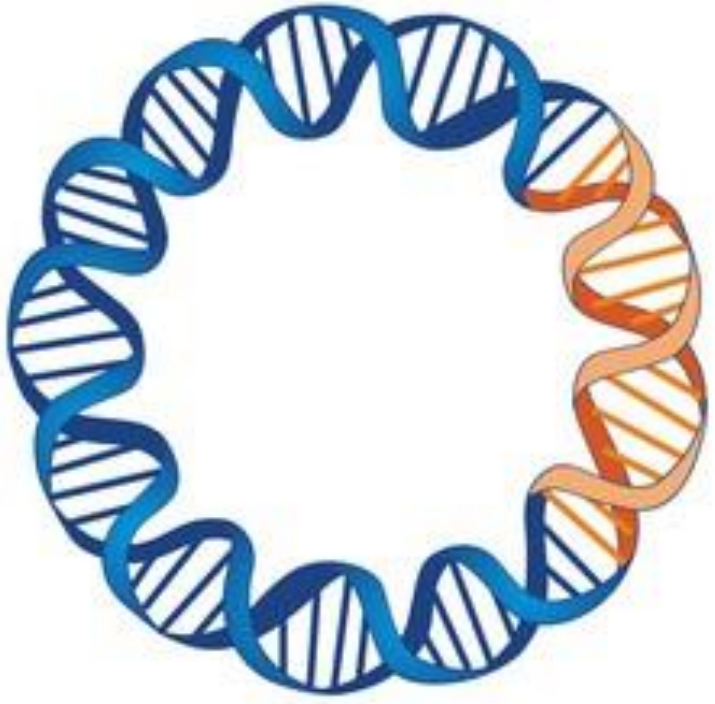
General characteristics of vectors used in GI. Classification. Basic properties. Capacity of vectors



Lecturer: Senior Lecturer, Department of Molecular Biology and Genetics, PhD, Smekenov I.T.
Subject: Genetic enginerring

(Lecture 3)

Lecture Goal:
To provide an overview of the general characteristics, classification, and basic properties of vectors used in genetic engineering.

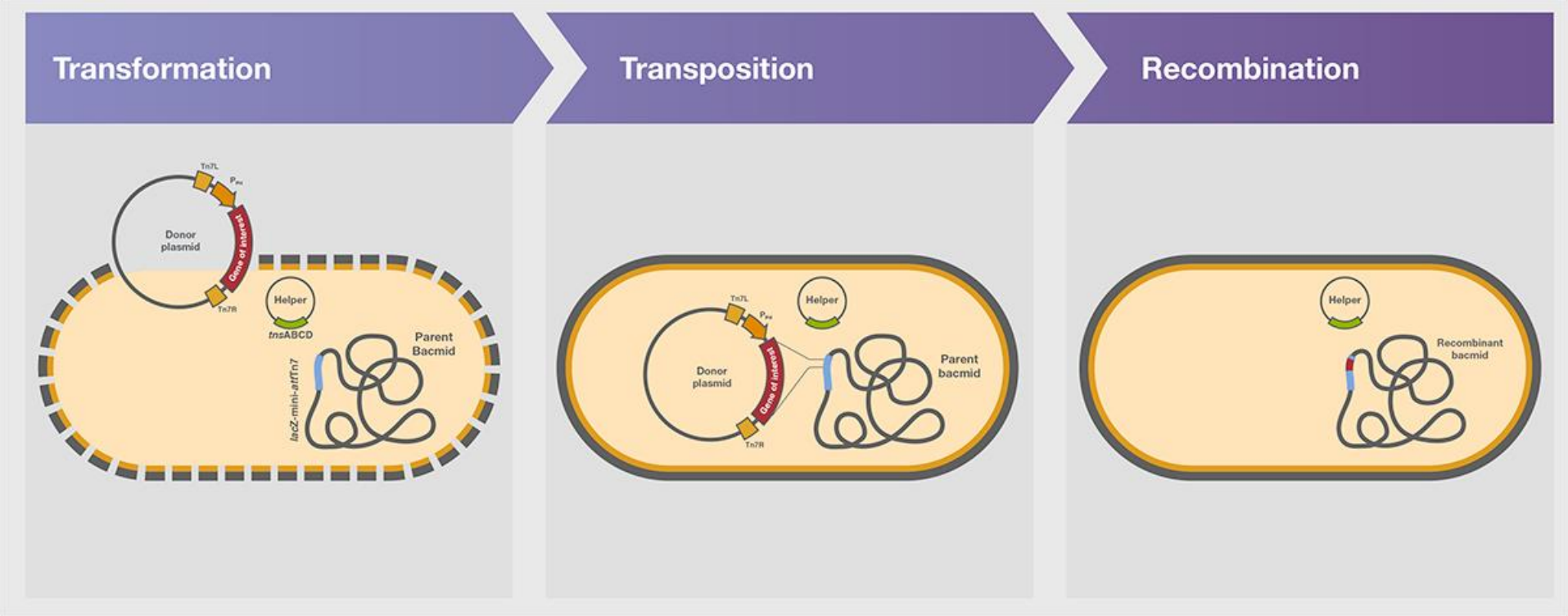
Tasks:

1. Classify the main types of vectors used in genetic engineering, such as plasmids, cosmids, bacteriophage vectors, and viral vectors.
2. Describe the basic properties of these vectors, including their structure and capacity for carrying genetic material.
3. Explain the concepts of transfection and competence, and their importance in introducing vectors into host cells.

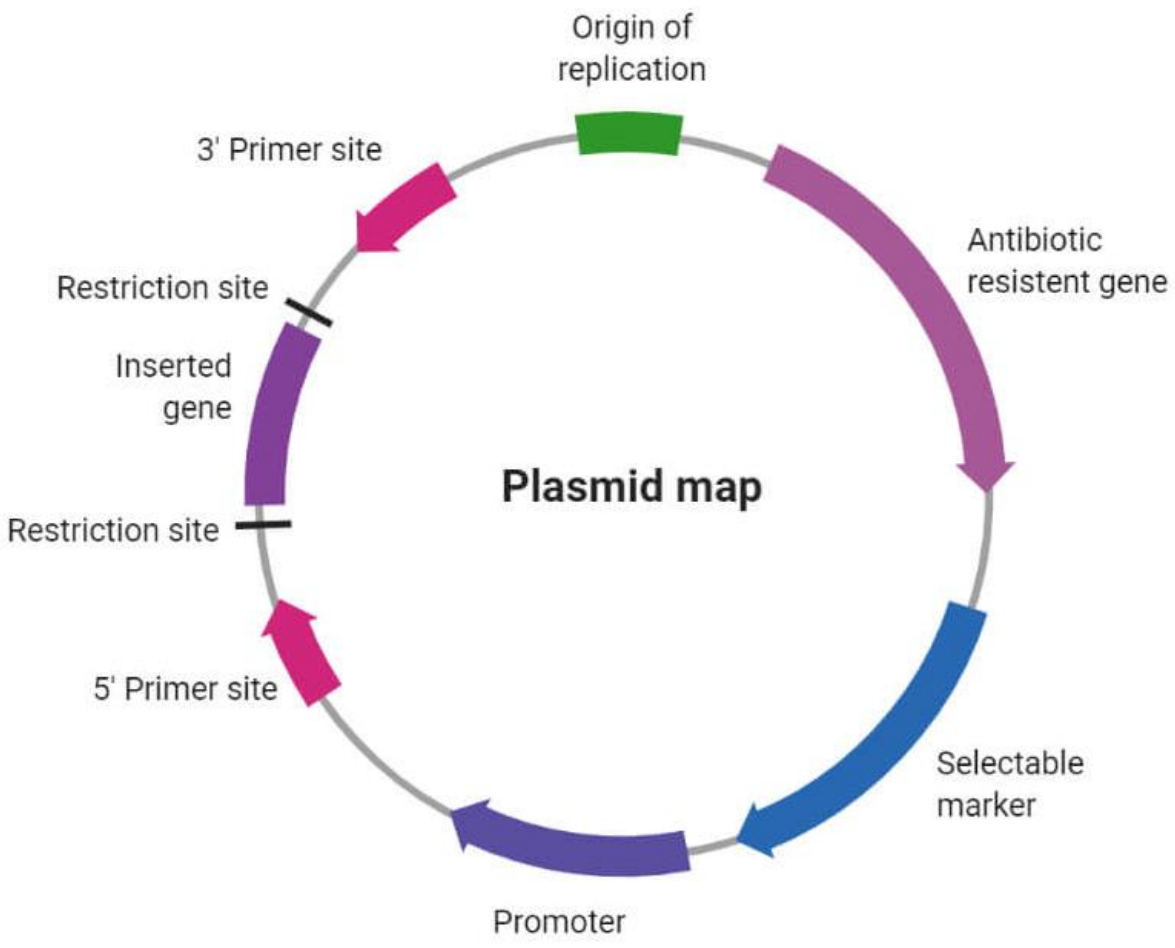
Keywords: *Vectors, plasmid vector, cosmid, bacteriophage vector, viral vectors, transfection, competence, vector capacity, cloning efficiency, gene delivery*

Definition

- A **vector** is a substance, usually a piece of DNA that carries a sequence of DNA or other genetic material and introduces it into a new cell.



Features



- Vectors act as **vehicles** to transfer genetic material from one cell to the other for different purposes like multiplying, expressing, or isolation.

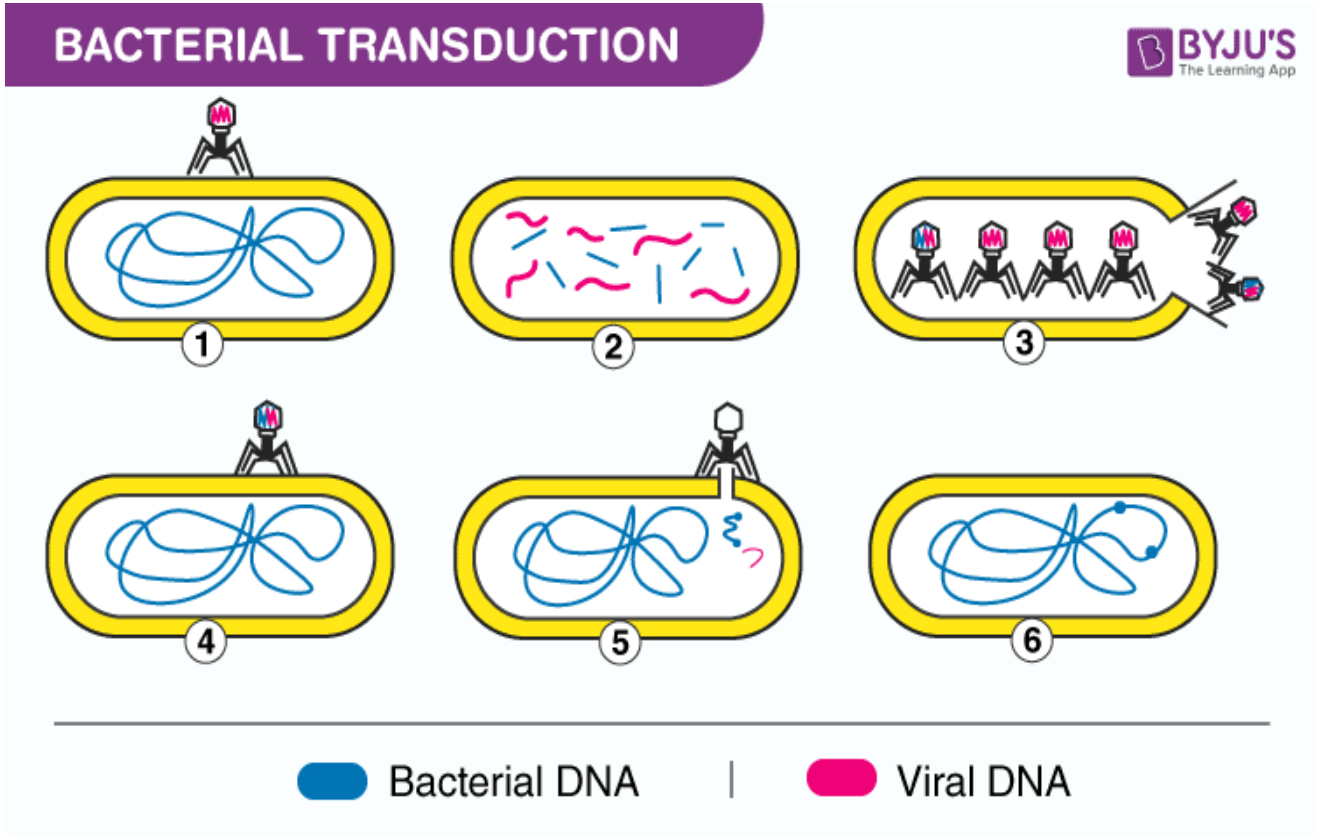
- Vectors are used as a **tool** in molecular cloning procedures so as to introduce the desired DNA insert into a host cell.

- Vectors usually have an **insert**, also known as a transgene, that carries the recombinant DNA and a larger sequence called the **backbone** of the vector responsible for the structure of the vector.

- Vectors can be classified into **different types** depending on different characteristics. The selection of vectors thus depends on the purpose of the process.

- Vectors are an important component of the genetic engineering process as these form the **basis** for the transfer of DNA fragments from one cell to another.
- Vectors have particular features that carry the gene sequences and enable them to **survive within** the host cell.
- Even though vectors are usually DNA sequences, viruses and other particles can also function as vectors in processes like **transduction**.
- Vectors can be **reused** for multiple processes as these can be recovered at the end of the process.

✓ **Transduction** is a mode of genetic transfer from one bacteria to another through a virus.



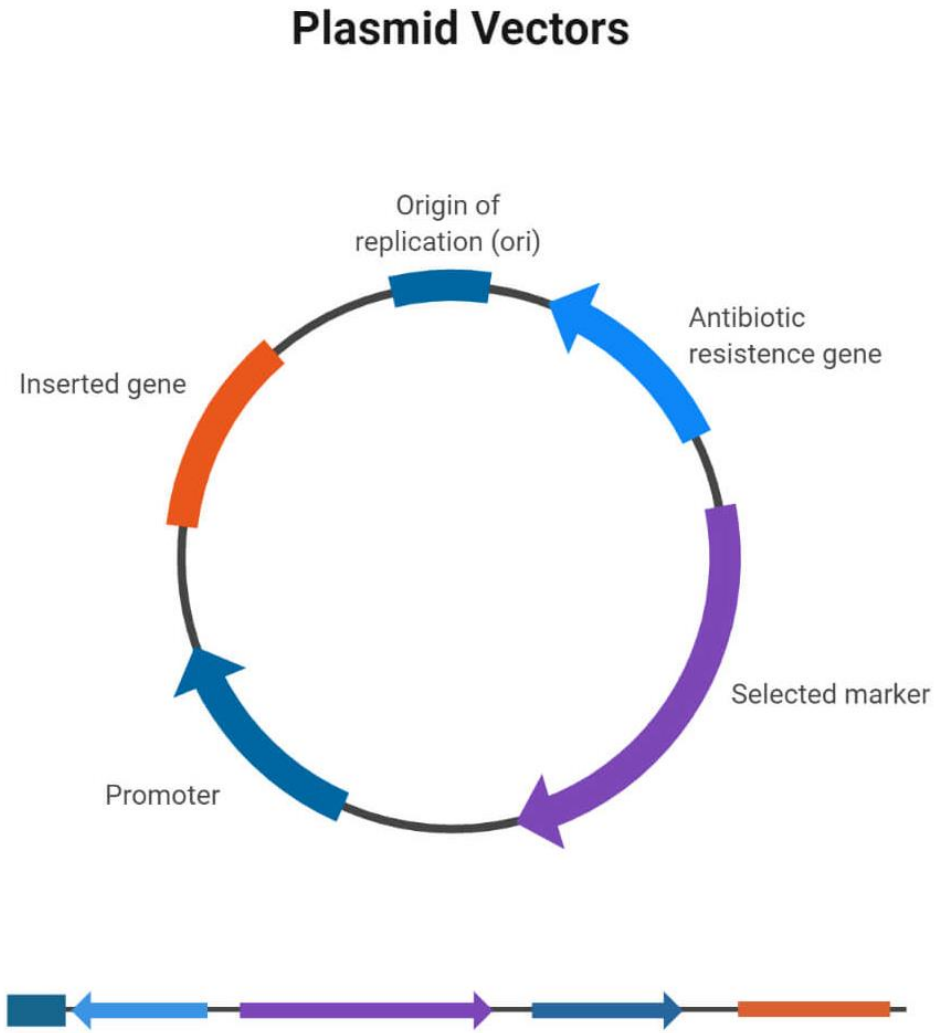
Some characteristic features of vectors

1. Vectors should be capable of replicating **autonomously**.
2. The size of an ideal vector should also be **small enough** for it to be incorporated into the host genome.
3. Vectors should be easy to isolate and purify as these need to be recovered and reused for **multiple processes**.
4. For a vector to be effective, these should also have certain components that facilitate the process of determining whether the host cell has received the vector (**resistance gene to an antibiotic or marker genes**).
5. Many vectors also require **unique restriction enzyme** recognition sites that enable the insertion of the vector DNA in the presence of specific restriction enzymes.
6. In the case of gene transfer processes, it is important that the vector **is capable of integrating** itself or the recombinant DNA into the genome of the host cell.
7. It is important that the introduction of recombinant DNA into the vector **doesn't affect** the replication cycle of the vector.

Types of vectors

1. Cloning vectors

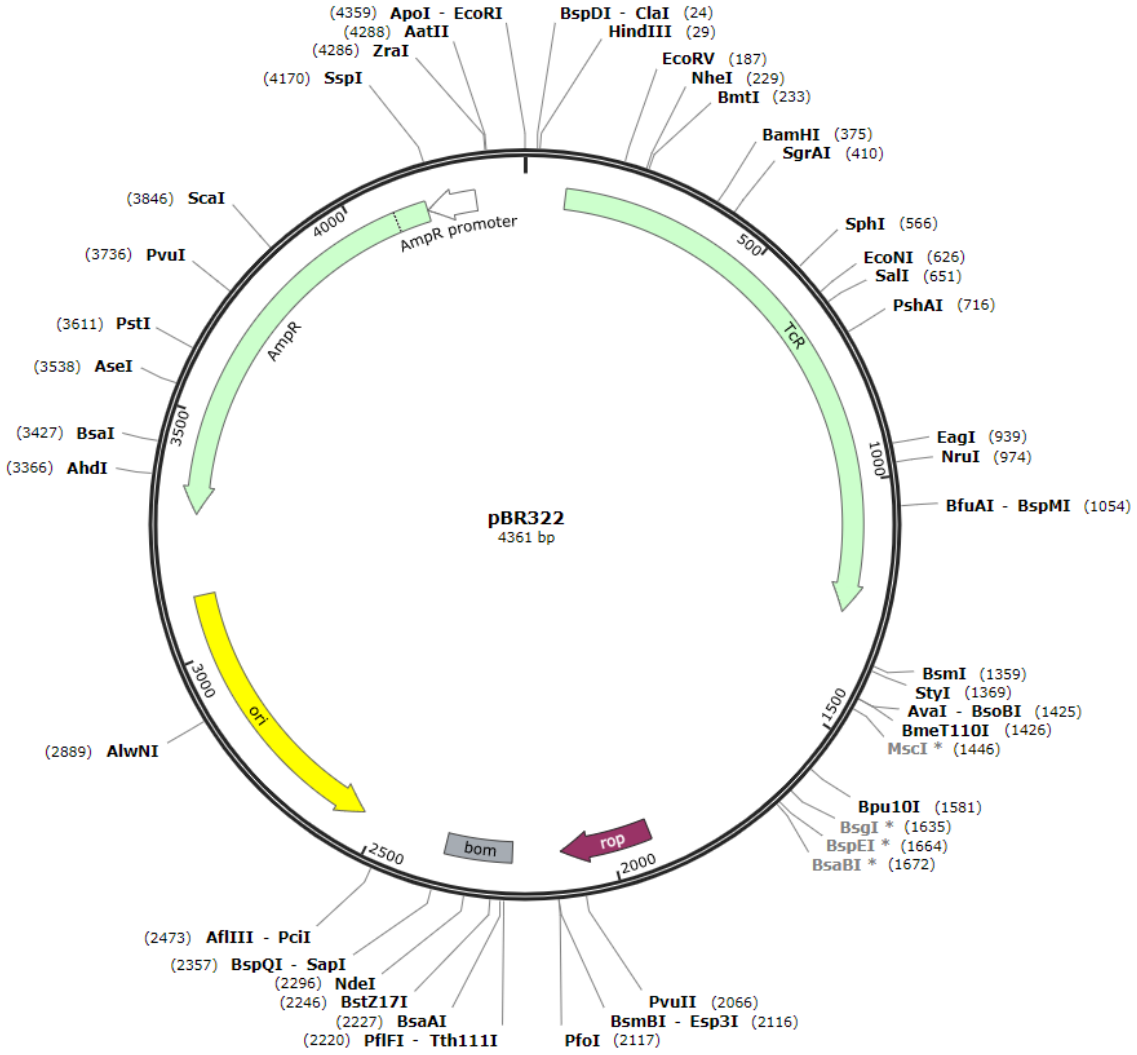
- Cloning vectors are vectors that are capable of replicating autonomously and thus are used for the replication of the recombinant DNA within the host cell.
- Cloning vectors are responsible for the determination of which host cells are appropriate for replicating a particular DNA segment.
- Cloning vectors are of further different types that are defined by different features unique to each type of vector.

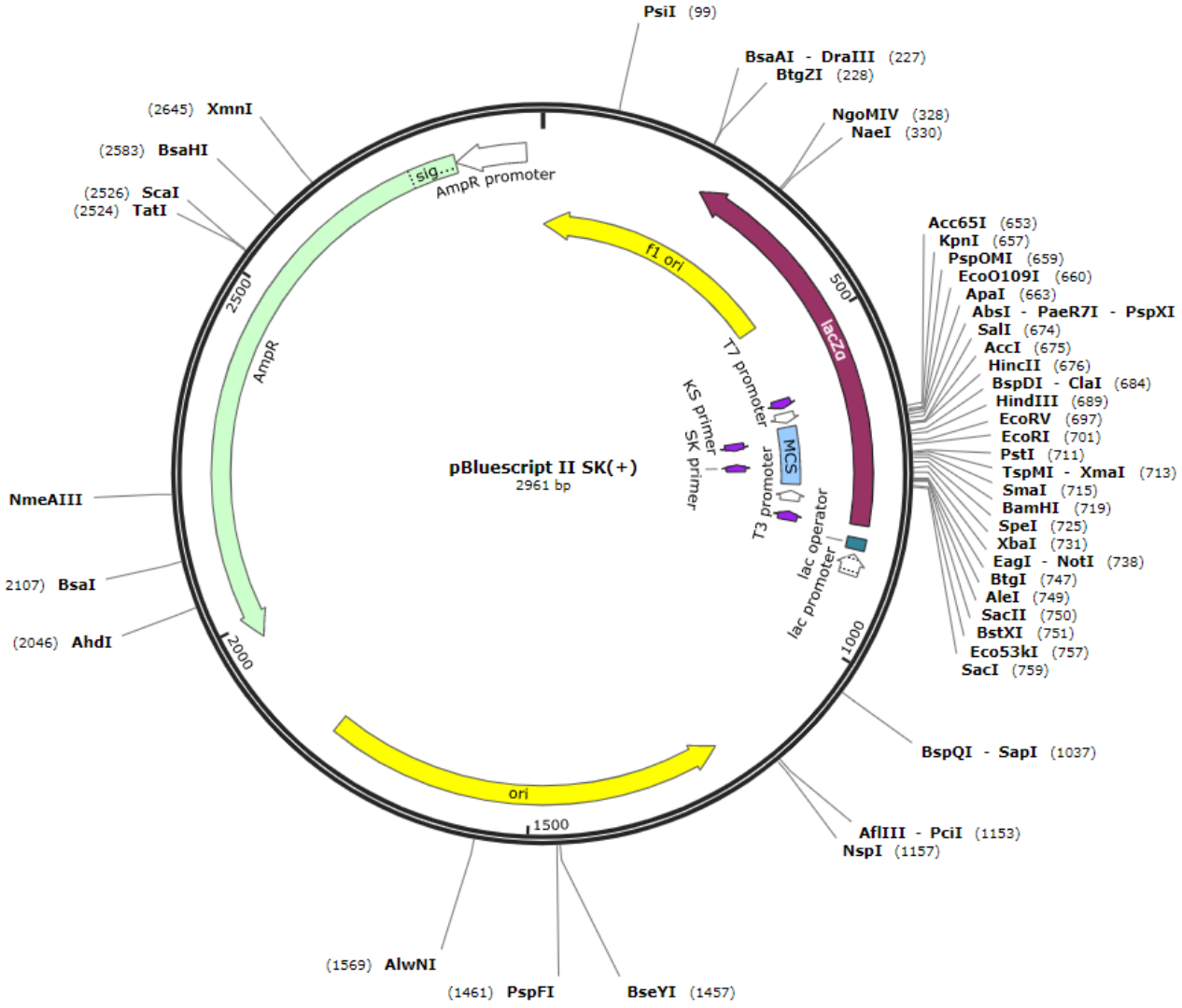


a. Plasmid vector

- **Plasmids** are extrachromosomal autonomous replicating double-stranded circular DNA molecules.
- From 1-500 kb
- 10-100 copies per bacterial cell
- Carry phenotypic traits
- Plasmids can carry insert DNA that is less than 20 kb as the cloning efficiency and plasmid stability decrease with the size of the vectors.
- Bacterial plasmids contain ori sequences that not only control plasmid replication but also determine the possibility of two plasmids coexisting within the same host cell.

Some of the most widely used plasmids are **pBR322**, **pUC**, and **pBluescript** vectors that use *E. coli* as the host.

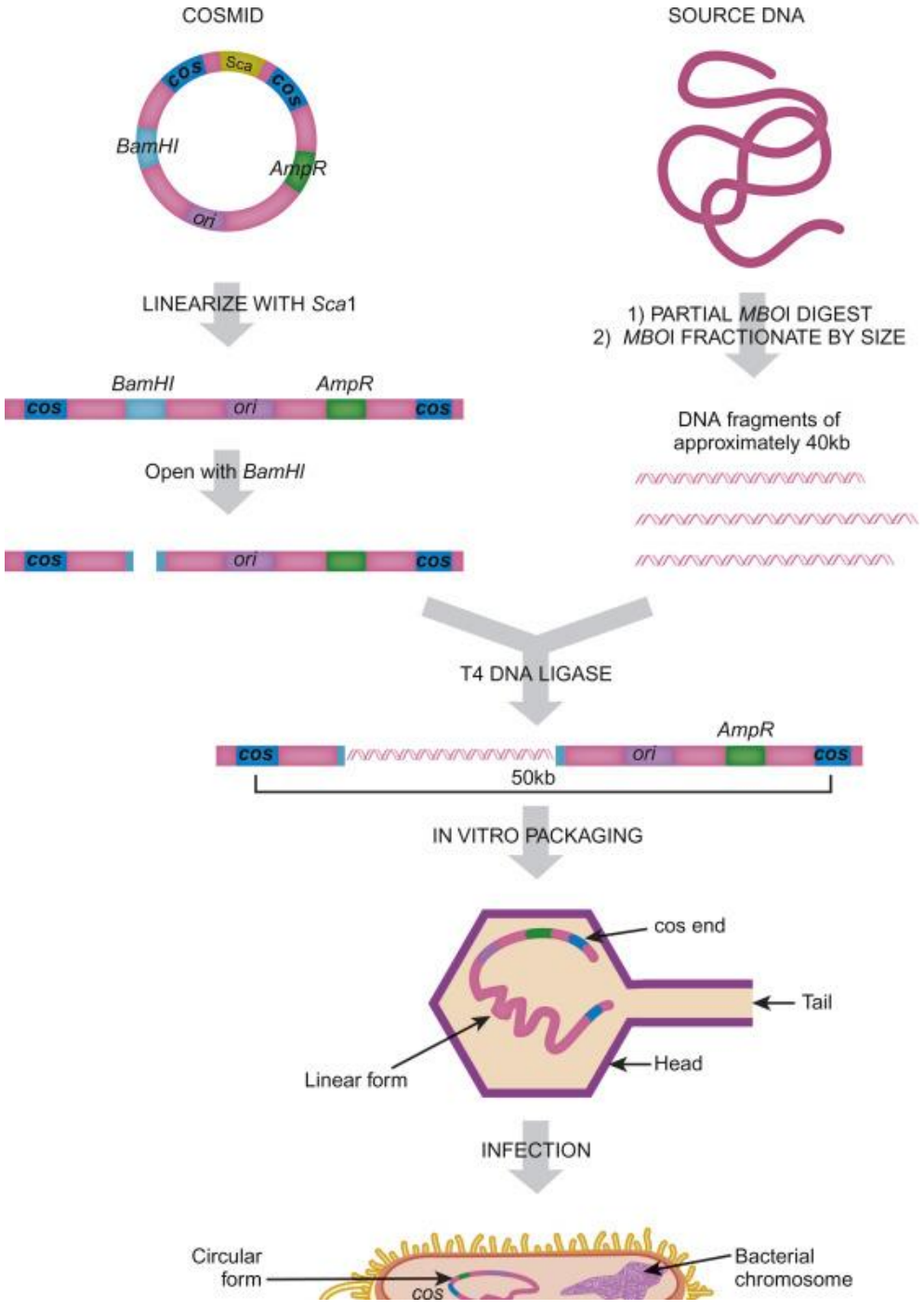


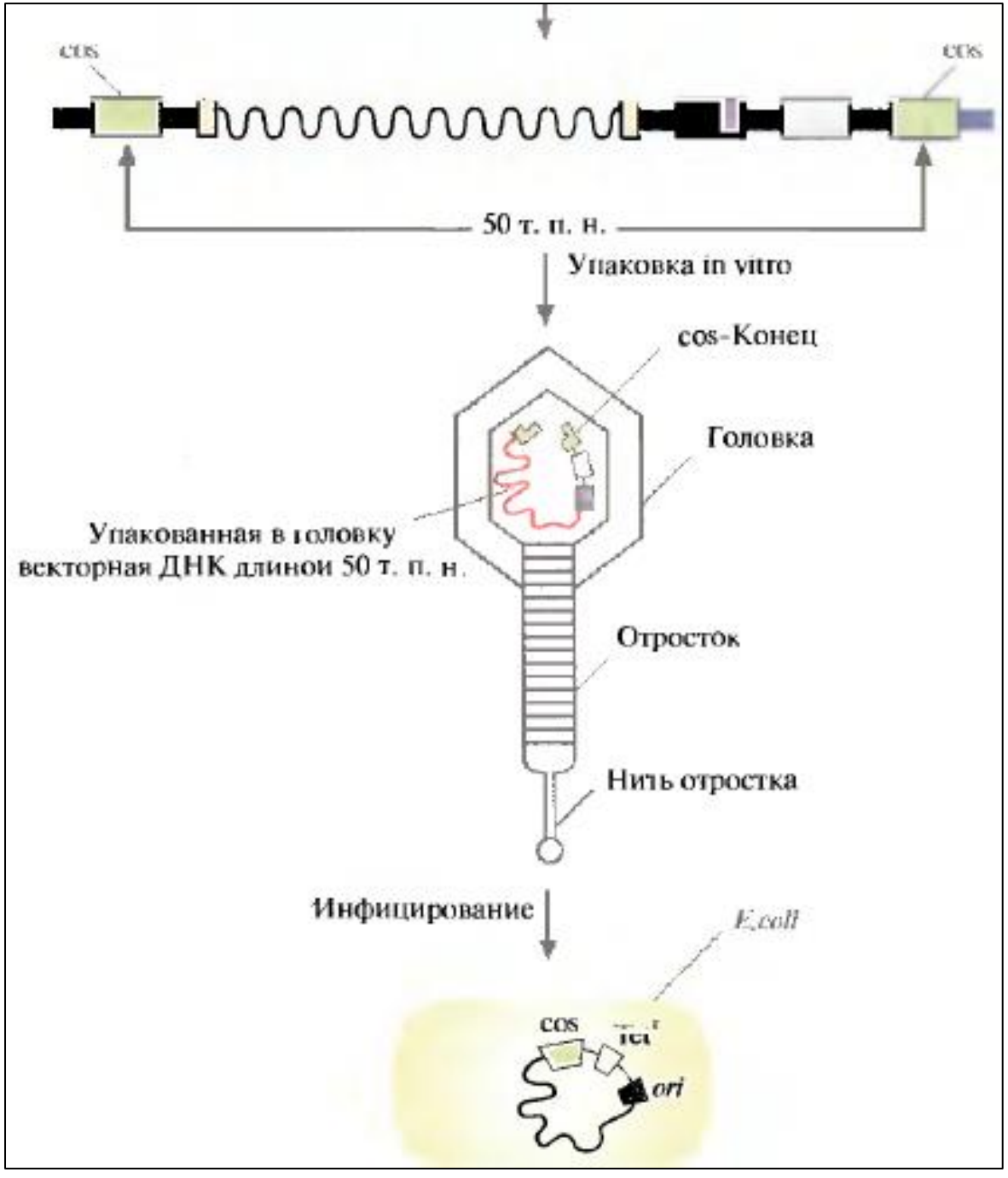


b. Cosmid

Cosmids are vectors in which a lambda phage region is inserted, which are capable of incorporating up to 40 kb of foreign DNA (pLFR-5, about 6 kb) and packing into capsids.

- ✓ Cosmid vectors are prepared by the insertion of the **cos region** of the phage vector into the plasmid vectors.
- ✓ It can carry DNA sequences having sizes ranging from **28 to 46 kb.**
- ✓ Cosmid vectors are created in order to incorporate **large-sized DNA** molecules that cannot be carried by plasmids.
- ✓ The hybrid structure of cosmid enables the **phage heads** to be incorporated within all donor DNA for transfer.
- ✓ One of the examples of the cosmid vectors prepared and used in practice are cosmid pHC79 which is a cos-containing derivative of the vector **pBR322**.


COSMID
SOURCE DNA
cos
Sca
cos
BamHI
AmpR
ori
LINEARIZE WITH Sca1
1) PARTIAL MBOI DIGEST
2) MBOI FRACTIONATE BY SIZE
BamHI
AmpR
cos
ori
cos
Open with BamHI
DNA fragments of
approximately 40kb
cos
ori
cos
T4 DNA LIGASE
AmpR
cos
ori
cos
50kb
IN VITRO PACKAGING
cos end
Tail
Linear form
Head
INFECTION
Circular
form
cos
Bacterial
chromosome


cos
cos
50 т. п. н.
Упаковка in vitro
cos-Конец
Головка
Упакованная в головку
вскторная ДНК длиной 50 т. п. н.
Отросток
Нить отростка
Инфицирование
E.coli
cos
ori

c. Bacteriophage vector

- **Phasmids** are molecular vectors that are artificial hybrids between a phage and a plasmid. Can develop as phages or plasmids.

❖Bacteriophage vectors are viruses that only infect bacteria and transform them efficiently while carrying large inserts.

❖The most important feature of a phage is the packaging system which enables the incorporation of large eukaryotic genes and their regulatory elements.

❖Some of the common phages used as vectors include M13 phages, λ phages, and P1 phages.

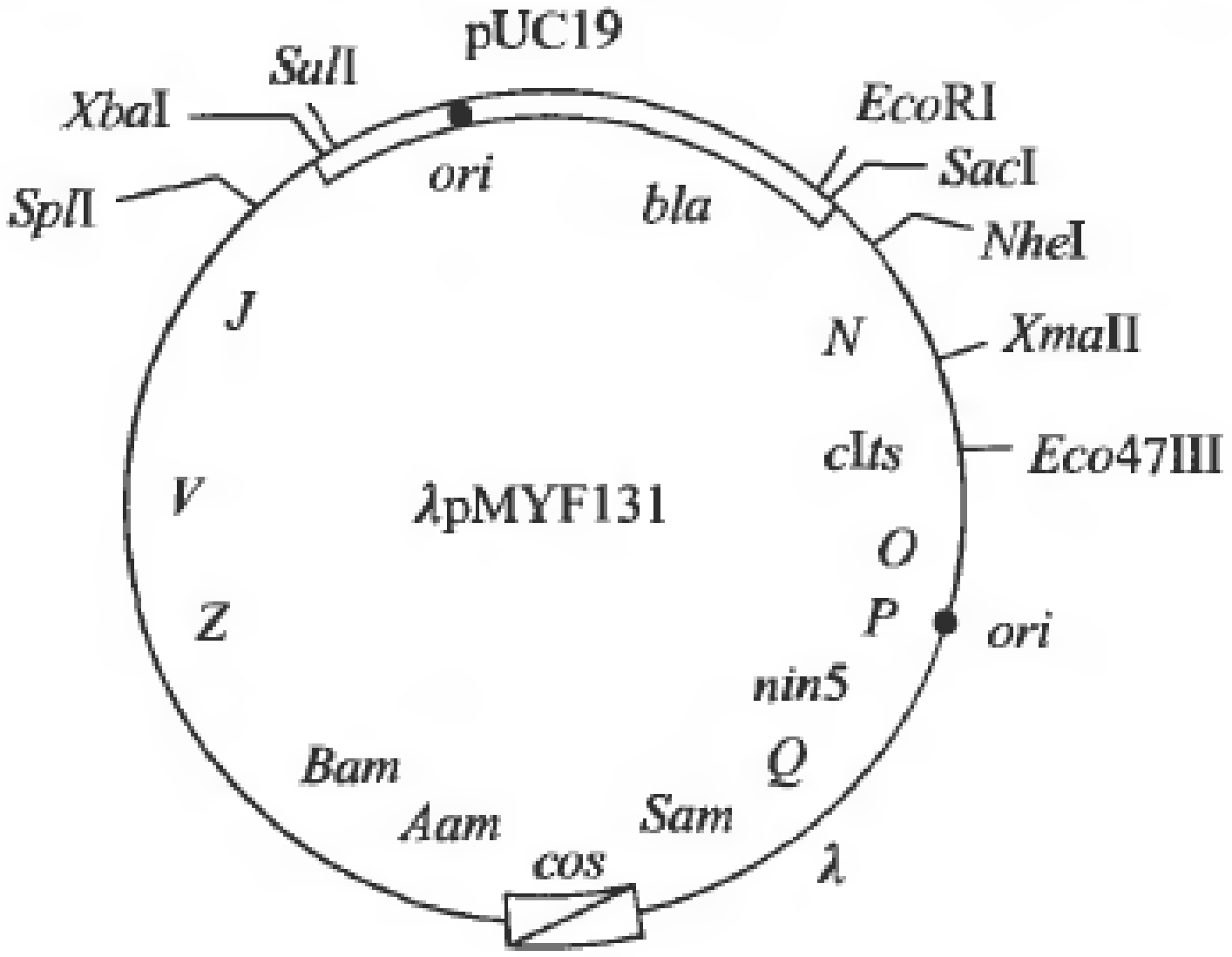
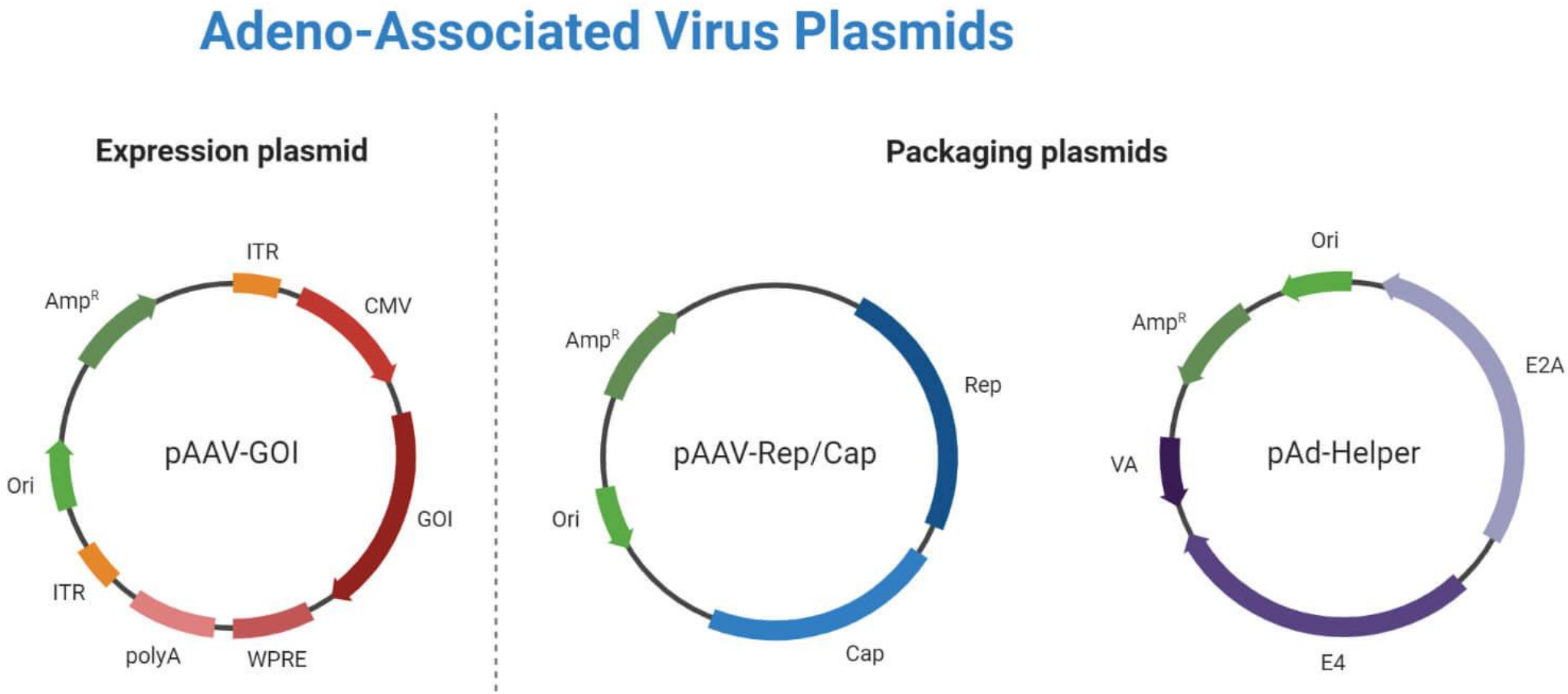
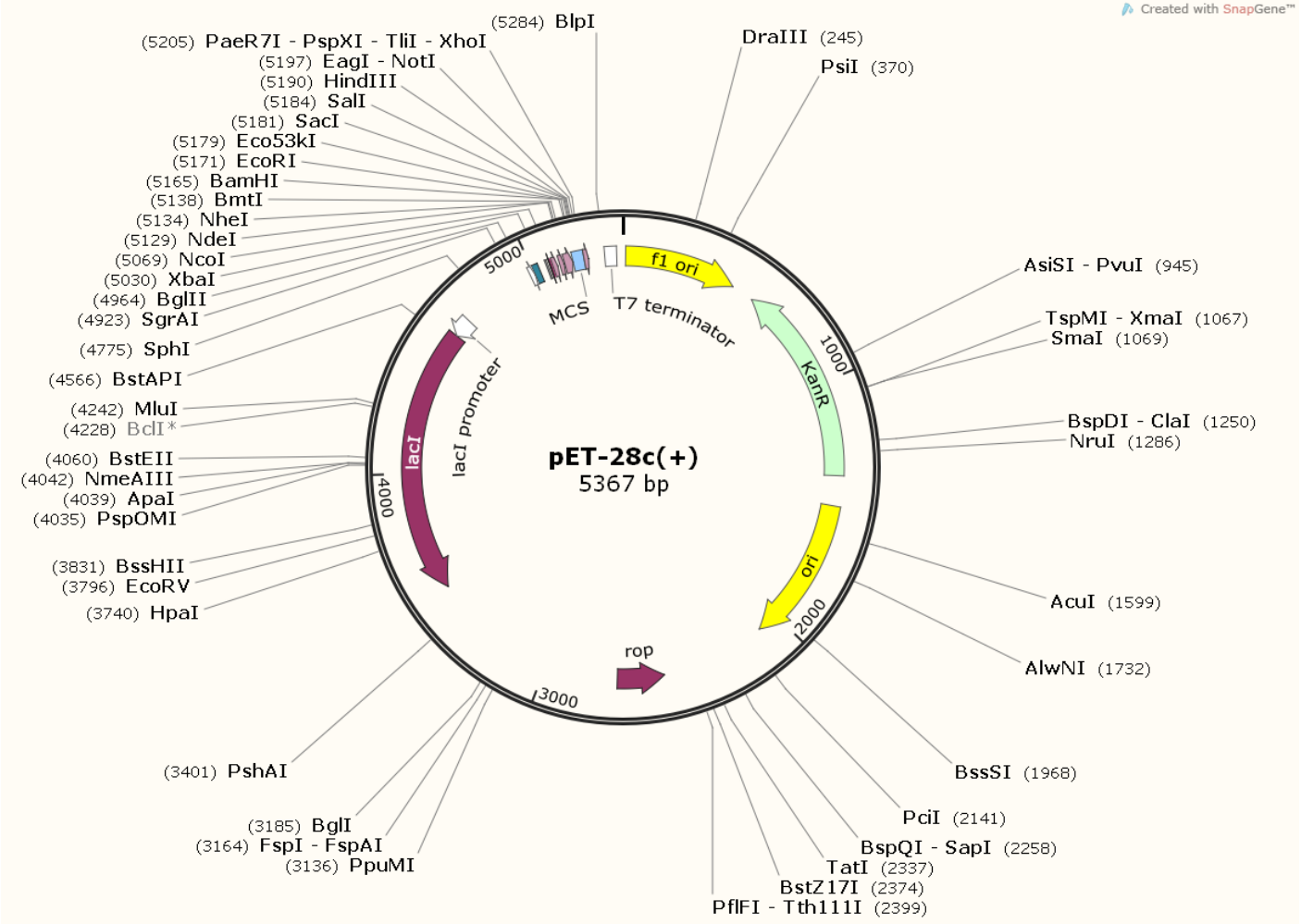


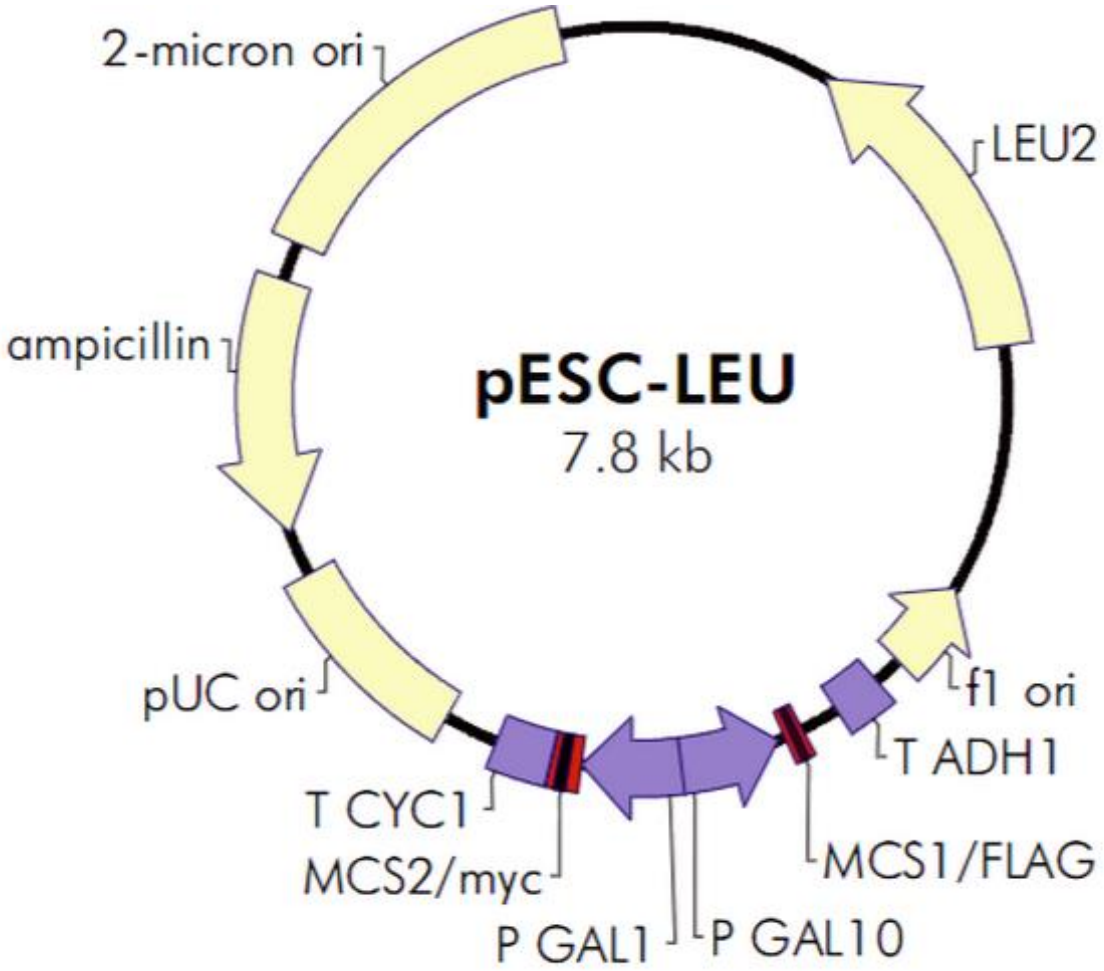
Рис. 2.24. Карта фазмиды λpMYF131

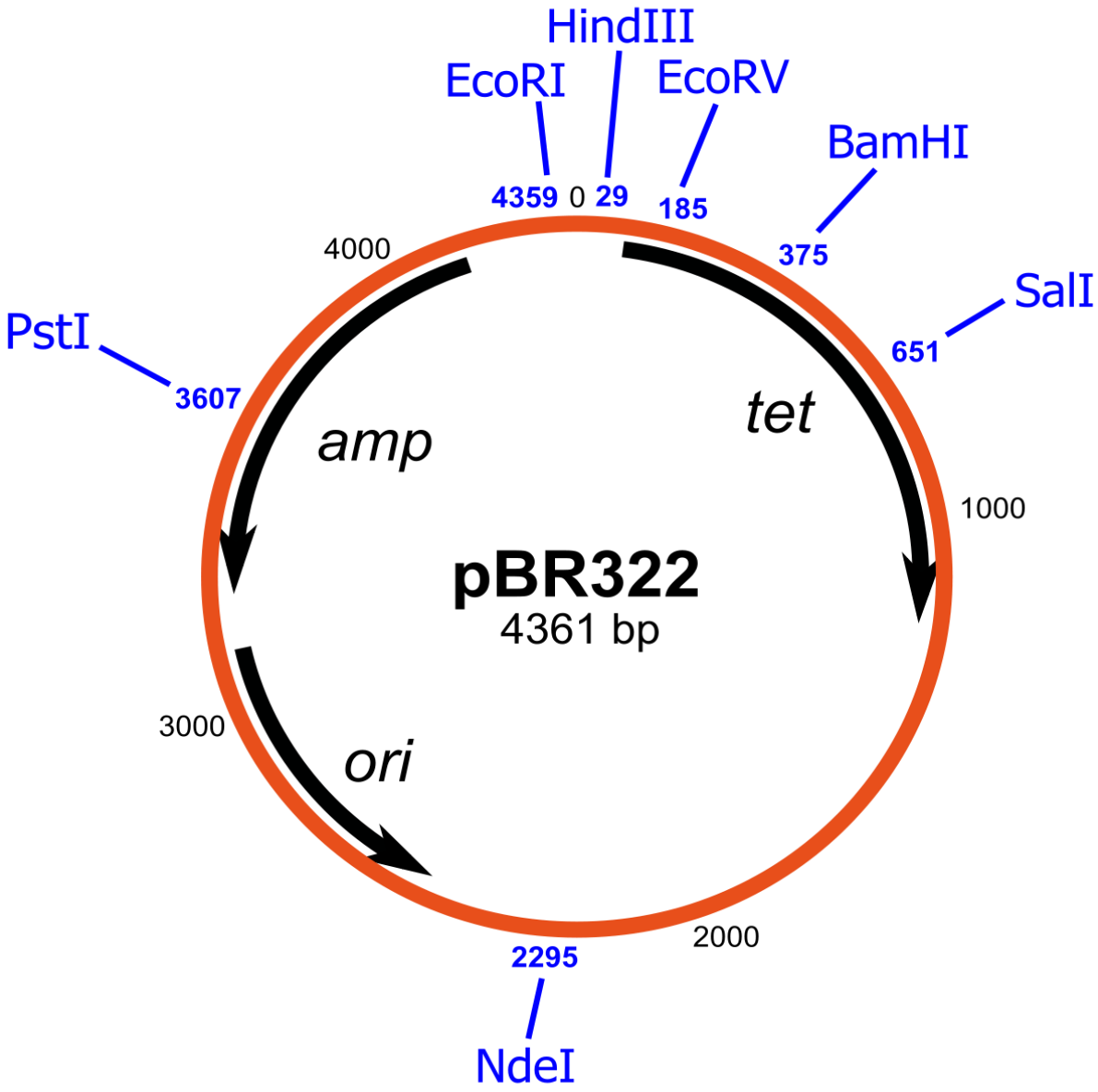
2. Viral vectors



3. Expression vector - Expression vectors are vectors that enable the expression of cloned genes in order to determine the successful cloning process. (pET28c)



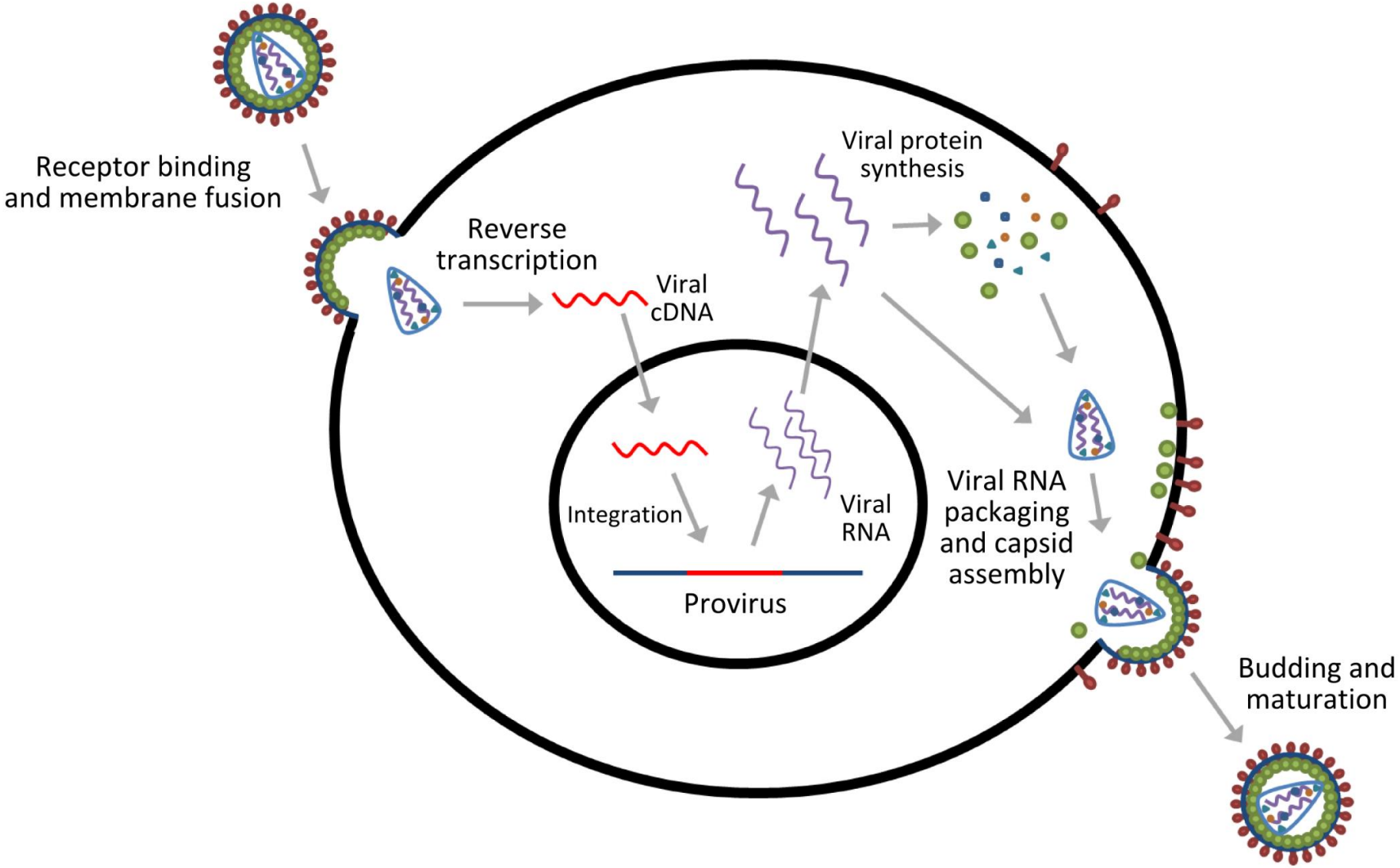




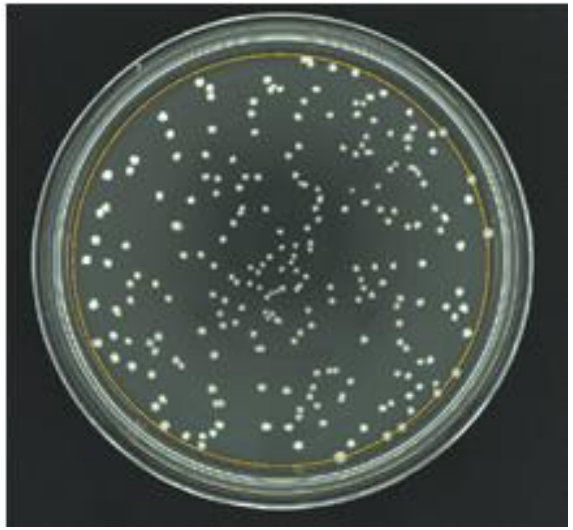
- Yeast plasmid

- Bacterial plasmid

Transfection is the introduction of virus DNA into cells, followed by the formation of viral progeny.

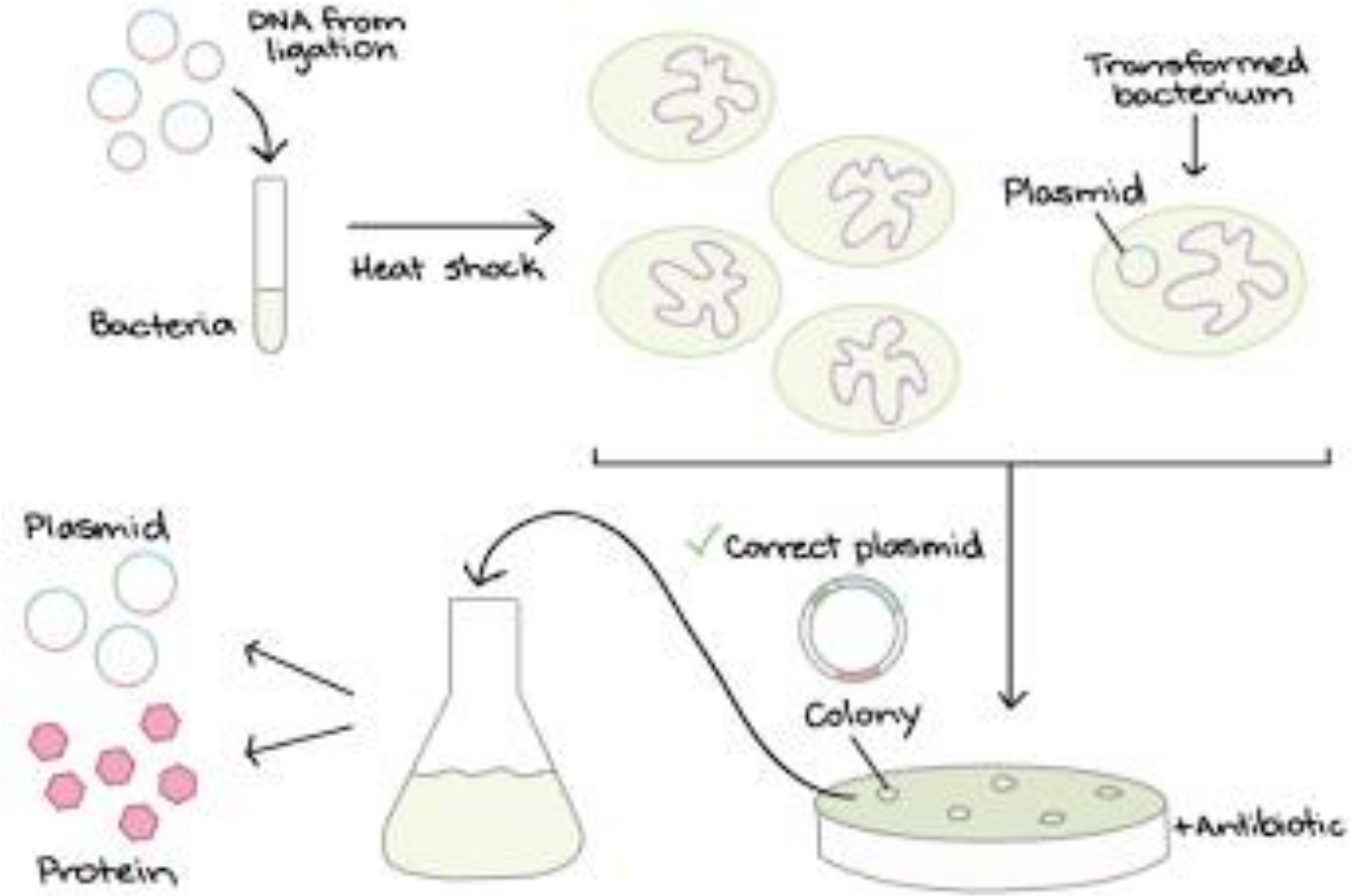


Transformation is the process by which exogenous NA enters the recipient cell and causes heritable changes in it.



Bacteria colonies

Yeast colonies



Competence is the physiological state of a cell when it is able to absorb NA from the environment.