraction of the desired product.

Isolation of DNA

- First step is lysis of the cell from which the DNA has to be obtained. For this purpose various lysing enzymes are being used.
- DNA is released along with other molecules like RNA, Proteins, Polysaccharides and lipids.
- DNA is separated by removing other molecules from it by treating them with specific enzymes like Ribonuclease (Remove RNA) and Proteases (Remove Proteins).

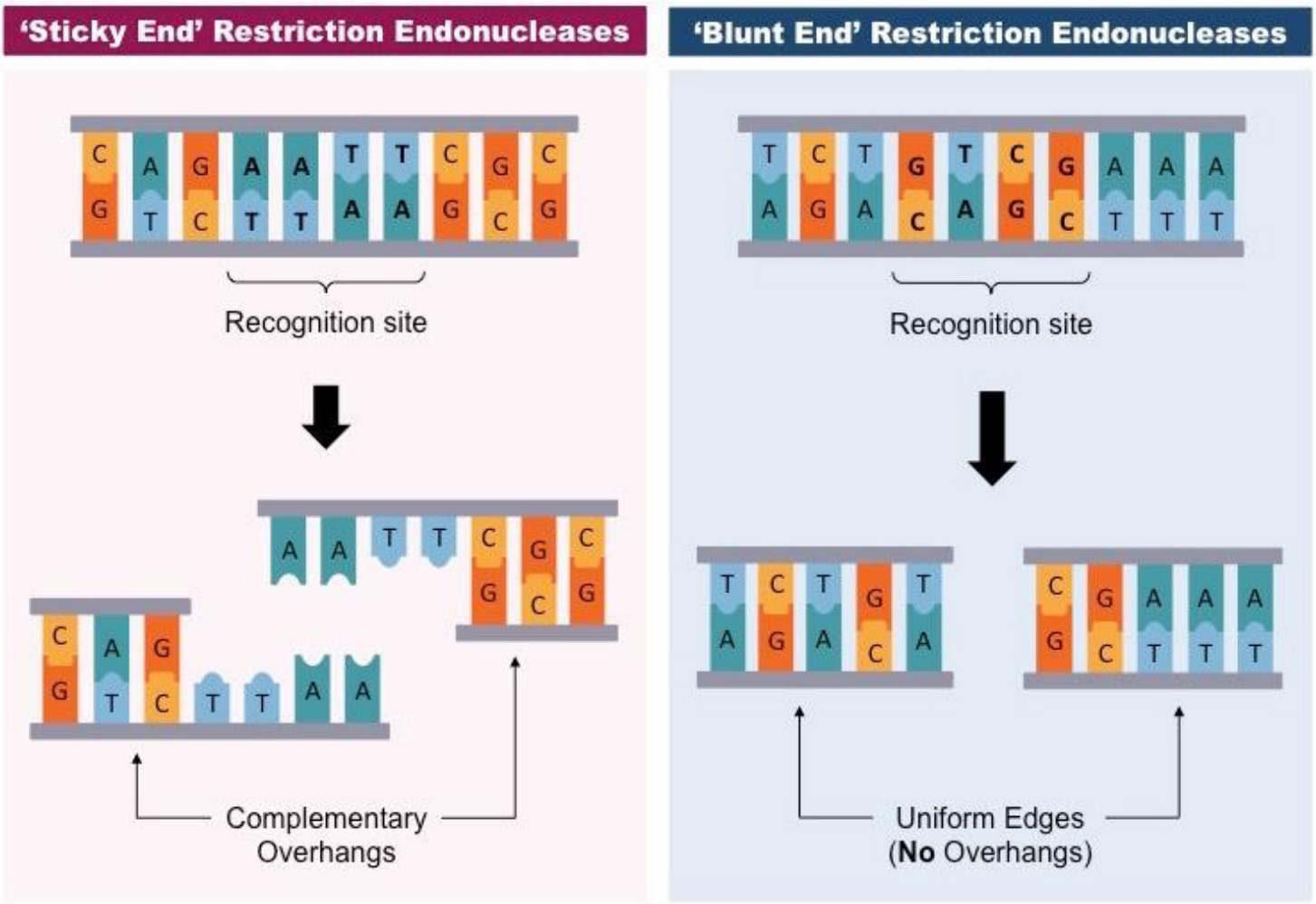
- Finally, the pure DNA molecules precipitate it out by adding chilled ethanol and collecting it in the suspension.

Tools of Genetic Engineering

- Enzymes
- Cloning Vectors
- Competent Host

Enzymes:

- **Lysing Enzymes:**
  - Plant Cells: Cellulase, Pectinase, Protease & Lipase
  - Animal Cells: Protease & Lipase
  - Fungal Cells: Chitinase, Protease & Lipase
  - Bacterial Cells: Lysozyme
- **Restriction Enzymes:**
  - In 1963, Scientist discovered two enzymes responsible for restricting the growth of bacteriophage in Escherichia coli.
  - Restriction enzymes are members of endonucleases.
  - Restriction enzymes belong to a larger class of enzymes called Nucleases. There are two types of nucleases enzymes:
    - Exonucleases: Cut of DNA nucleotides from ends. E.g.
    - Endonucleases: Cut DNA at any point except the ends. E.g. Hind II
- **Naming of Restriction Enzymes:**
  - First letter comes from the Genus.
  - Second & Third letters come from the species.
  - Fourth letter comes from a bacterial strain.
  - Roman numbers after the name show the order in which the enzymes were isolated from the bacterial strain.
- **Mechanism of Restriction Enzymes:**
  - Each restriction endonuclease recognises a specific palindromic nucleotide sequences in the DNA
  - Palindrome in DNA is a sequence of base pairs that reads the same on the two strands when orientation of reading is kept the same.
- **Plane of Cutting:**
  - **Blunt End**
    - Cut both strands of DNA through the centre
    - These are known as symmetric cuts.
    - As a result, two blunt ends are formed.
  - **Sticky End**
    - Cut in a way producing protruding and recessed ends.
    - These are known as asymmetric cuts.
    - As a result, DNA fragments with single strand extensions formed.



Vectors:

Vectors are the DNA molecules that can carry a foreign DNA segment into the host cell. Vectors are also known as vehicle DNA or Gene carriers. Vectors are of two types:

- **Cloning Vector:** used for the cloning of DNA inserted inside the suitable host cell.
- **Expression Vector:** used to express the DNA insert for producing specific protein inside the host.

Major Vectors used in rDNA technology:

- **Plasmids:** Plasmids are small, circular, double stranded and extrachromosomal DNA present in bacterial cells. They can replicate independently due to the presence of an origin of replication. Plasmids are 1kbp-200kbp in size and have a limited number of genes. Plasmid vectors created from wild plasmid are called constructed vectors or artificial plasmid vectors. During construction some unwanted portion removed and desired sequences are inserted. Examples of Plasmid Vectors are:
    - **pUC Vectors**
        - This plasmid vector created by University of California are termed pUC vectors.
        - pUC vectors consist of an Ori Site & Lac Z gene as a selectable marker.

        - Structure of all pUC vectors is the same, but they differ from each other in their multiple cloning sites.
    - **pBR322**
        - This is the first artificial cloning vector
        - pBR322, where p indicates that is a plasmid, B and R stands for Boliver and Rodriquez. 322 is specific number to distinguish from others
        - It consist of 8 restriction sites
        - pBR322 has two selectable marker genes i) Tet^r gene ii) amp^r gene
    - **Ti Plasmid**
        - Ti plasmids are tumor inducing plasmids present in *Agrobacterium tumefaciens* bacteria.
        - The plasmid carries a transfer gene which helps to transfer T-DNA from one bacterium to another bacterial or plant cell.
        - Ti plasmids have been used for the introduction of genes of desirable traits into plants.
- **Bacteriophage Vectors**
    - These are bacterial viruses that carry a desired gene to a host cell.
    - Copy number is very high, that's why it is used as a cloning vector.
    - g. M13 Phage, Lambda Phage & P1 Phage.
- **Cosmids**
    - Cosmids are hybrid vectors created from Plasmid + Lambda Phage.
    - Cosmids gets circular DNA like Plasmid and they have cos sies like Lambda Phage.
    - In 1978, Casmid was first constructed by Collins and Hohn.

Host:

The cell receiving the rDNA is called Host. Many types of host cells are available E.g. E.coli, yeast, animal or plant cells. Coli is the most widely used organism as its genetic engineering. DNA is a hydrophilic molecule and it can't pass through the plasma membrane. DNA is a hydrophilic molecule, it can't pass through cell membranes so the bacterial cells must first be made "competent" to take up DNA. Therefore, certain treatments are made in the host self to become competent to take up rDNA.

- **Chemical Method:** Treatment of bacterial cells with specific concentration of divalent calcium. This increases the chances of rDNA entry into the bacterial cell wall through the tiny pores. Incubation of bacterial cells with recombinant DNA on ice. Thereafter these are placed briefly at 42'C after this again transferred on ice. This process enables the bacteria to take up rDNA.
- **Microinjection Method:** In this method, the rDNA solution is directly injected into the nucleus of animal cells. Capillary class micropipettes or micro injections help to inject the rDNA into host cells. This is most common in case of animal cells.
- **Gene Guns Method:** This technique is also known as a biolistic technique. High velocity particles of Gold or Tungsten coated with rDNA are bombarded on Host Cells. This method is mostly used in plant cells.
- **Electroporation method:** Electroporation is a technique used to change the permeability of cell membrane of cells to uptake rDNA in the medium. Electroporator instruments used to apply suitable voltage to make permeability of the cell. Electroporation is helpful to transform bacteria, fungi, plant cells and animal cells.

DNA Cloning

DNA cloning is the process of making multiple, identical copies of a piece of DNA. This process requires cloning vectors which possess the following properties:

- It should be smaller in size but should be able to carry a large DNA insert.
- The cloning vector should have the origin of replication so that it can autonomously replicate in the host organism.
- It should have a restriction site.
- It should have a selectable marker to screen recombinant organisms.
- It should possess multiple cloning sites.

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