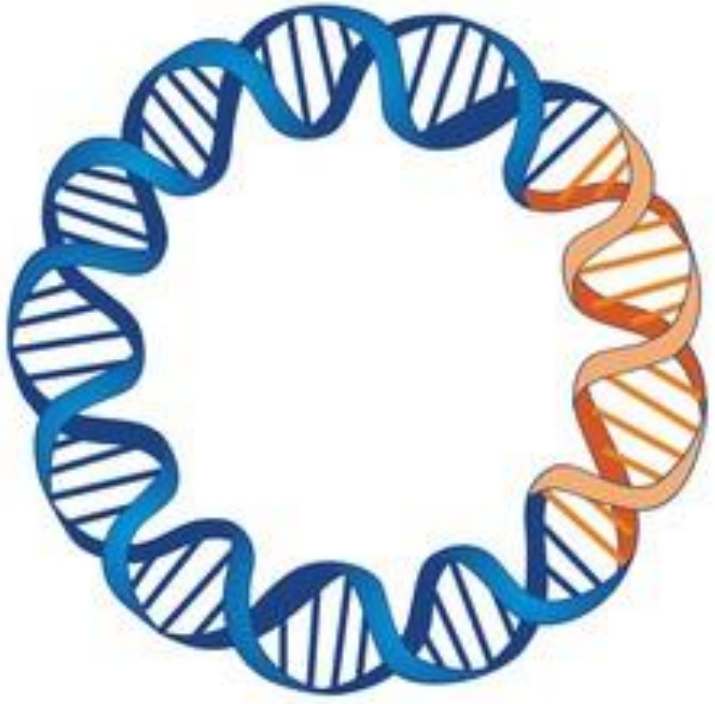
General characteristics of vectors used in DNA recombination technologies. Classification. Basic properties. The capacity of vectors.



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*

Summary

1. Vectors

- Vectors are DNA molecules used as vehicles to transfer foreign genetic material into host cells.
- They must contain an origin of replication, a selectable marker, and at least one cloning site.

2. Plasmid vectors

- Small, circular, double-stranded DNA molecules that replicate independently of chromosomal DNA.
- Commonly used due to their stability, ease of manipulation, and availability of selectable markers (e.g., antibiotic resistance genes).

3. Cosmids

- Hybrid vectors that combine plasmid and bacteriophage λ features.
- Can carry larger DNA inserts (up to 45 kb) and are packaged into phage particles for efficient infection of bacteria.

4. Bacteriophage vectors

- Based on bacterial viruses such as λ phage or M13.
- Used for cloning relatively large DNA fragments and for constructing genomic libraries.

5. Viral vectors

- Derived from animal or plant viruses (e.g., adenovirus, retrovirus, lentivirus).
- Applied in gene delivery, gene therapy, and vaccine development due to their ability to infect eukaryotic cells efficiently.

6. Transfection

- The process of introducing foreign DNA into eukaryotic cells using physical, chemical, or viral methods.
- Efficiency depends on the vector type and the method used (e.g., lipofection, electroporation, viral infection).

7. **Competence**

- The physiological state of a cell that allows uptake of external DNA.
- In bacteria, competence can be natural (as in Bacillus subtilis) or induced artificially (via $CaCl_2$ treatment or electroporation).

8. Vector capacity

- The maximum size of foreign DNA that a vector can accommodate.
- Varies among vector types: plasmids (~10 kb), cosmids (~45 kb), bacteriophage λ (~50 kb), BACs (~300 kb), YACs (~1 Mb).

9. Cloning efficiency

- Reflects the success rate of obtaining recombinant clones after ligation and transformation.
- Affected by vector design, insert-to-vector ratio, and host cell competence.

10. Gene delivery

- The process of introducing genetic material into target cells for expression or modification.
- Viral vectors are most efficient, but non-viral systems are safer and easier to control.

Key questions

1. What are the essential features that define a DNA vector?
2. How do plasmid vectors differ from cosmids and bacteriophage vectors?
3. What determines the **vector capacity** and why is it important in cloning?
4. Describe the difference between **transformation** and **transfection**.
5. What factors influence the **cloning efficiency** in recombinant DNA experiments?
6. Why are viral vectors widely used for **gene delivery** in gene therapy?
7. What is meant by **bacterial competence**, and how can it be induced artificially?

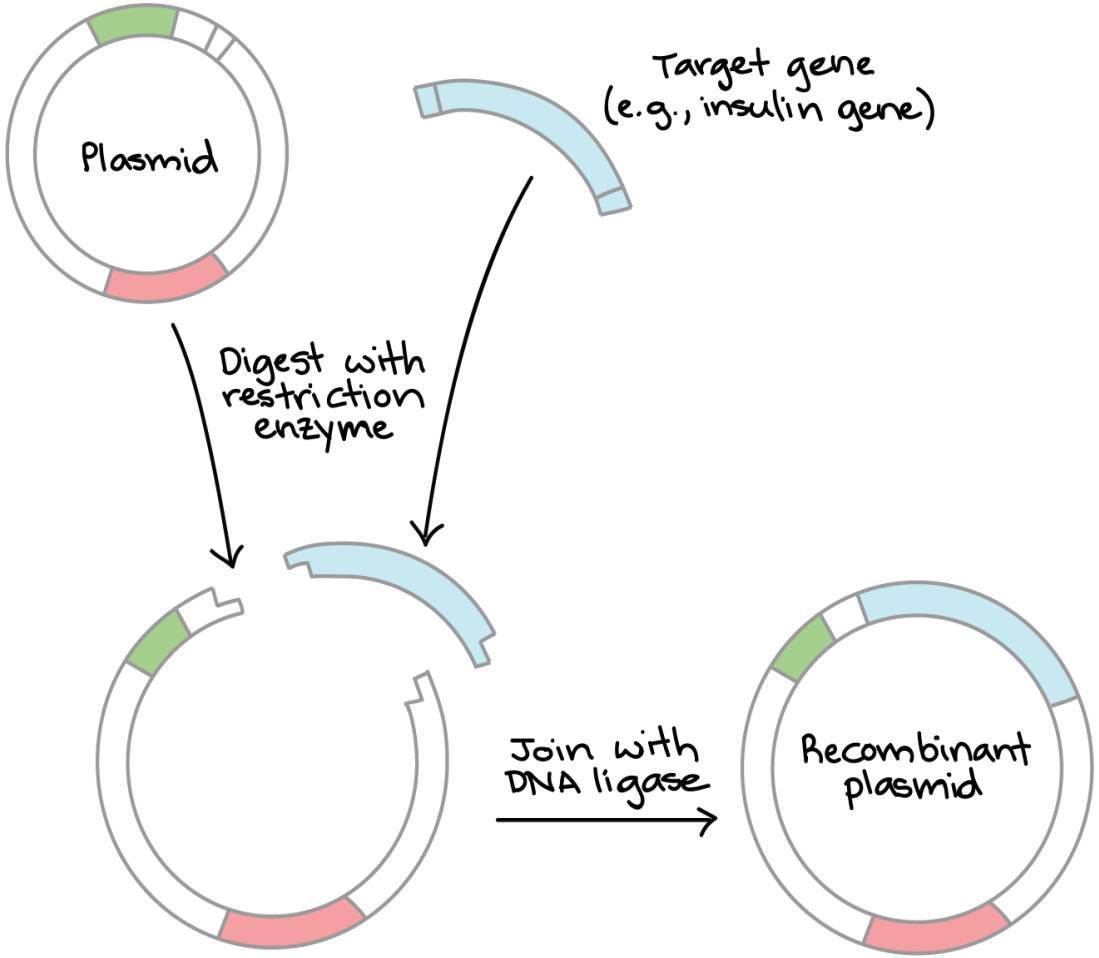
Definition

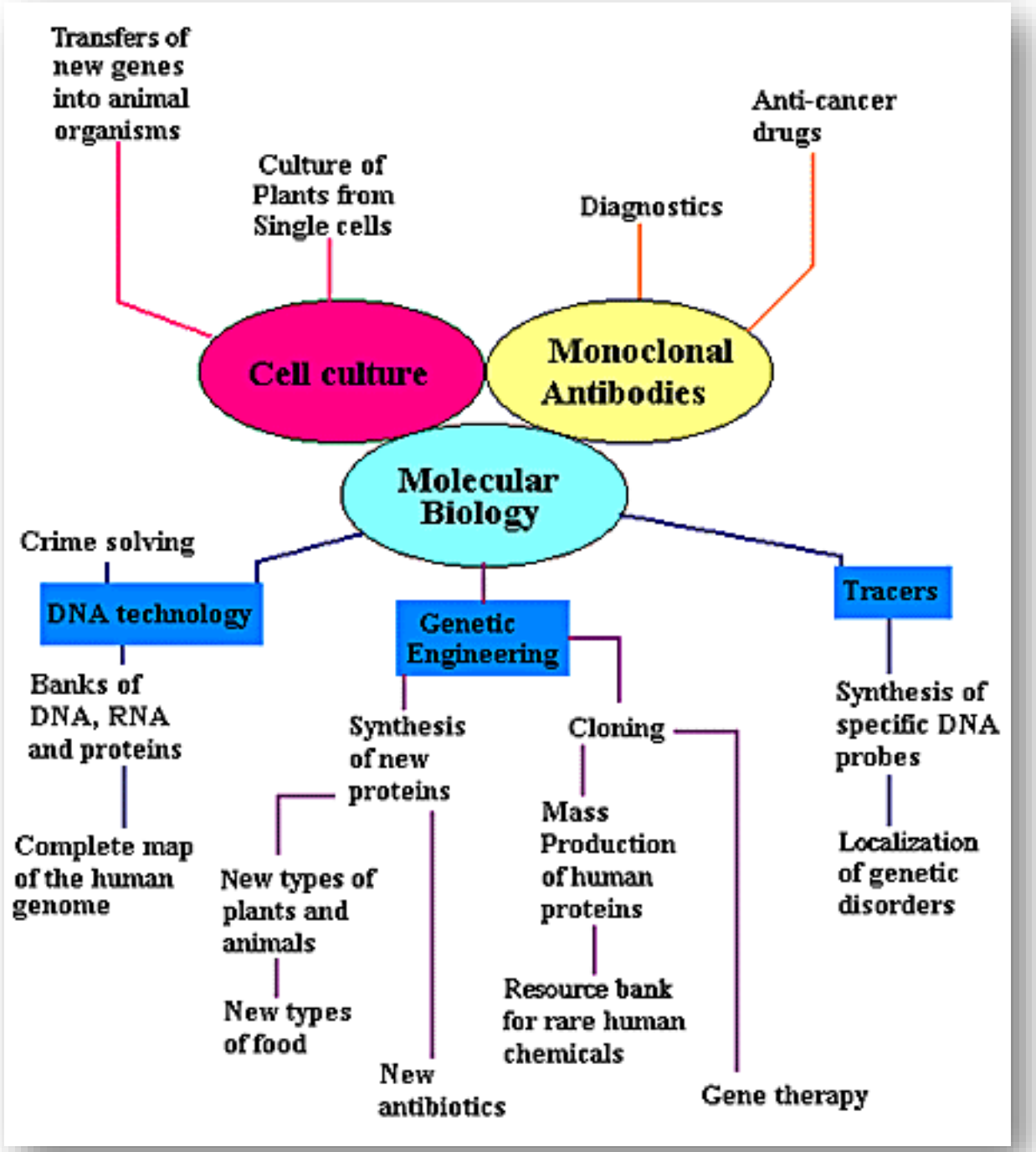
- A **vector** is a substance, usually a piece of DNA that carries a sequence of DNA or other genetic material and introduces it into a new cell.

✓ The **term "vector"** is typically used in science to emphasize the existence of a specific direction.

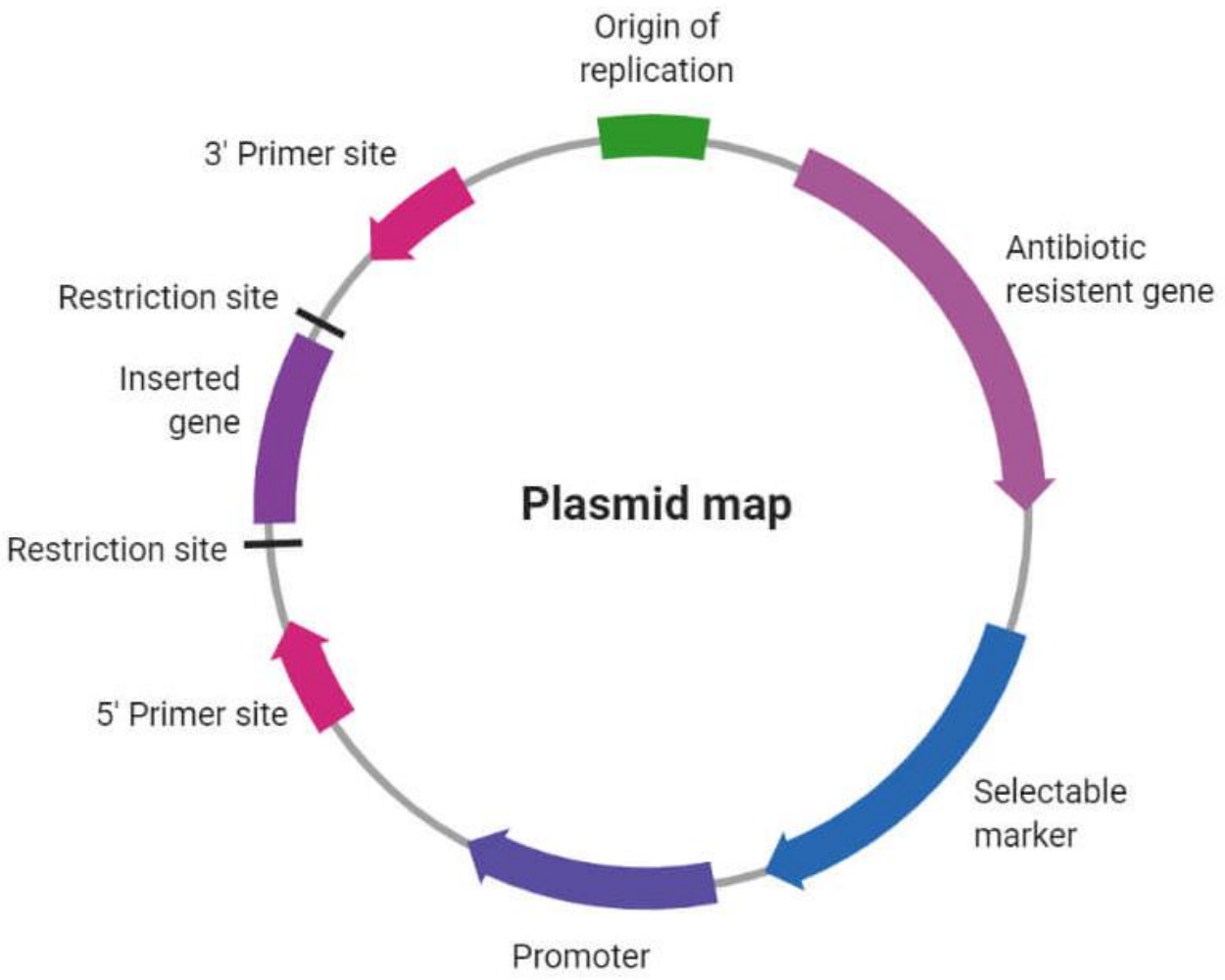
✓ So, just by the meaning of the term, it's clear that biological vectors direct **artificially introduced DNA** into intact target cells.

DNA cloning


Plasmid
Target gene
(e.g., insulin gene)
Digest with restriction enzyme
Join with DNA ligase
Recombinant plasmid


Transfers of new genes into animal organisms
Culture of Plants from Single cells
Diagnostics
Anti-cancer drugs
Cell culture
Monoclonal Antibodies
Molecular Biology
Crime solving
DNA technology
Genetic Engineering
Tracers
Banks of DNA, RNA and proteins
Complete map of the human genome
Synthesis of new proteins
New types of plants and animals
New types of food
New antibiotics
Cloning
Mass Production of human proteins
Resource bank for rare human chemicals
Gene therapy
Synthesis of specific DNA probes
Localization of genetic disorders

Features



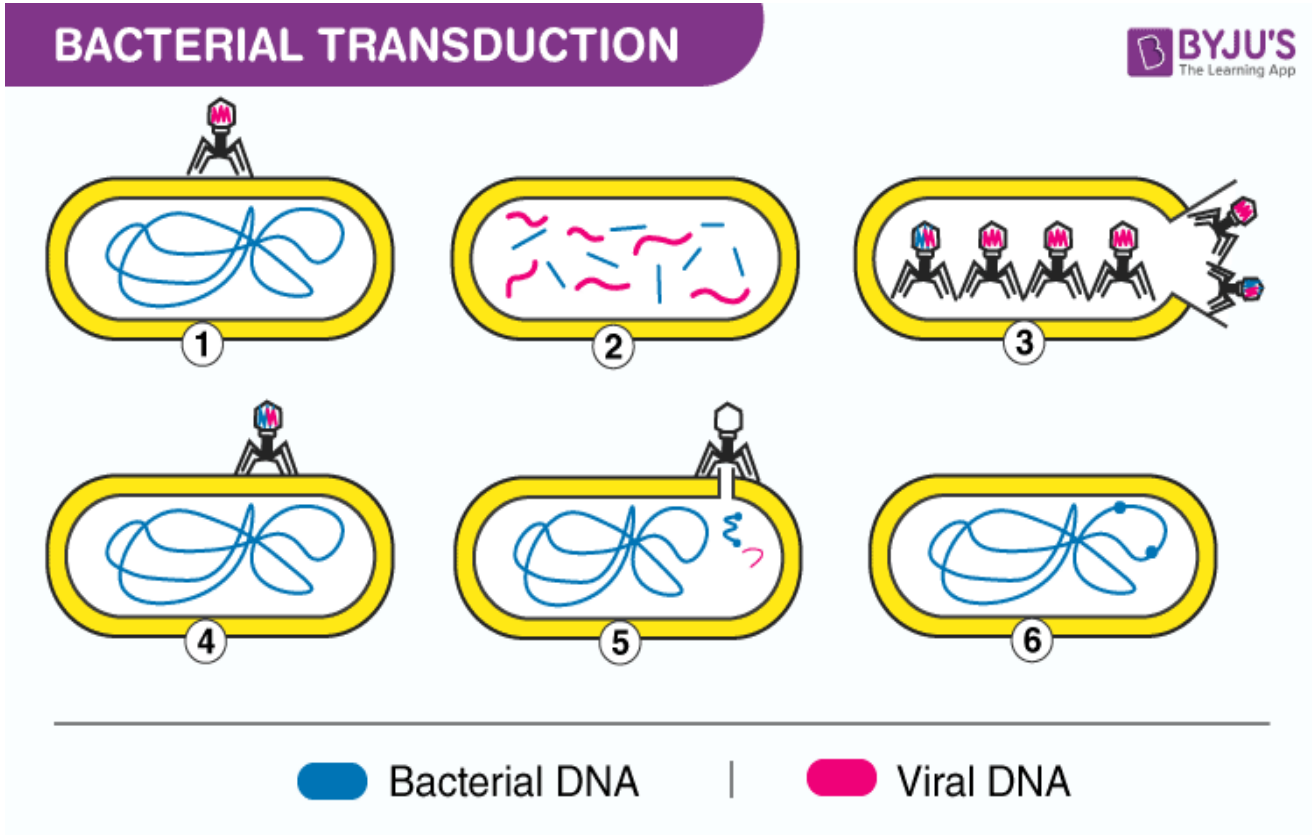
- Vectors act as **vehicles** to transfer genetic material from one cell to the other for different purposes like multiplying, expressing, or isolation.
- Vectors are used as a **tool** in molecular cloning procedures so as to introduce the desired DNA insert into a host cell.
- Vectors usually have 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.

Some characteristic features of vectors

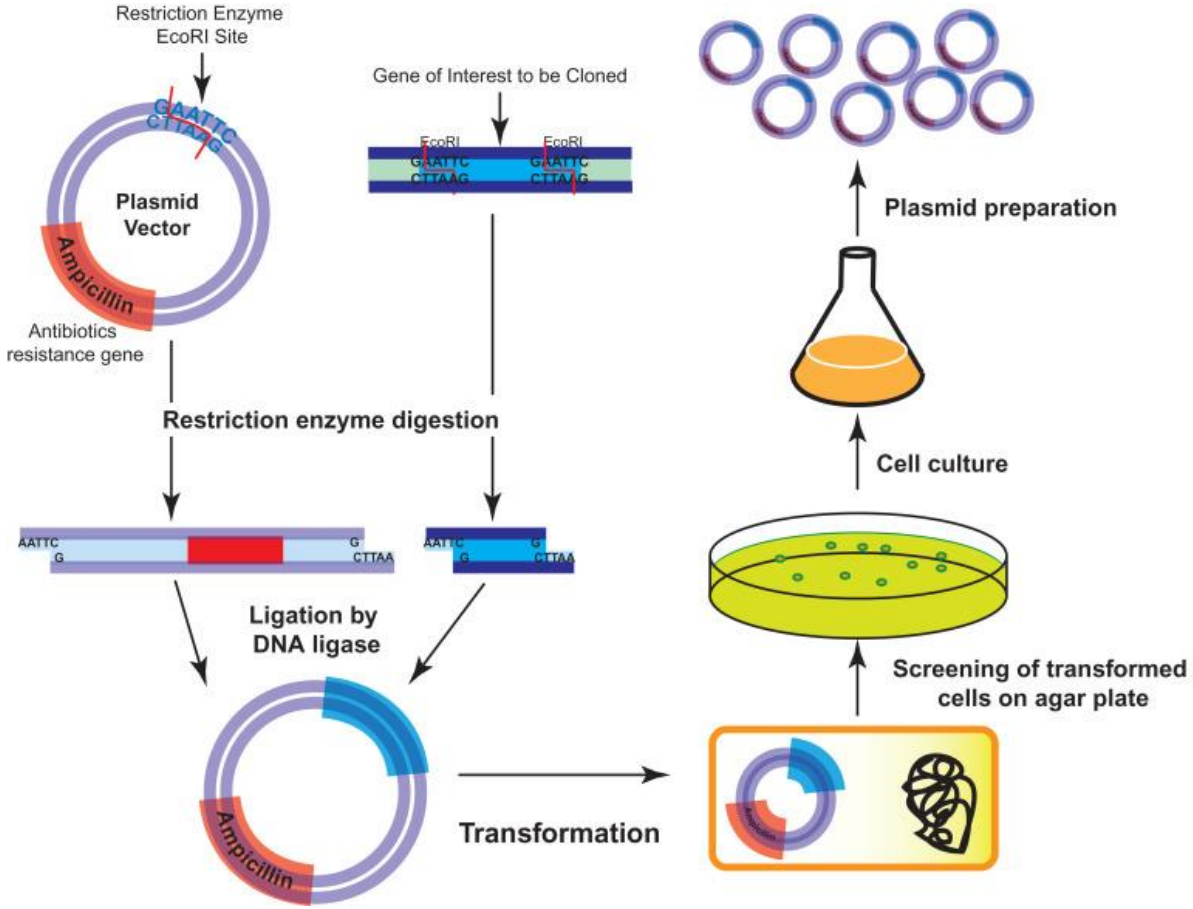
1. Vectors should be capable of replicating **autonomously**.
2. The size of an ideal vector should also be **small enough** for it to be incorporated into the host genome.
3. Vectors should be easy to isolate and purify as these need to be recovered and reused for **multiple processes**.
4. For a vector 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.

- 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.



Plasmids



- **Plasmids** are hereditary factors located in cells outside of chromosomes.
- Plasmids include genetic factors of cellular organelles (mitochondria, plastids, etc.) and genetic factors that are not essential cellular components.

Among the latter, the best-studied are the so-called kappa factor in paramecia, which produces the antibiotic substance paramecia, the CO2 sensitivity factor, and the agent that causes malelessness in Drosophila, as well as a number of bacterial plasmids.

In bacteria, plasmids can control drug resistance and the synthesis of bactericins, enterotoxin, hemolysin, and some antigens.

Sex factors, also known as sex factors, determine sexual differentiation in bacteria.

. **Many plasmids have been shown to consist of circular molecules of double-stranded DNA with a molecular weight of 10^{-6} to 10^{-8} daltons.**

Types of bacterial plasmids

Typically, it is circular DNA, largely independent of the host chromosome, and is commonly found in bacteria and some other cell types. Naturally occurring plasmids typically replicate independently of the bacterial chromosome. Plasmids can contain genes up to 10 kb in size. Many different types of plasmids are found in bacteria. The most useful classification of natural plasmids is based on the key characteristics encoded by the plasmid genes. According to this classification, the five main types of plasmids are listed below:

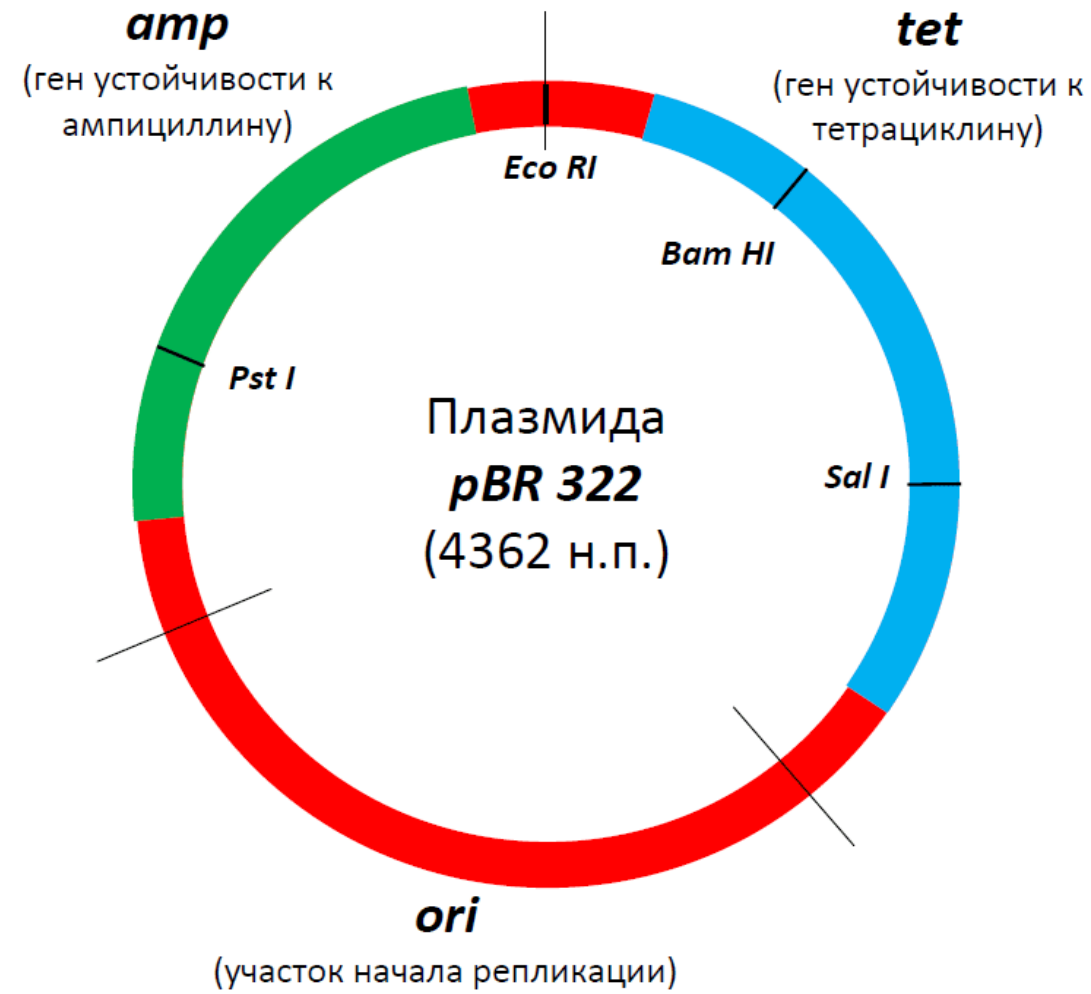
a. **F –** plasmids. Fertility plasmids carry only tra genes and have no other characteristics other than the ability to facilitate conjugative plasmid transfer. For example, the F plasmid from E. Coli.

b. **R -** plasmids. Resistance plasmids contain genes that confer resistance to one or more antibacterial drugs, such as chloramphenicol, ampicillin, and others, on the host bacteria. This type of plasmid is primarily used in recombinant DNA technology.

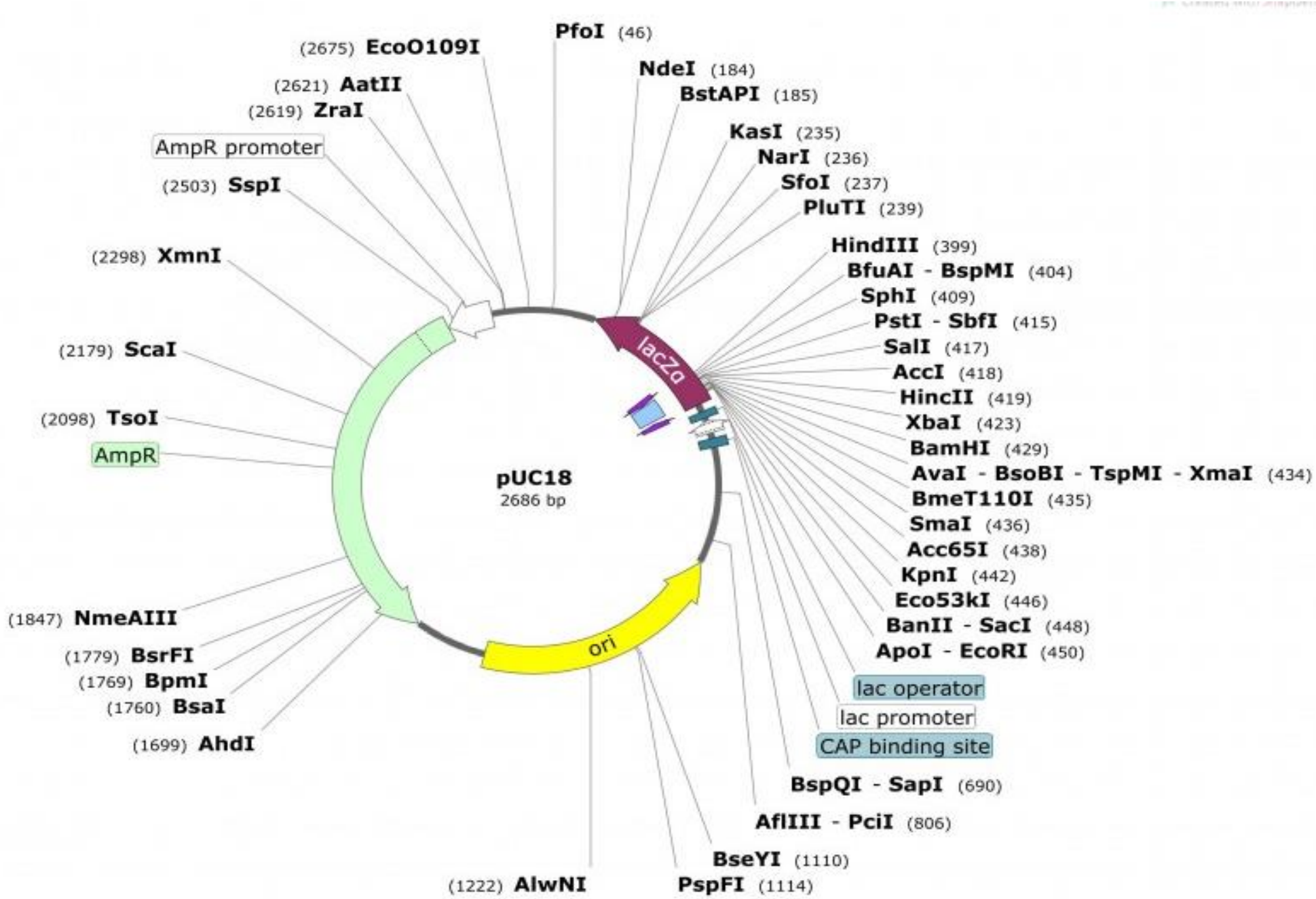
c**. Col Plasmids.** These plasmids encode colicins (proteins) that inhibit the growth of other bacteria, such as ColE1 from E. coli.

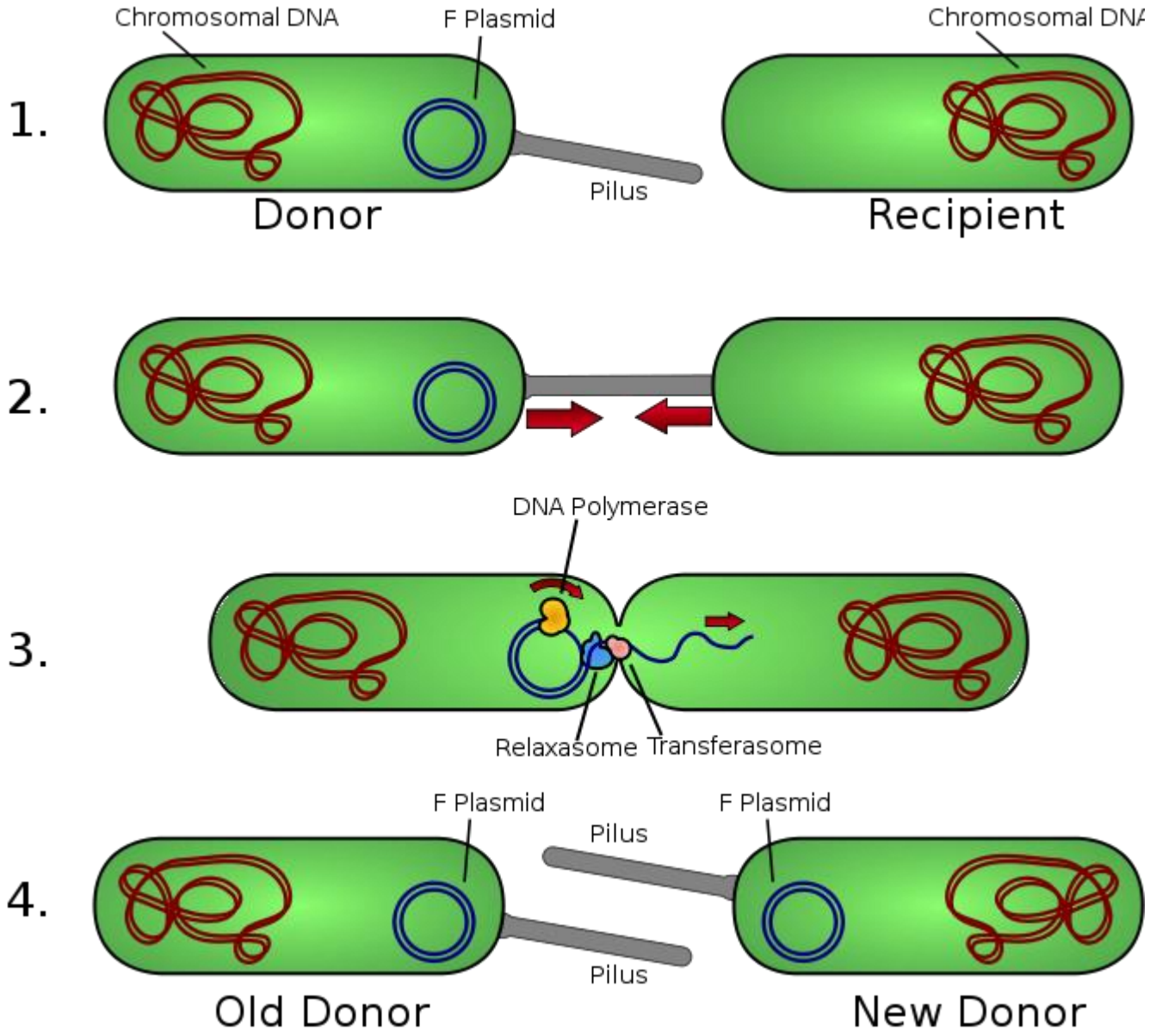
d. **Degradable plasmids.** These plasmids allow the host bacteria to metabolize unusual molecules such as toluene and salicylic acid.

e. **Virulence plasmids.** These confer pathogenicity to host bacteria, such as the Ti plasmids of Agrobacterium tumefaciens, which cause crown yellows in dicotyledonous plants.

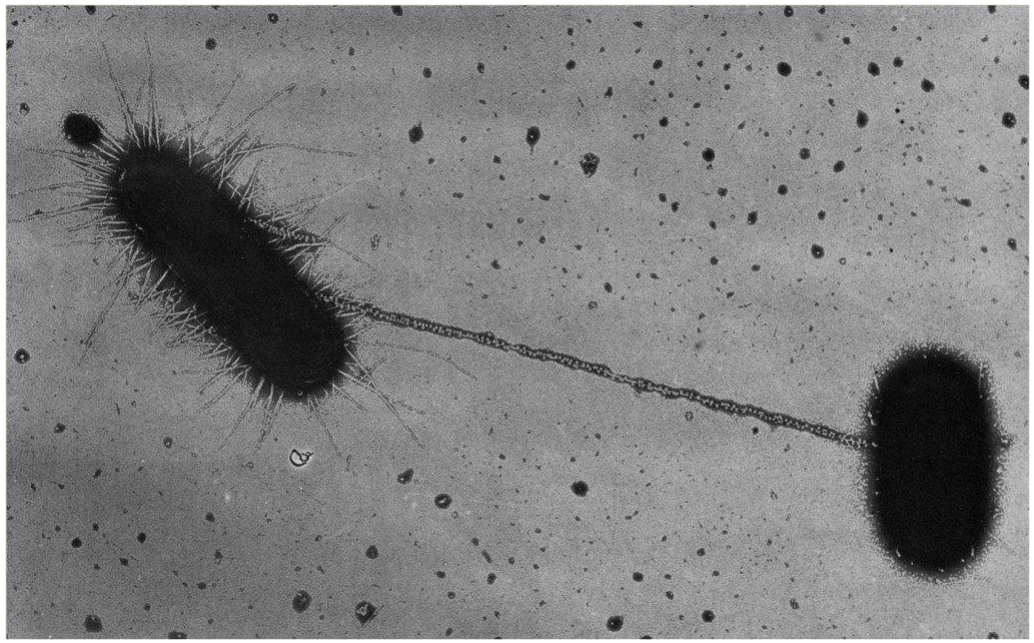


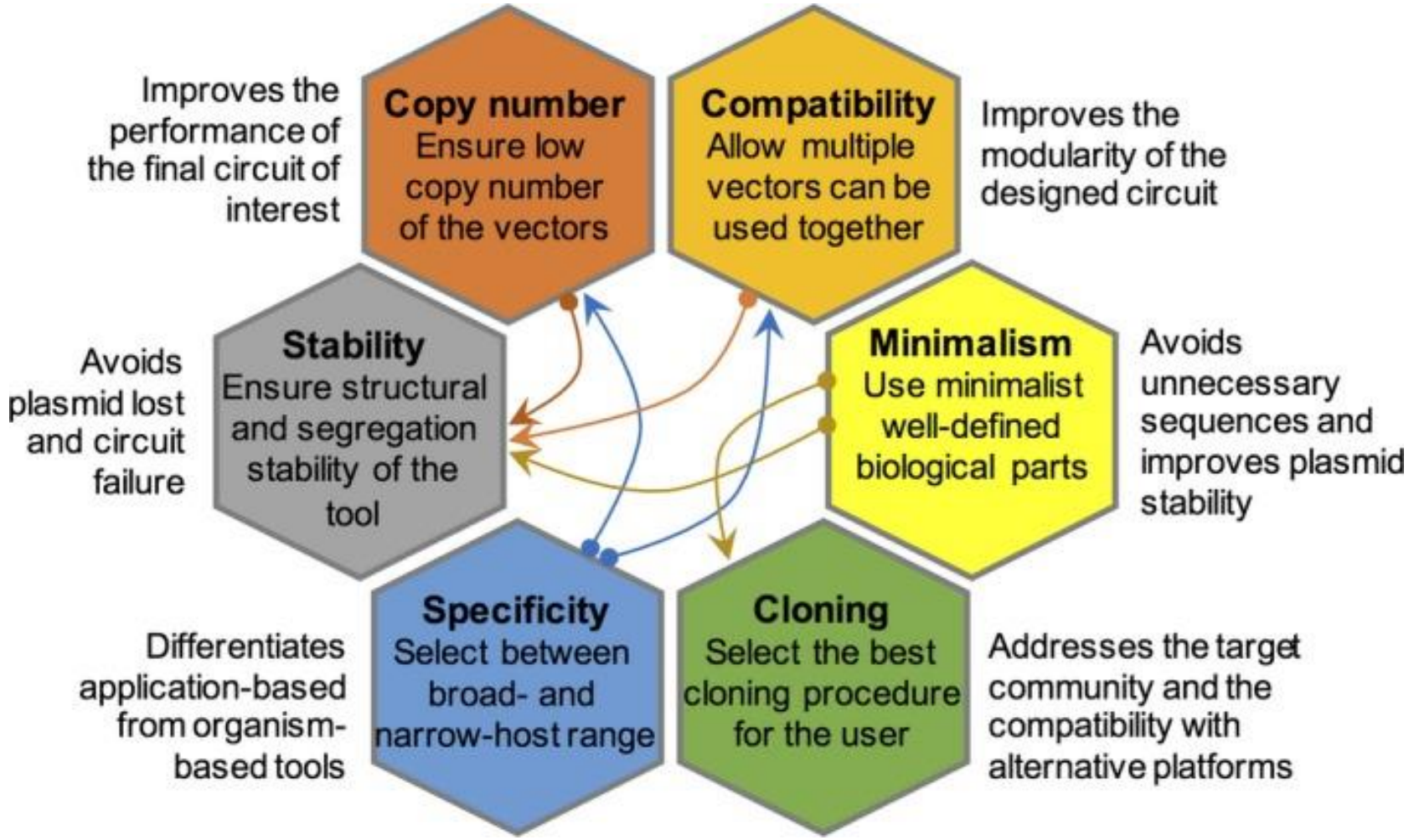
The "p" indicates that this is indeed a plasmid.The "BR" designates the laboratory where the vector was originally created. The "BR" stands for Bolívar and Rodríguez, the two researchers who developed pBR322.The "322" distinguishes this plasmid from others developed in the same laboratory.


pUC18
2686 bp
lacZα
ori
AmpR promoter
AmpR
lac operator
lac promoter
CAP binding site
PfoI (46)
NdeI (184)
BstAPI (185)
KasI (235)
NarI (236)
SfoI (237)
PluTI (239)
HindIII (399)
BfuAI - BspMI (404)
SphI (409)
PstI - SbfI (415)
SalI (417)
AccI (418)
HincII (419)
XbaI (423)
BamHI (429)
AvaI - BsoBI - TspMI - XmaI (434)
BmeT110I (435)
SmaI (436)
Acc65I (438)
KpnI (442)
Eco53kI (446)
BanII - SacI (448)
ApoI - EcoRI (450)
BspQI - SapI (690)
AflIII - PciI (806)
BseYI (1110)
PspFI (1114)
(1222) AlwNI
(1699) AhdI
(1760) BsaI
(1769) BpmI
(1779) BsrFI
(1847) NmeAIII
(2098) TsoI
(2179) ScaI
(2298) XmnI
(2503) SspI
(2619) ZraI
(2621) AatII
(2675) EcoO109I



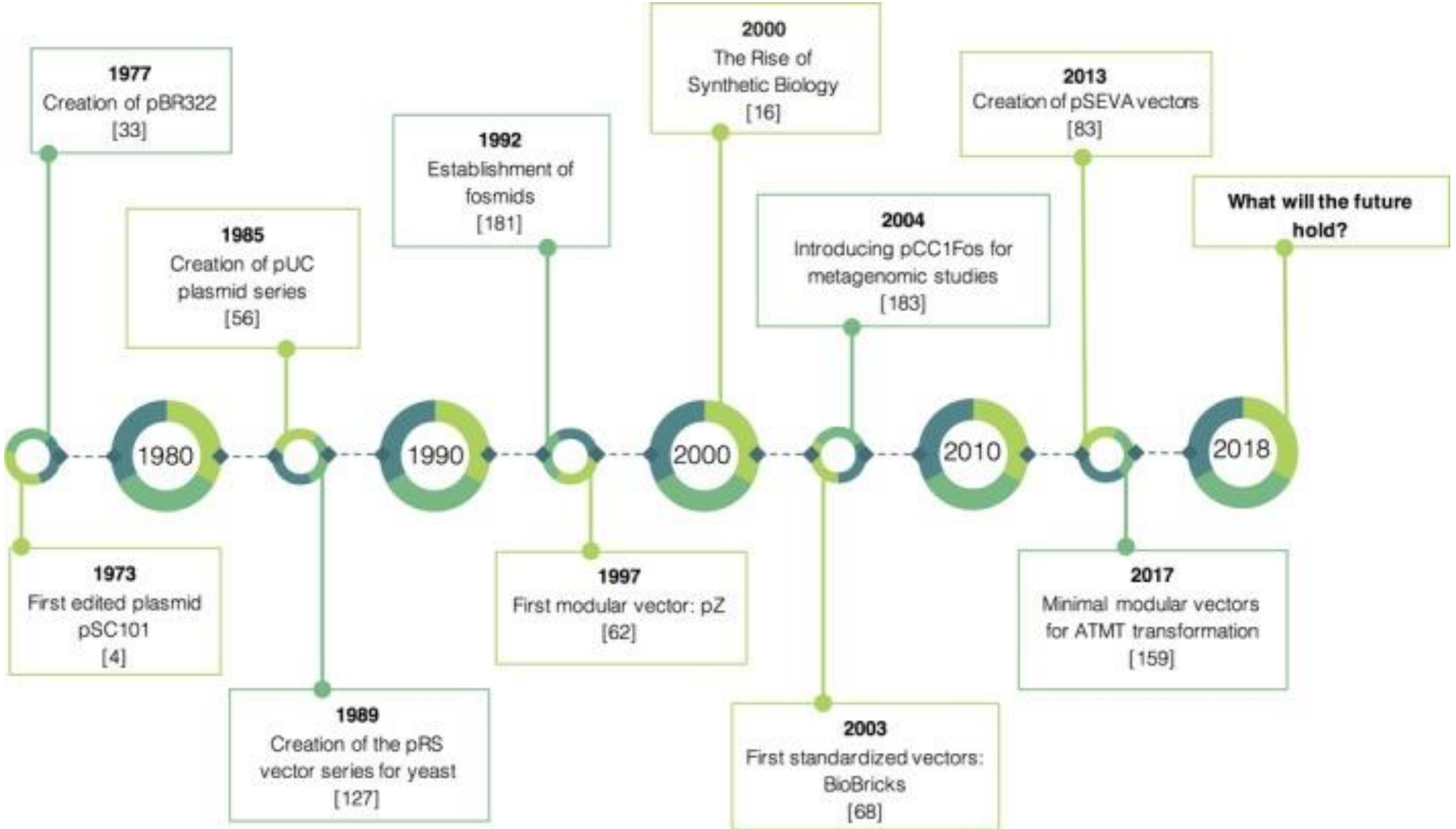
Conjugation in bacteria





The most important elements to consider for effective vector design.

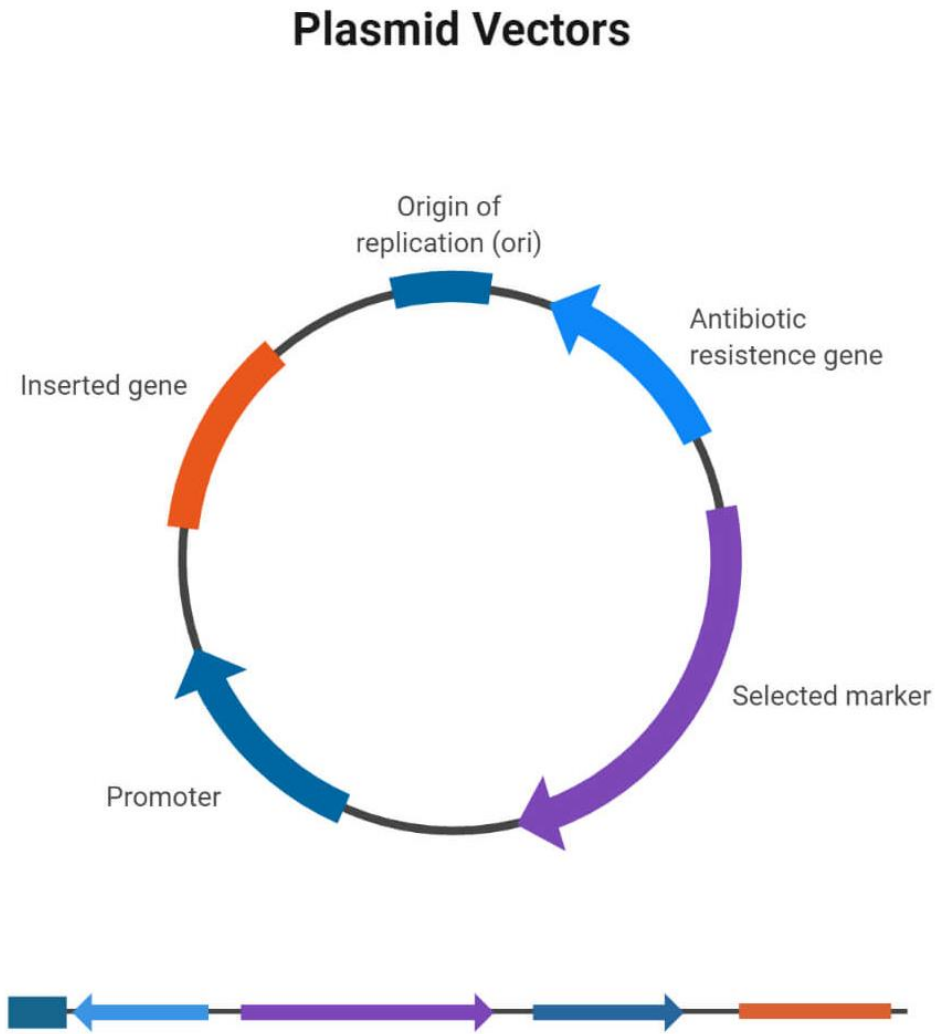
In this schematic illustration, the outer hexagons represent the primary impact of each step on the tool's effectiveness. The arrows connecting the hexagons indicate which elements significantly influence each other.



A timeline showing the most important advances in vector technology and design from 1970 to the present.

Types of vectors

Vectors can be divided into different groups depending on the purpose of the process and the type of particles used in the process. Below are the most commonly studied groups of vectors used for various purposes.



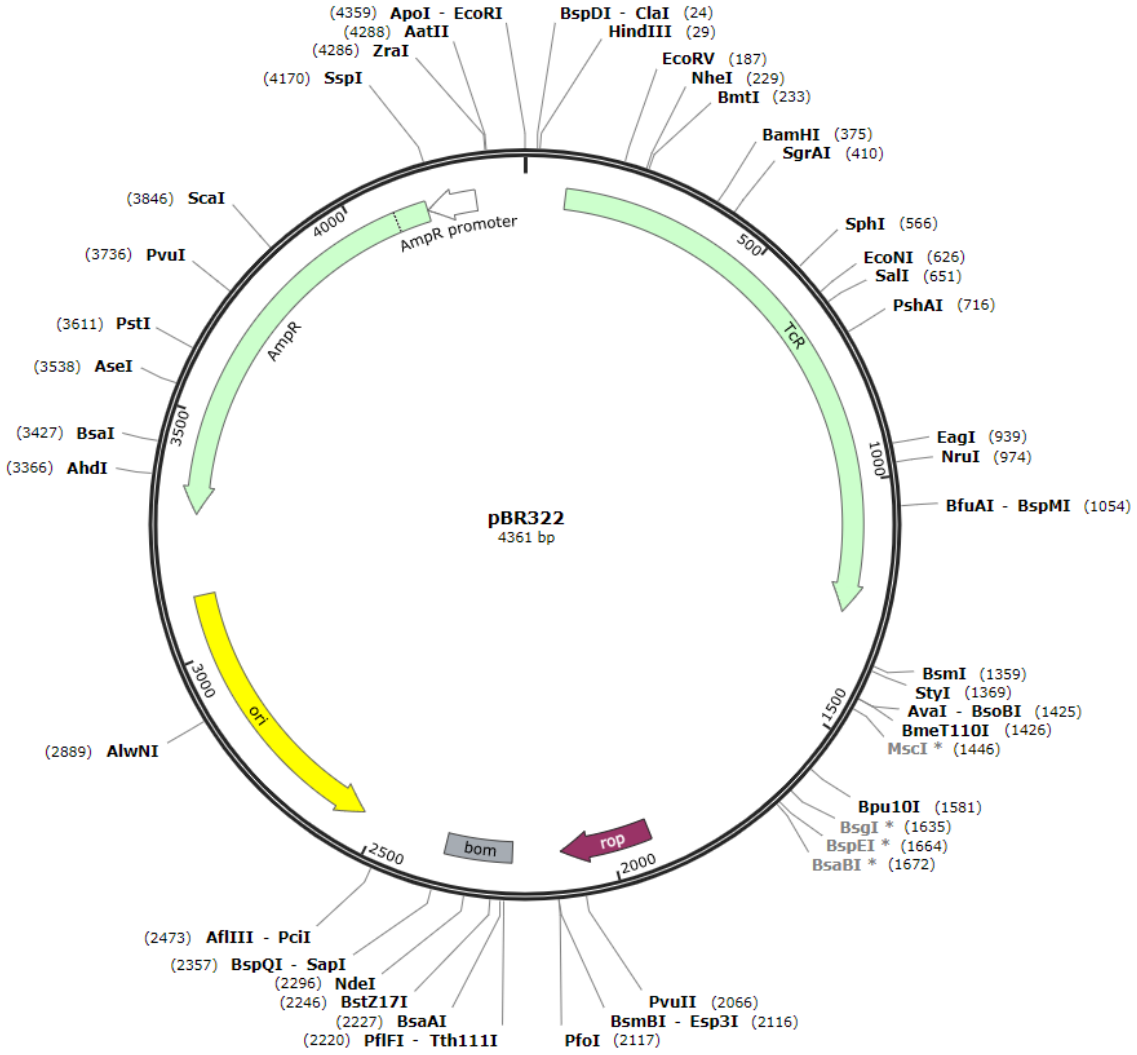
1. Cloning vectors

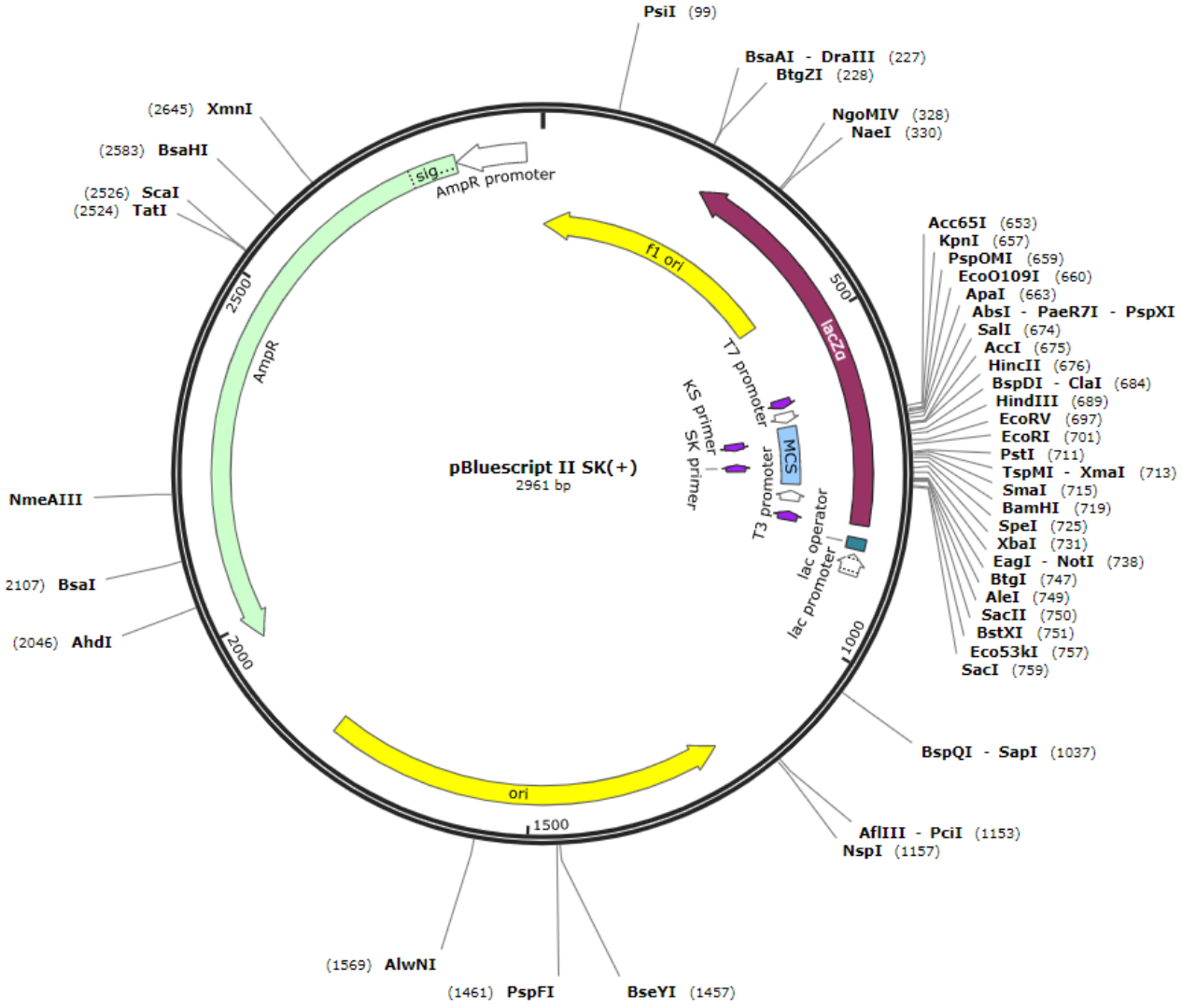
- Cloning vectors are vectors that are capable of replicating autonomously and thus are used for the replication of the recombinant DNA within the host cell.
- Cloning vectors are responsible for the determination of which host cells are appropriate for replicating a particular DNA segment.
- Cloning 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.





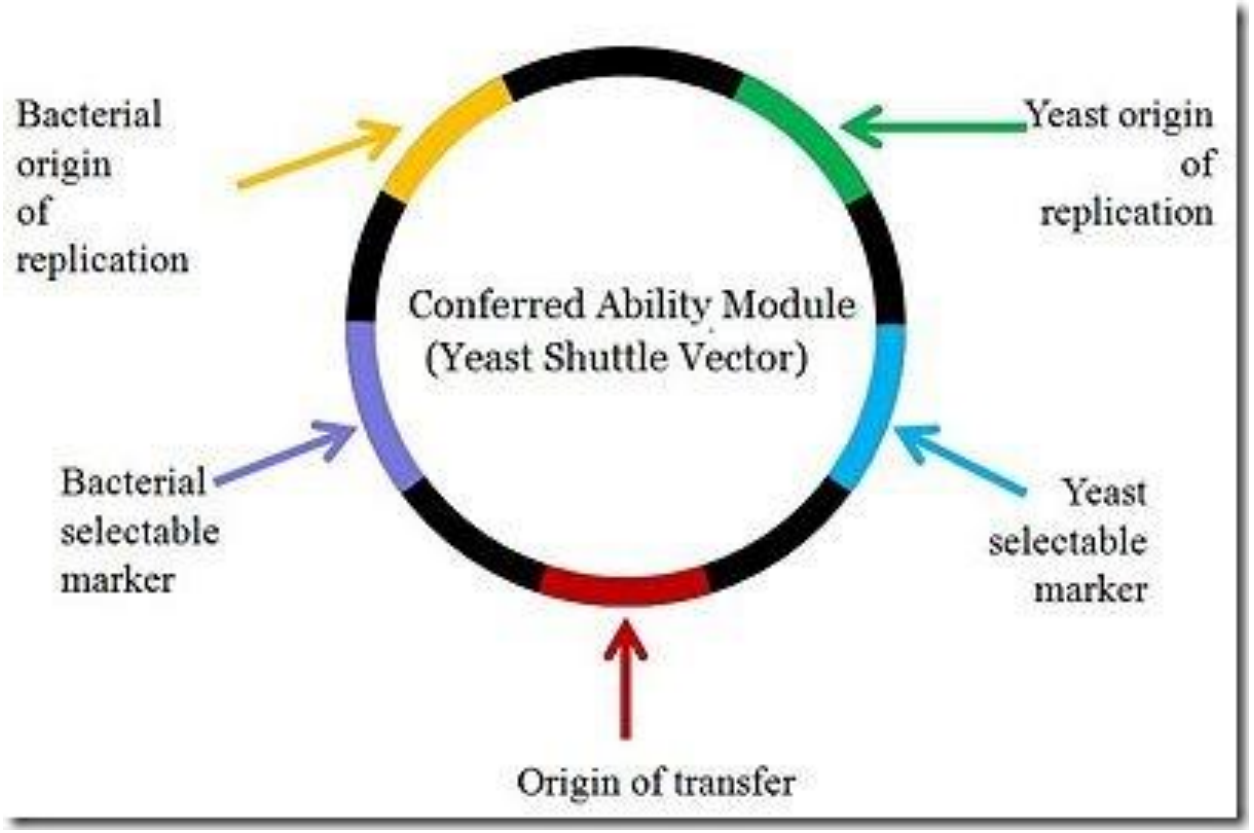
Shuttle vectors are vectors that carry replication origins from two different hosts, allowing them to "move" between the two.

These vectors contain DNA plasmids that can typically replicate in both mammalian and bacterial cells.

Shuttle vectors function as hybrid vectors, containing DNA sequences from bacterial plasmids and mammalian viruses.

The vectors contain three functional DNA sequences involved in the cloning process: a viral replication gene, a bacterial replication gene, and a drug resistance gene.

The presence of various replication sites and repair sequences allows these vectors to be restored and maintained in bacterial cells.

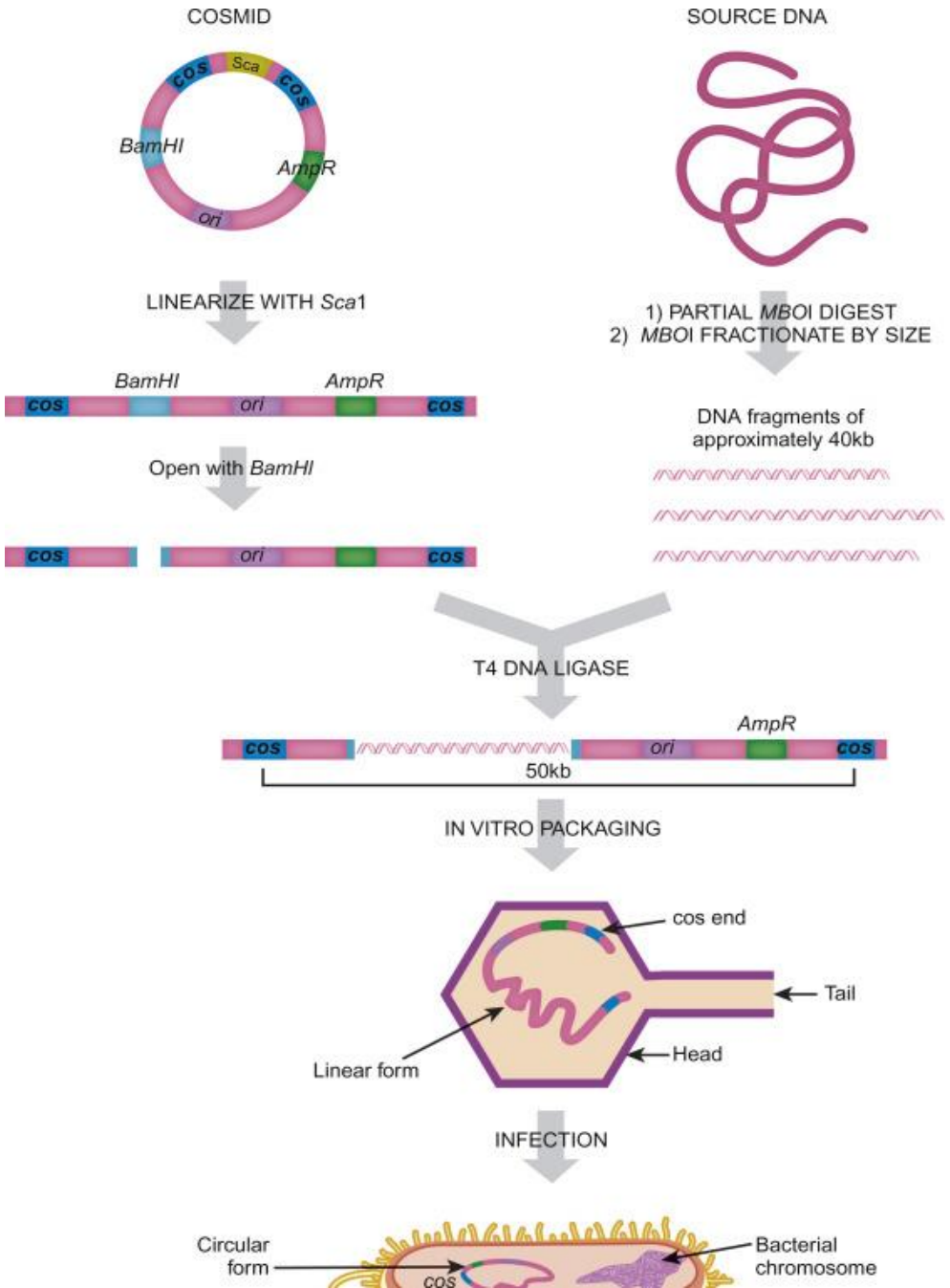


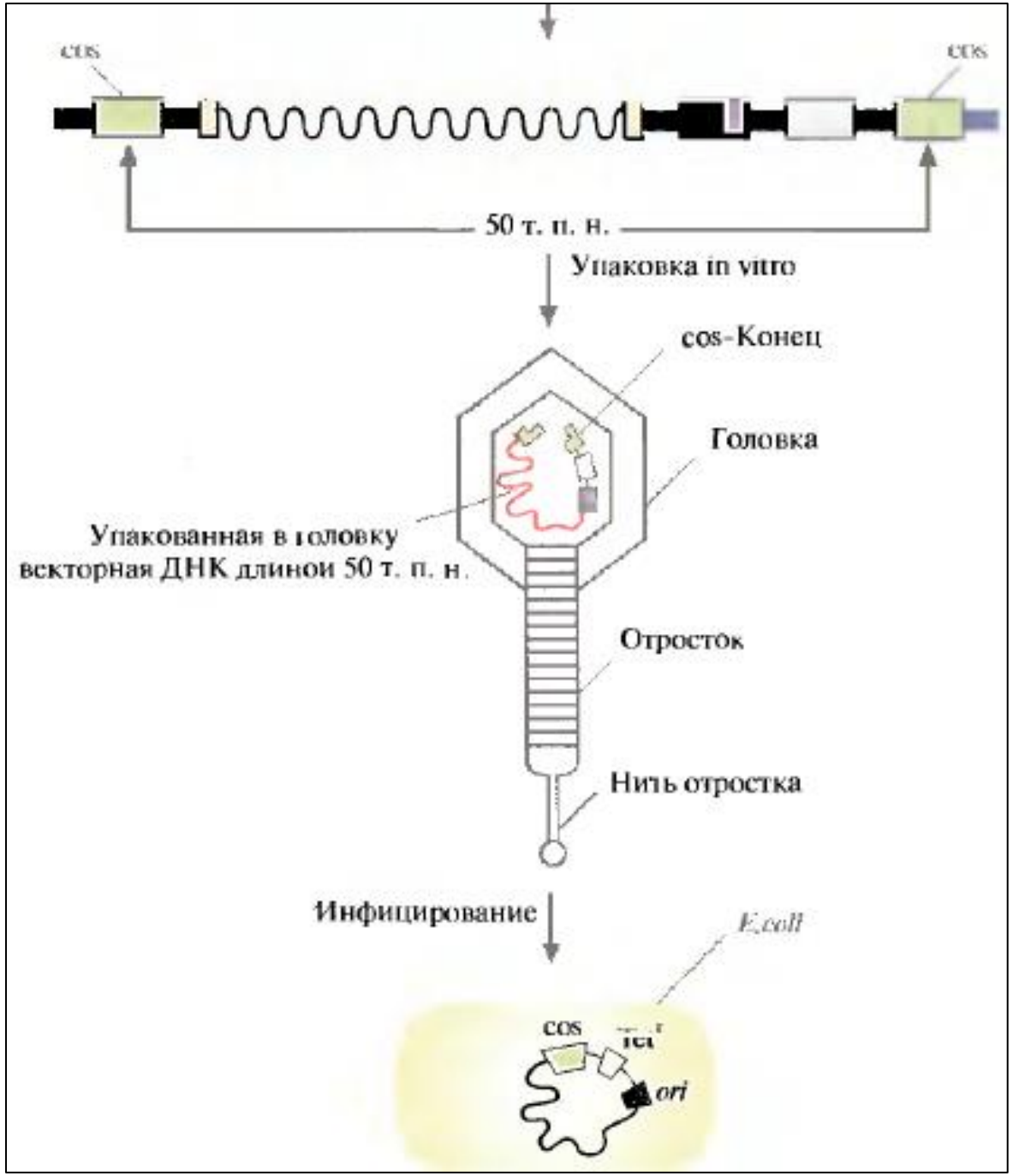
b. Cosmid

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**.

Scheme for creating a cosmid vector





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.

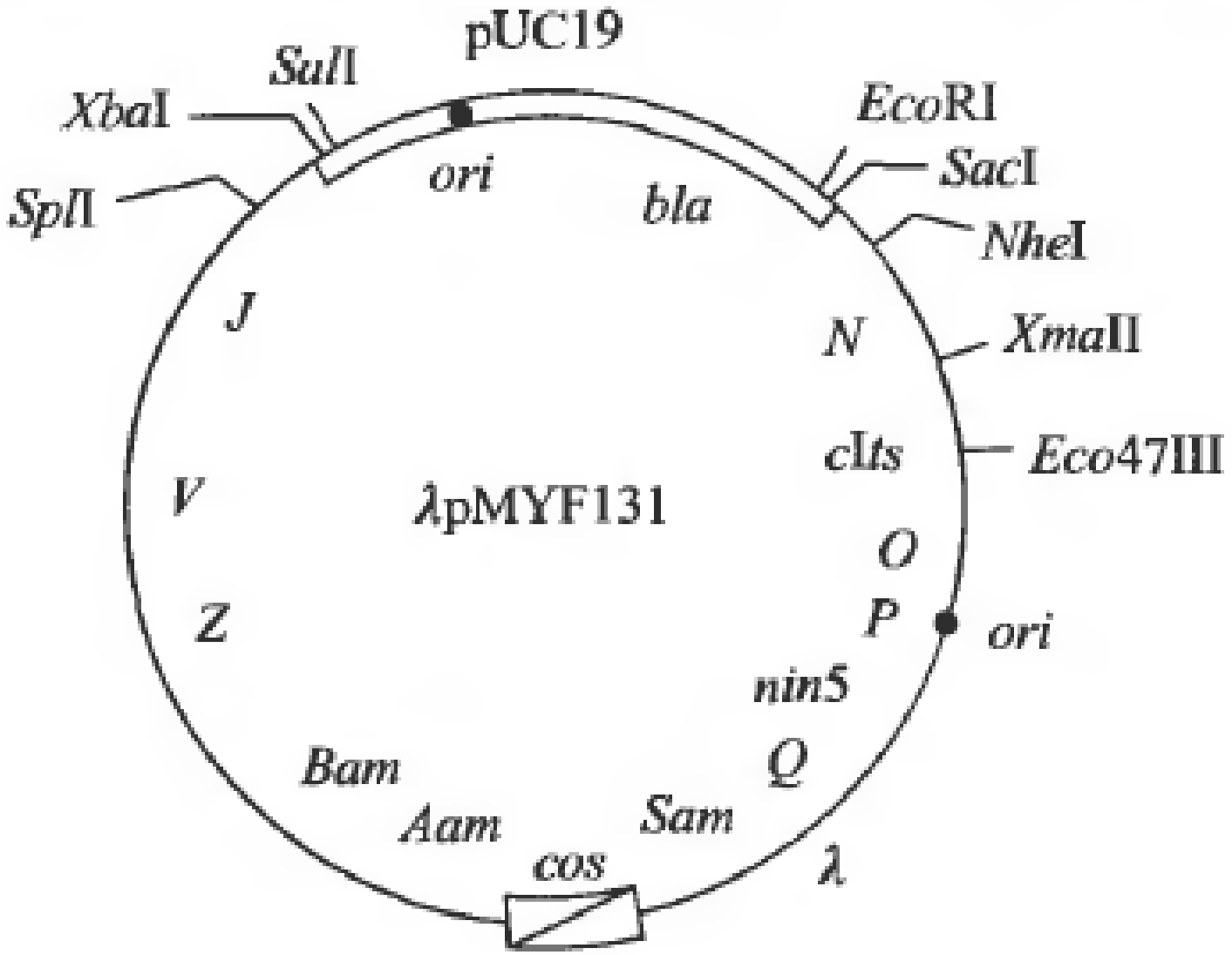
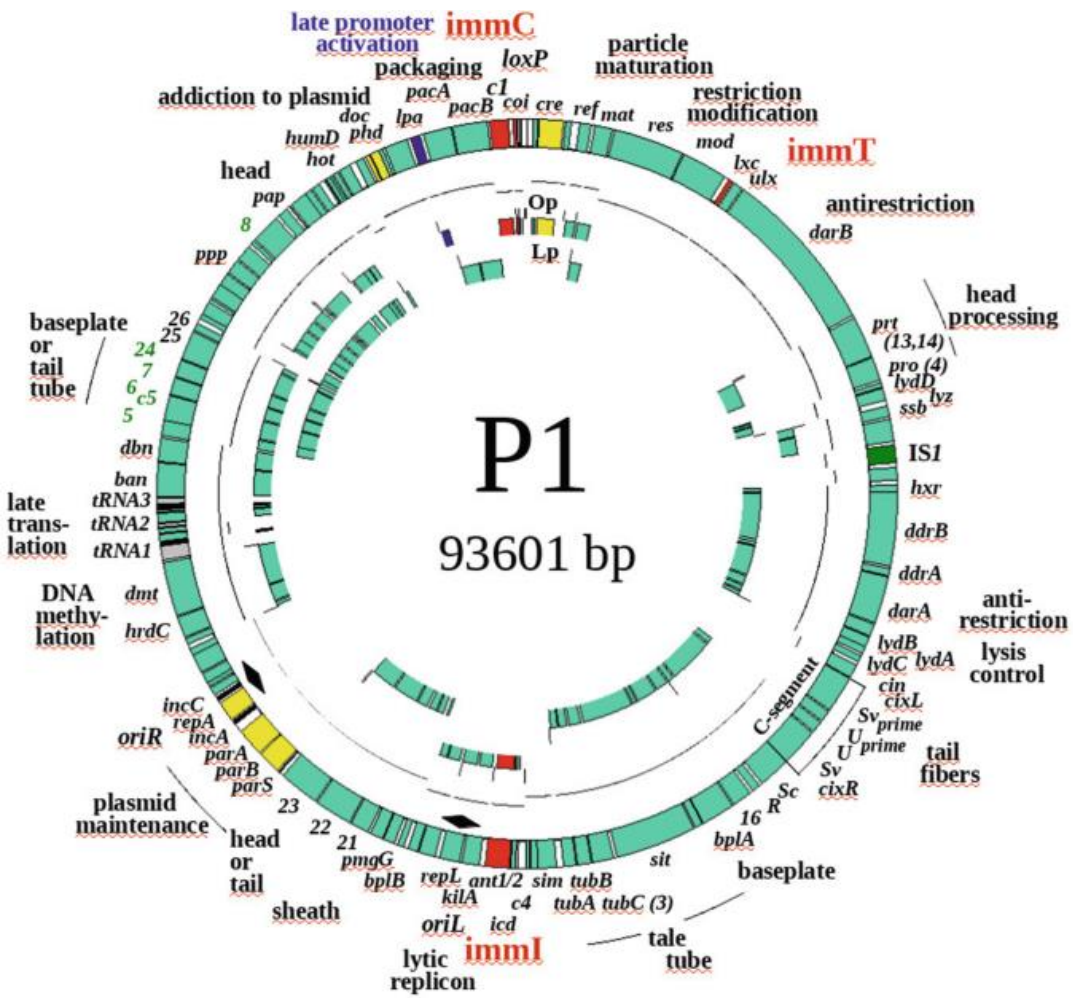


Рис. 2.24. Карта фазмиды λpMYF131

Bacteriophage vector



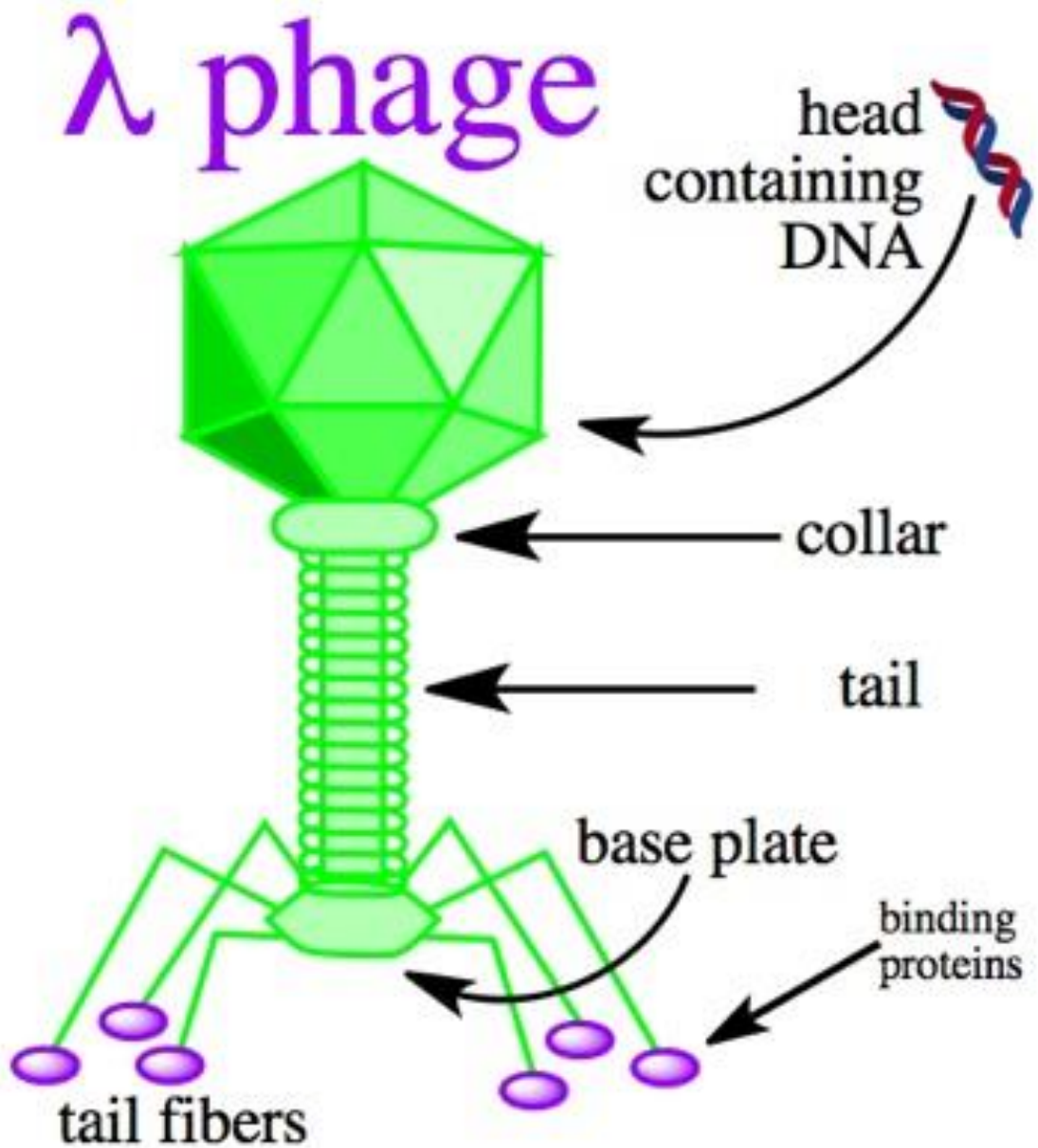
Bacteriophage vectors are viruses that infect only bacteria and efficiently transform them, carrying large inserts.

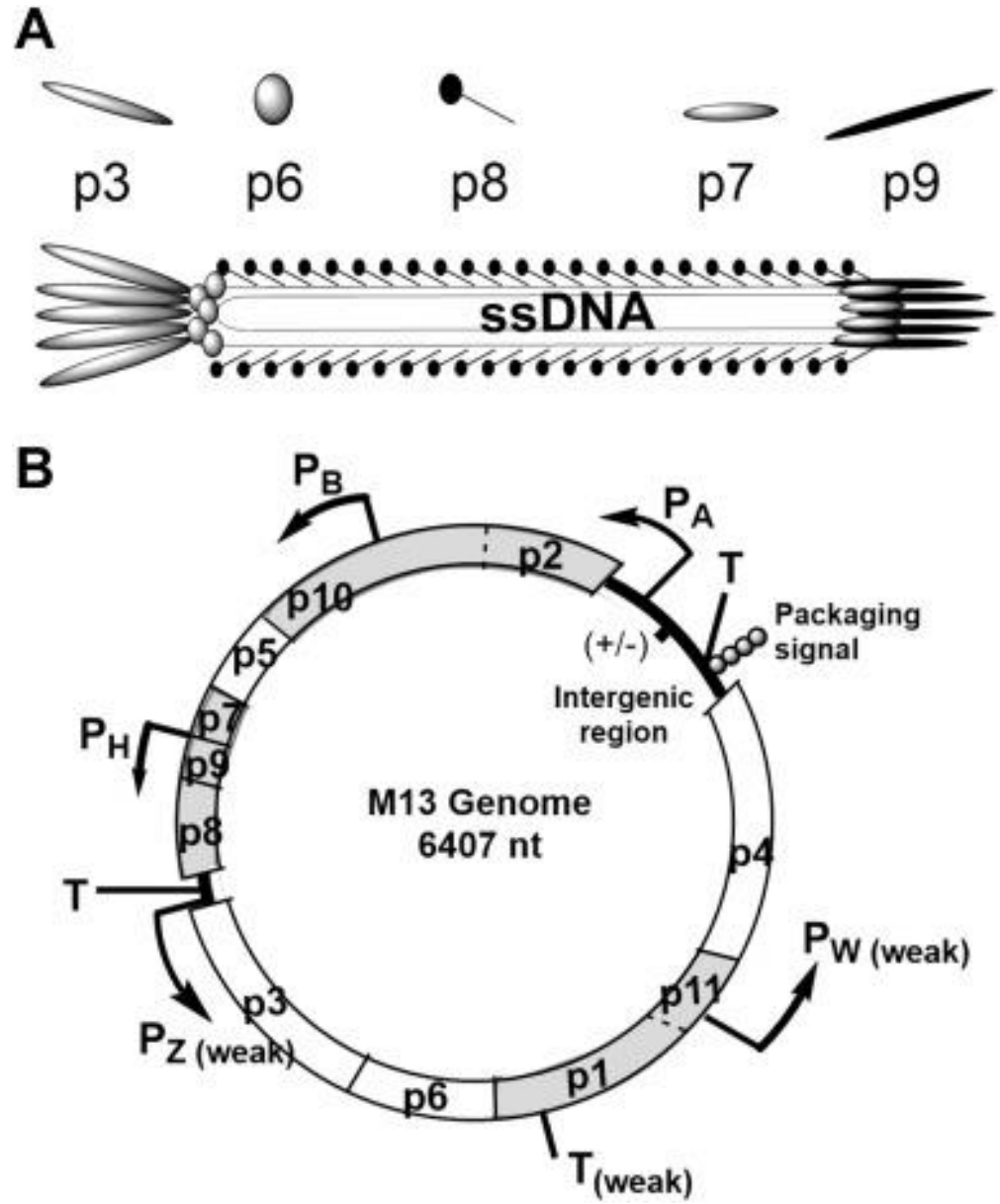
Bacteriophages, or vector phages, have higher transformation efficiency, increasing the chances of obtaining a clone containing recombinant DNA segments.

The most important feature of the phage is the packaging system, which allows for the insertion of large eukaryotic genes and their regulatory elements.

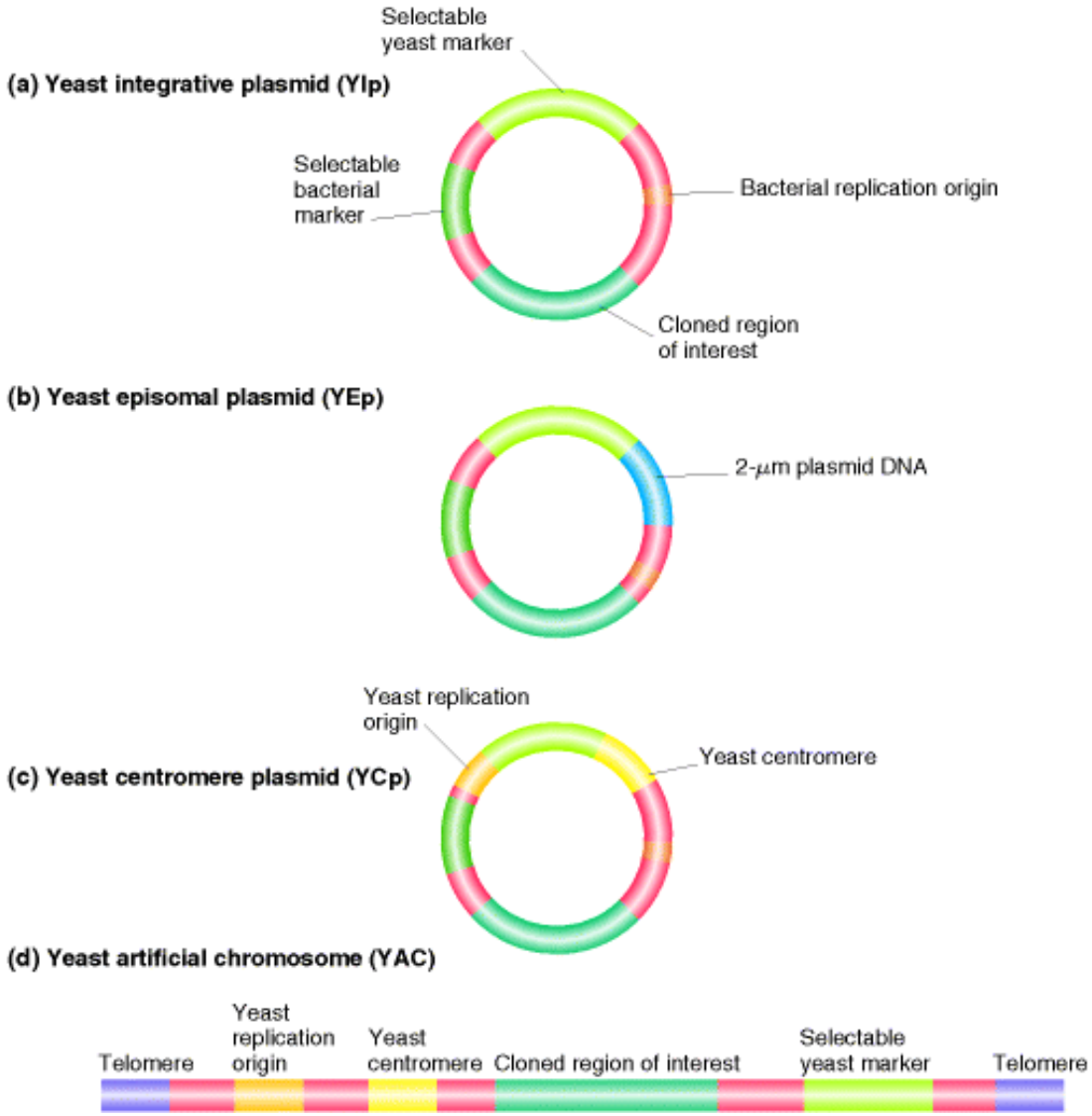
The use of phages also facilitates the isolation of large quantities of DNA, which can be used for insertion analysis. Although a number of phages exist that can be used as vectors, the λ phage is the most convenient vector for cloning.

It can selectively package a chromosome approximately 50 kb in length, and the phage size can be adjusted by removing the central portion of the genome, as this is not necessary for replication or packaging of the donor DNA.


λ phage
head containing DNA
collar
tail
base plate
binding proteins
tail fibers


A
p3
p6
p8
p7
p9
ssDNA
B
PB
PA
T
Packaging signal
(+/-)
Intergenic region
p2
p10
p5
p7
p9
p8
PH
T
M13 Genome
6407 nt
p4
PW (weak)
p11
p3
PZ (weak)
p6
p1
T(weak)

Yeast plasmid-based vectors

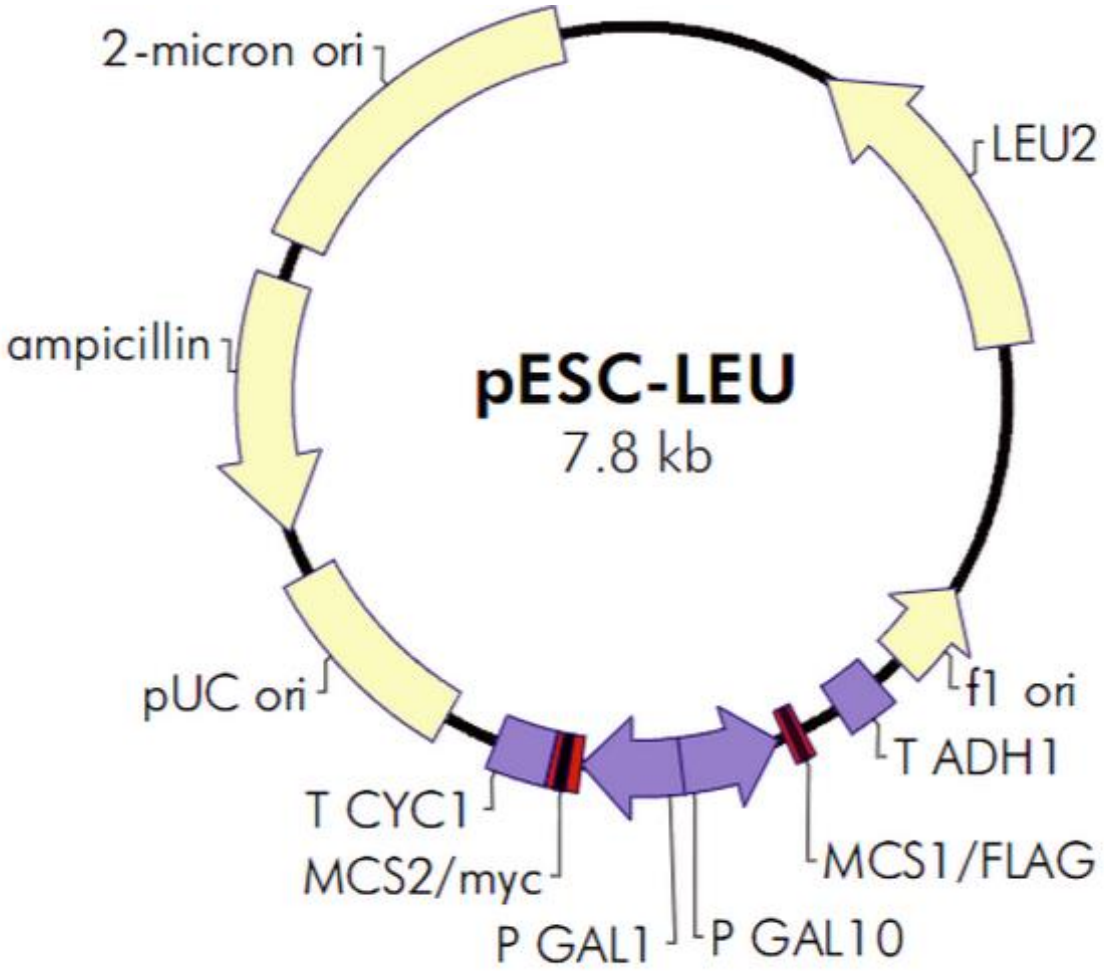


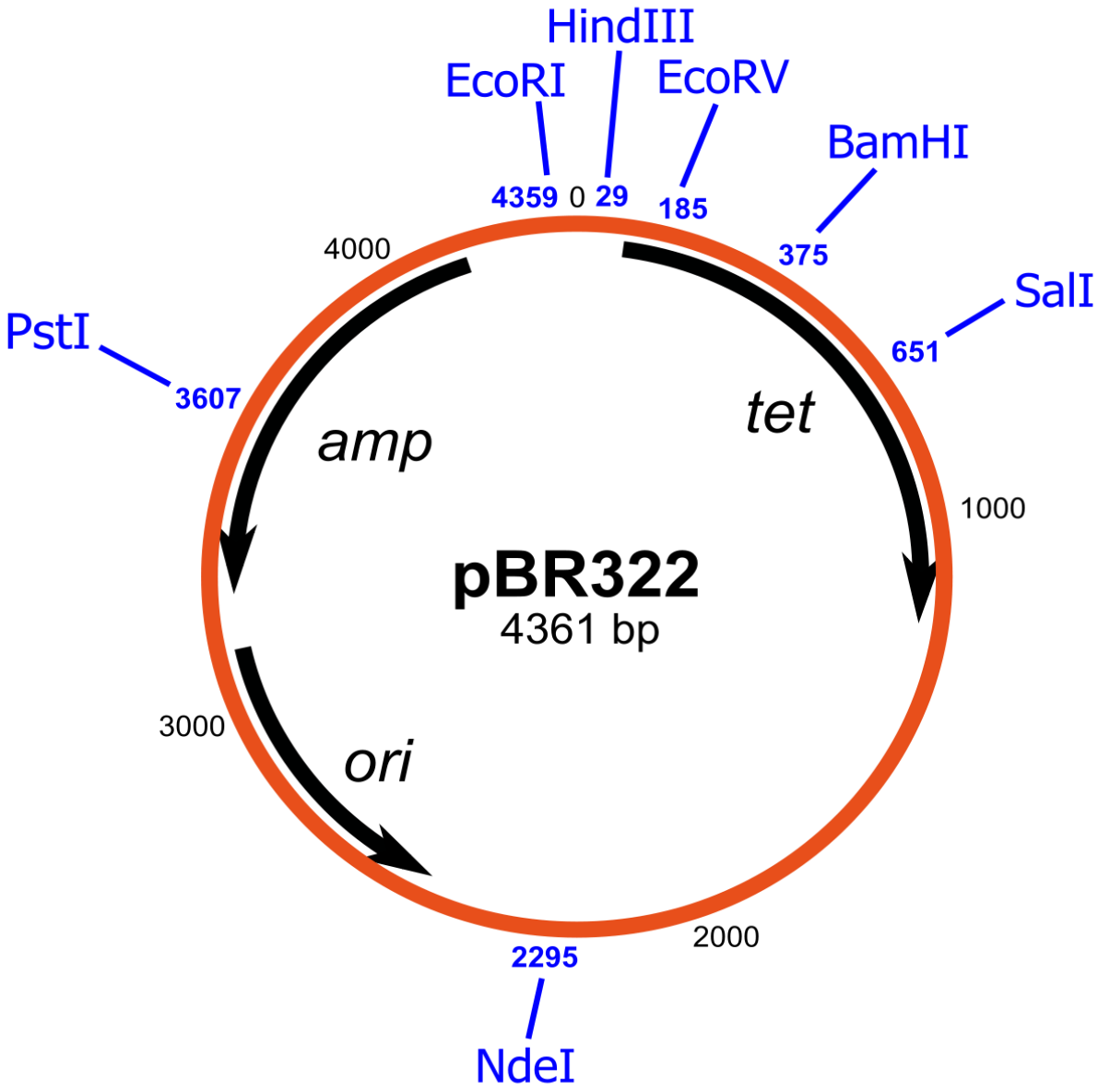
1) Yeast Integrative Plasmid (YIp) - Integrative Plasmid Vector
Characteristic: Does not contain an autonomous replication mechanism, so it integrates into the yeast genome.
Components: Contains selectable markers (for yeast and bacteria), a bacterial replication site, and a cloned gene.
Advantages: High cellular stability.
Disadvantages: Low transformation efficiency.

2) Yeast Episomal Plasmid (YEp) - Episomal Plasmid Vector
Characteristic: Contains a 2-micron plasmid, allowing autonomous replication in yeast.
Components: Same elements as YIp, but with a yeast replication origin.
Advantages: High copy number → High gene expression.
Disadvantages: Less stable, possible plasmid loss during division.

3) Yeast Centromere Plasmid (YCp) – Centromere Plasmid Vector
Feature: Contains a centromere, making it more stable, replicating one copy per cell.
Components: Like YEp, but with a centromere sequence (CEN).
Advantages: Increased stability.
Disadvantages: Low copy number → Less efficient expression.

4) Yeast Artificial Chromosome (YAC) – Yeast Artificial Chromosome
Feature: Functions like a full-fledged chromosome, allowing cloning of very large DNA fragments (up to 1 million base pairs).
Components: Telomeres, centromere, replication site, selectable markers, cloned DNA.
Advantages: Suitable for studying large genomic regions, used in genomic libraries.
Disadvantages: Complexity of construction and maintenance.

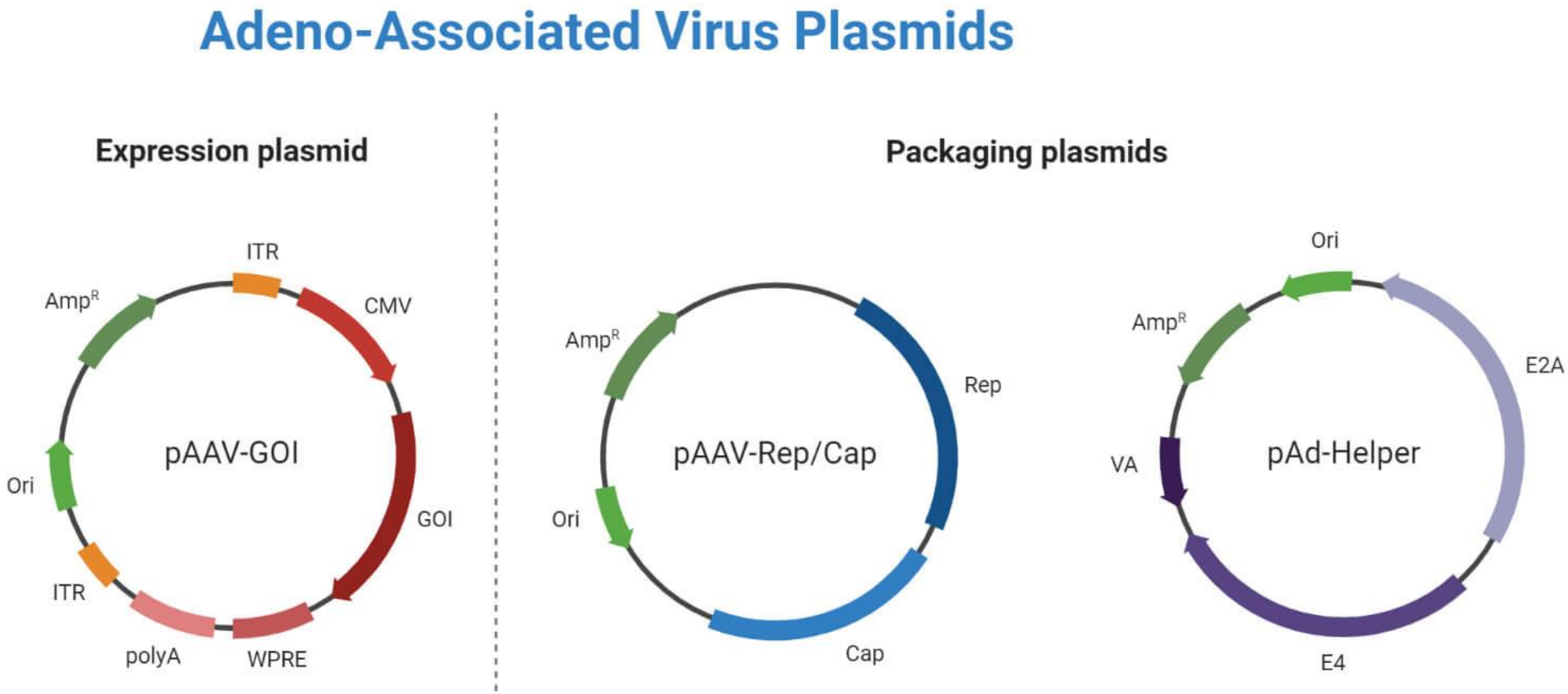




- Yeast plasmid

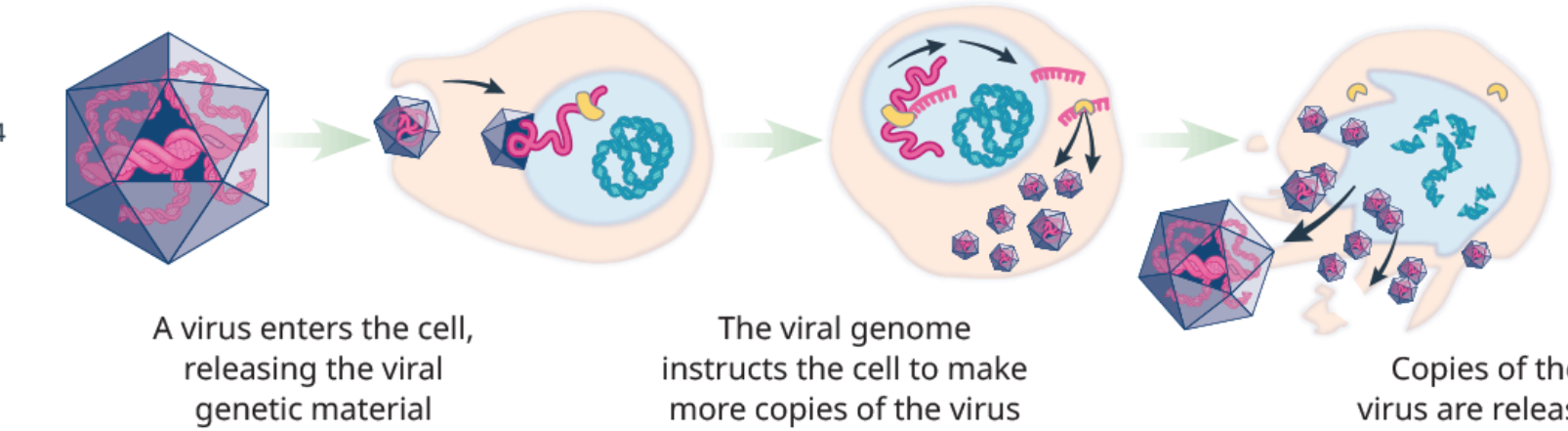
- Bacterial plasmid

2. Viral vectors

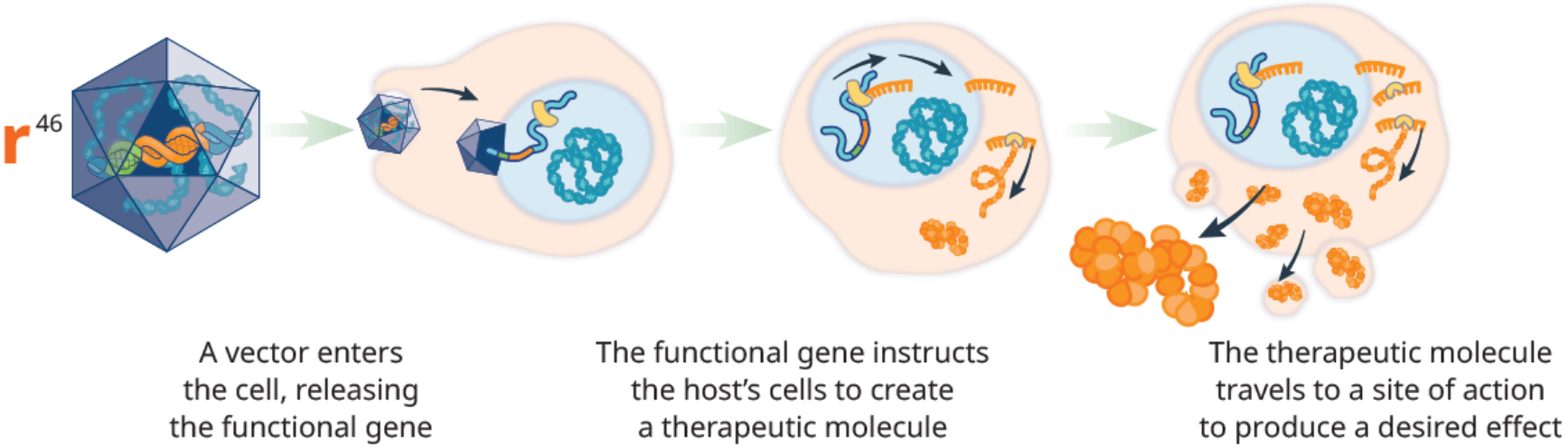


Virus-based vectors

Viruses use their genetic material to replicate.[44]



Vectors don't replicate; they carry the functional gene to cells.[46]



Virus protein shell

- Simple viruses have 2 parts: a genome (DNA or RNA) and a protein shell that protects and delivers the genome to the cells it is targeting[44]
- The genome instructs the cell machinery to make more copies of the virus[44]

Virus

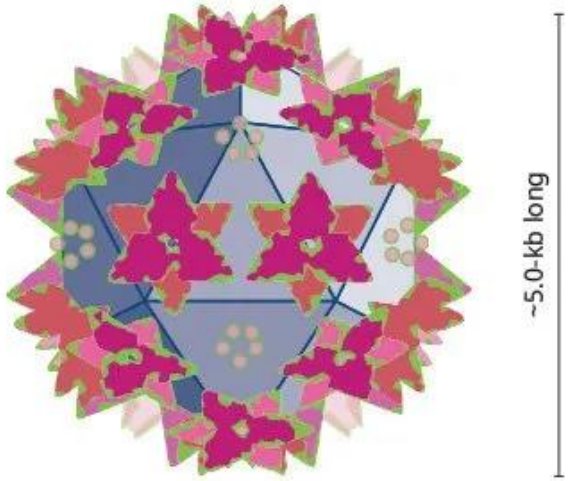
Genome

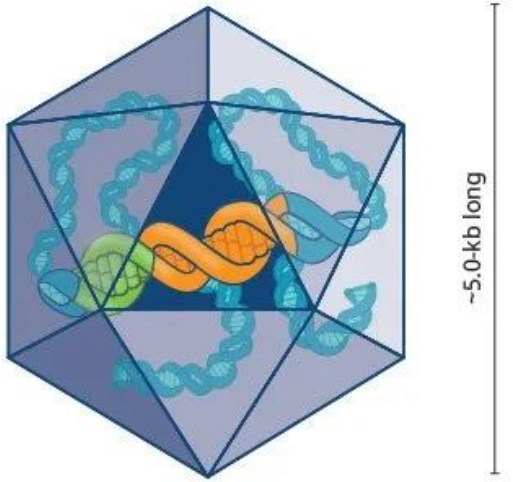
Vector

Functional gene

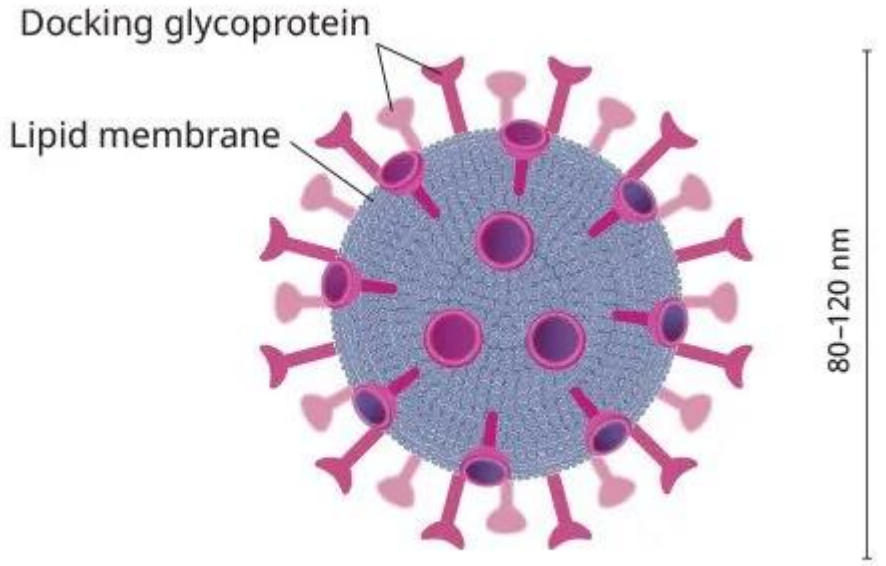
Vector protein shell

- A vector is the carrier construct containing the functional gene to be delivered to a cell[44]
- Vectors are made up of a functional gene and a viral-derived protein shell that protects and delivers the functional gene to the cells it is targeting[44]
- The functional gene instructs cell machinery to make a therapeutic molecule[44]

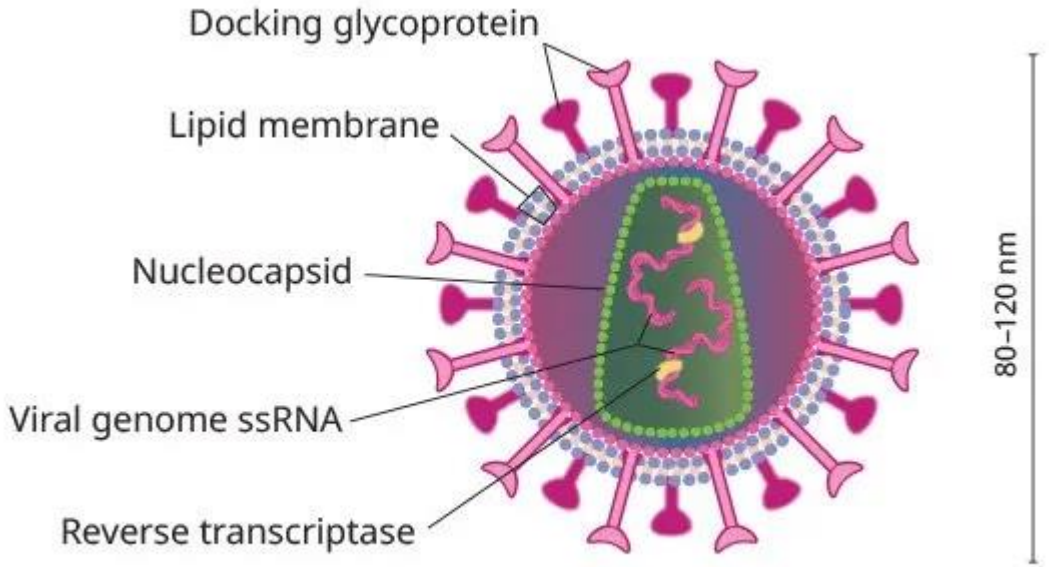




Adeno-associated virus



Lentivirus



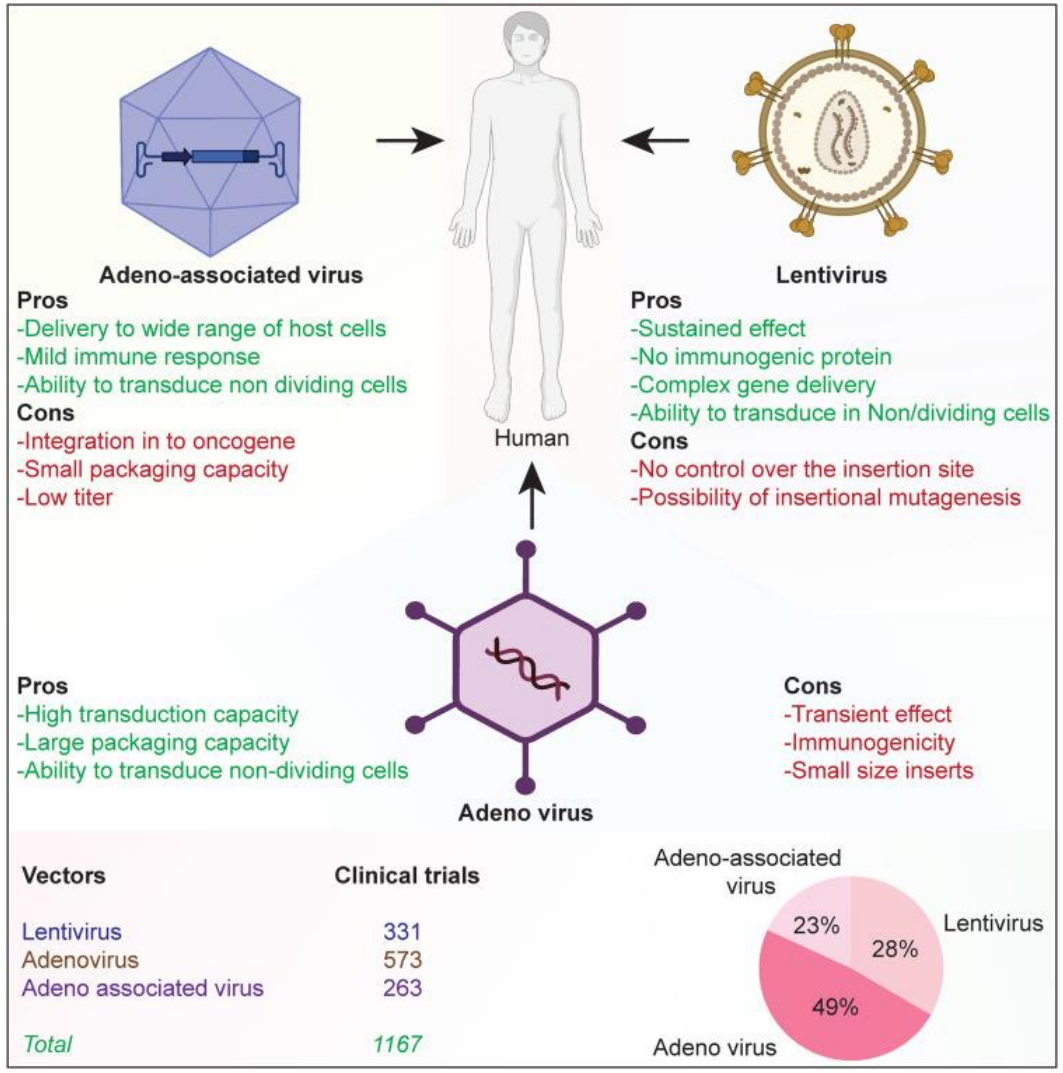
Lentiviral vector

Недостатки использование вирулентных векторов в генной терапии

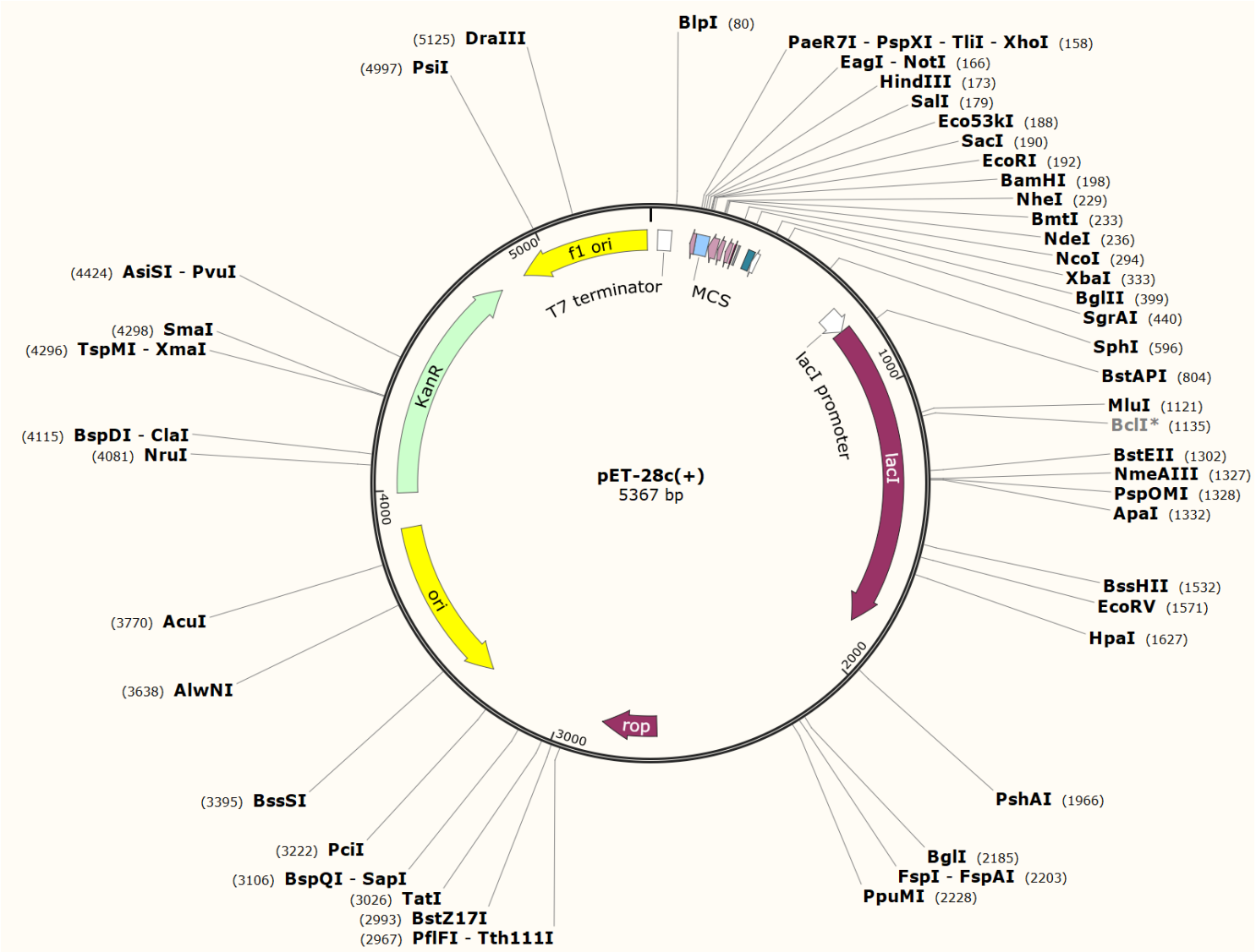
Gene vectors typically include viral and non-viral vectors.

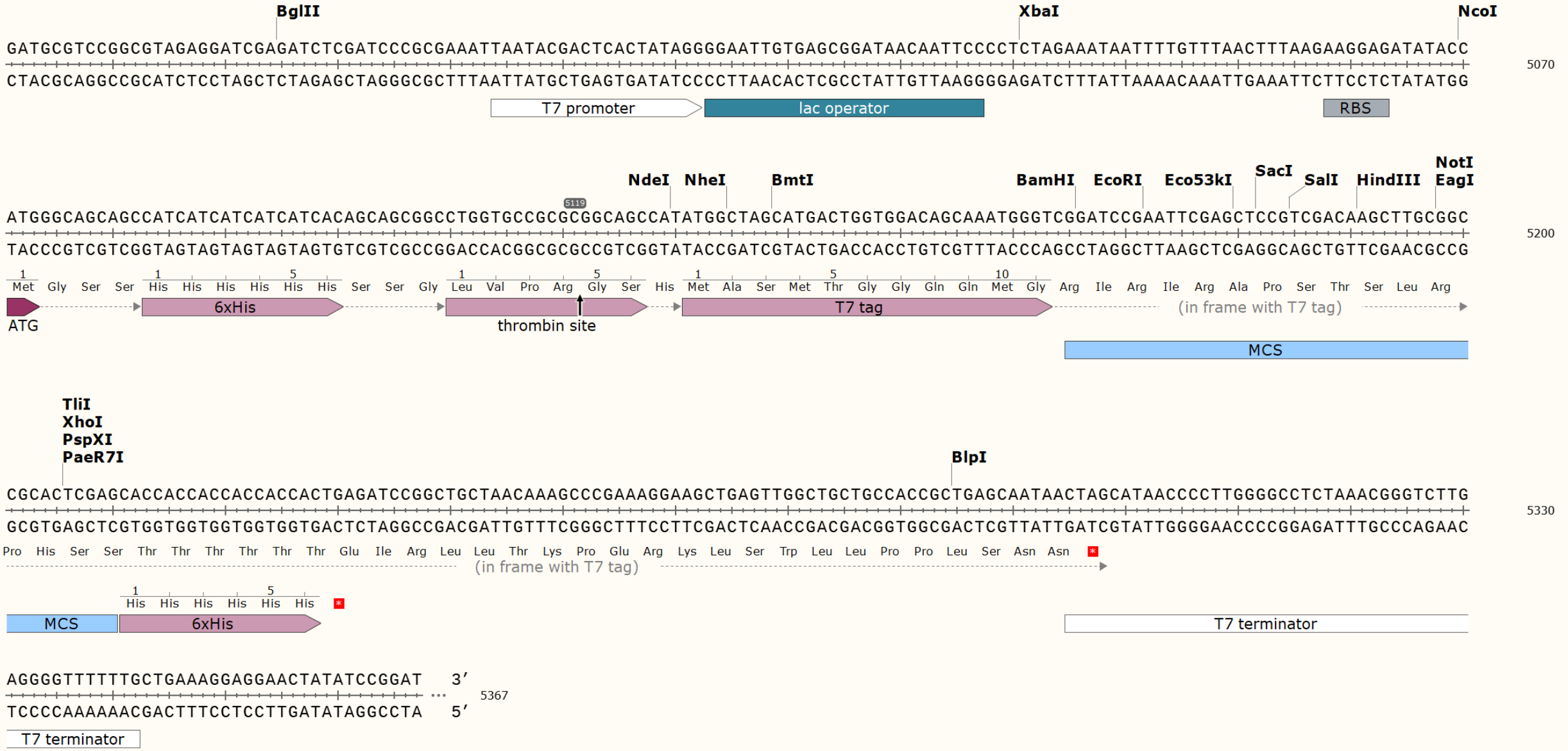
Currently, approximately **70% of gene** therapy clinical trials utilize viral vectors, which include retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses.

- Due to their exceptional infectivity, viral vectors generally offer excellent gene transfection capabilities.
- However, the clinical safety of viral vectors has been questioned due to their tendency to stimulate immunogenic responses and induce mutations associated with transgene insertion.
- Furthermore, viral vectors have several limitations, including low gene loading capacity, inability to deliver large genes, complex preparation procedures, and the inability to re-administer to patients.



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




BglII
XbaI
NcoI
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CTACGCAGGCCGCATCTCCTAGCTCTAGAGCTAGGGCGCTTTAATTATGCTGAGTGATATCCCCTTAACACTCGCCTATTGTTAAGGGGAGATCTTTATTAAAACAAATTGAAATTCTTCCTCTATATGG
5070
T7 promoter
lac operator
RBS
NdeI
NheI
BmtI
BamHI
EcoRI
Eco53kI
SacI
SalI
HindIII
NotI
EagI
5119
ATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGC
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5200
Met Gly Ser Ser His His His His His His Ser Ser Gly Leu Val Pro Arg Gly Ser His Met Ala Ser Met Thr Gly Gly Gln Gln Met Gly Arg Ile Arg Ile Arg Ala Pro Ser Thr Ser Leu Arg
ATG
6xHis
thrombin site
T7 tag
(in frame with T7 tag)
MCS
TliI
XhoI
PspXI
PaeR7I
BlpI
CGCACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTG
GCGTGAGCTCGTGGTGGTGGTGGTGGTGACTCTAGGCCGACGATTGTTTCGGGCTTTCCTTCGACTCAACCGACGACGGTGGCGACTCGTTATTGATCGTATTGGGGAACCCCGGAGATTTGCCCAGAAC
5330
Pro His Ser Ser Thr Thr Thr Thr Thr Thr Glu Ile Arg Leu Leu Thr Lys Pro Glu Arg Lys Leu Ser Trp Leu Leu Pro Pro Leu Ser Asn Asn
(in frame with T7 tag)
His His His His His His
MCS
6xHis
T7 terminator
AGGGGTTTTTTGCTGAAAGGAGGAACTATATCCGGAT 3′
TCCCCAAAAAACGACTTTCCTCCTTGATATAGGCCTA 5′
5367
T7 terminator

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5) http://molbiol.ru/