Cloning VectorS

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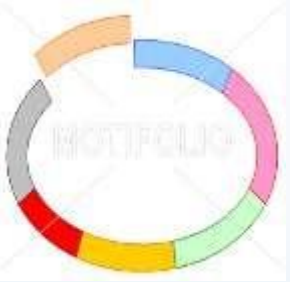
Objectives

After the end of the presentation we'll know -

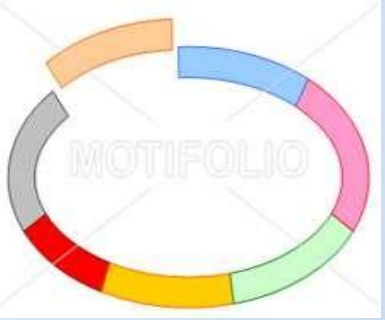
- What is cloning vector?
- Why cloning vector?
- History
- Features of a cloning vector
- Types of cloning vector
  - Plasmid
  - Bacteriophage
  - Cosmid
  - Bacterial Artificial Chromosome (BAC)
  - Yeast Artificial Chromosome (BAC)
  - Human Artificial Chromosome (HAC)
  - Retroviral Vectors
- What determines choice of vector?
- Vector in molecular gene cloning

Cloning Vector



- The molecular analysis of DNA has been made possible by the cloning of DNA. The two molecules that are required for cloning are the **DNA to be cloned** and a **cloning vector**.
- A **cloning vector** is a **small piece of DNA** taken **from a virus**, **a plasmid** or the **cell of a higher organism**, that can be **stably maintained** in an organism and into which a foreign DNA fragment can be inserted for cloning purposes.
- Most vectors are **genetically engineered**.
- The cloning vector is chosen according to the **size and type** of DNA to be cloned.

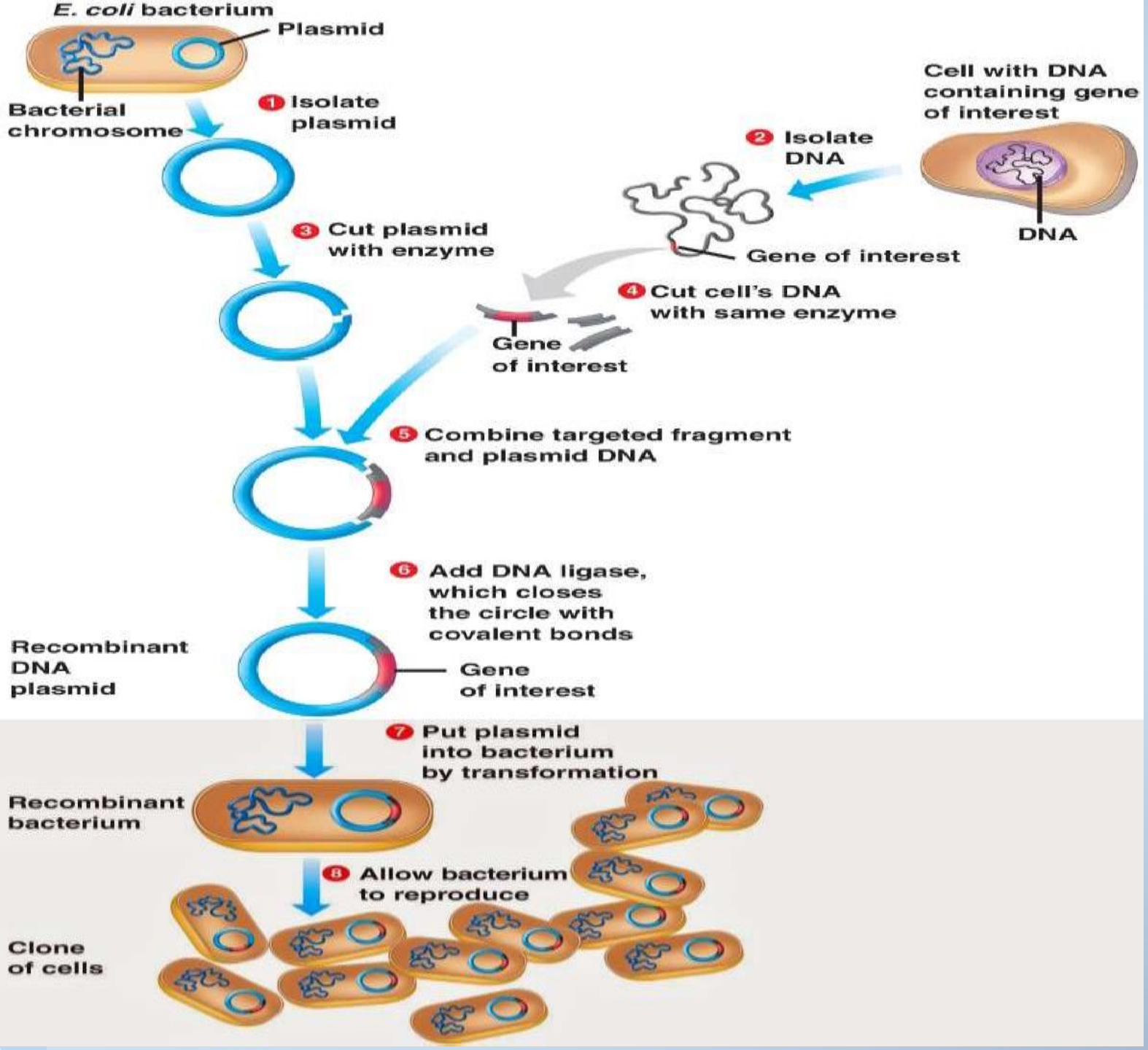
Cloning Vector

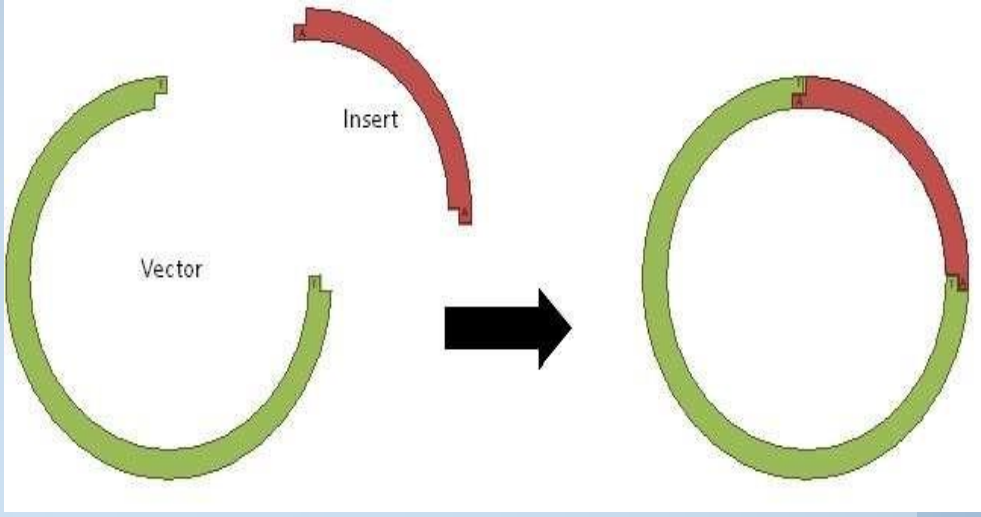


- The vector therefore **contains features** that allow for the convenient insertion or removal of DNA fragment **in or out** of the vector, for example by treating the vector and the foreign DNA with a **restriction enzyme and then ligating** the fragments together.
- After a DNA fragment has been cloned into a cloning vector, it may be further **subcloned** into another vector designed for more specific use.

Why Cloning Vector?

- **Cloning vector** is used as a **vehicle to artificially carry** foreign genetic material into another cell, where it can be replicated and expressed.
- It is **used to amplify** a single molecule of DNA into many copes.
- Cloning vectors are DNA molecules that are used to **"transport"** cloned sequences between **biological hosts and the test tube**.
- **Without Cloning Vector, Molecular Gene Cloning is totally impossible.**

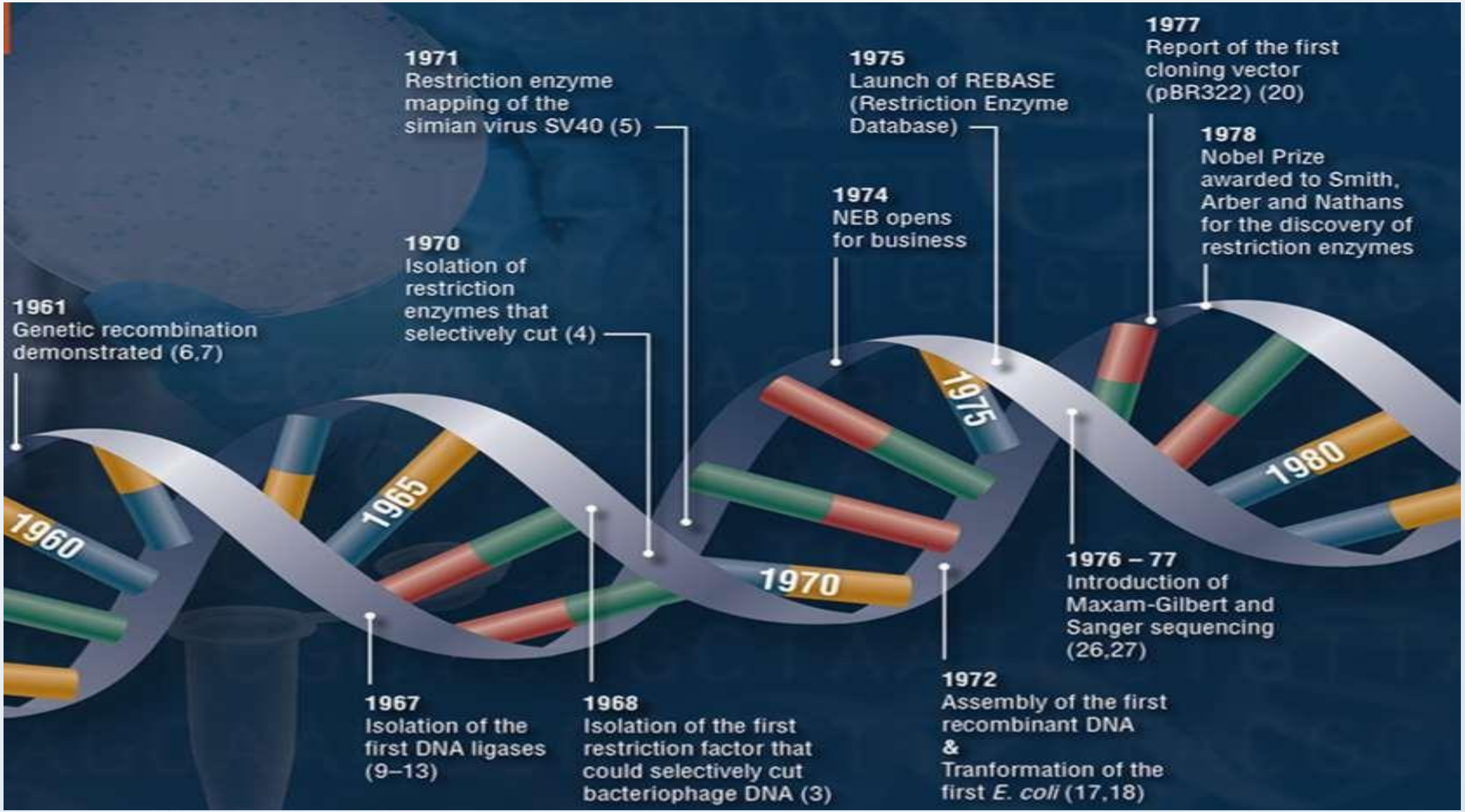




Vector in Gene Cloning

History

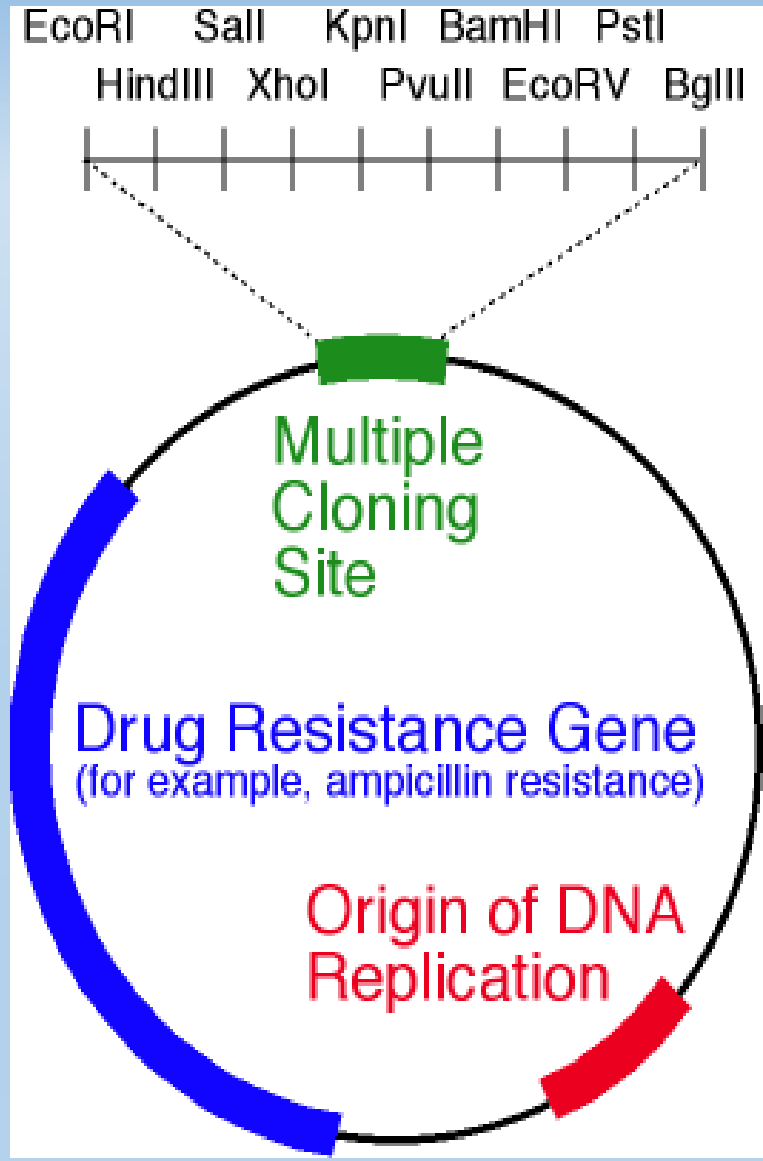
- Scientists (**Herbert Boyer, Keiichi Itakura** and **Arthur Riggs**) working in **Boyer's lab (University of California)** recognized a general cloning vector with unique restriction sites for cloning in foreign DNA and the expression of antibiotic resistance genes for selection of transformed bacteria.
- **In 1977**, they described the first vector designed for cloning purposes, pBR322 – a plasmid.
- This vector was small, ~4 kb in size, and had two antibiotic resistance genes for selection.


1961
Genetic recombination demonstrated (6,7)
1960
1965
1967
Isolation of the first DNA ligases (9–13)
1968
Isolation of the first restriction factor that could selectively cut bacteriophage DNA (3)
1970
Isolation of restriction enzymes that selectively cut (4)
1971
Restriction enzyme mapping of the simian virus SV40 (5)
1972
Assembly of the first recombinant DNA
&
Tranformation of the first E. coli (17,18)
1974
NEB opens for business
1975
Launch of REBASE (Restriction Enzyme Database)
1976 – 77
Introduction of Maxam-Gilbert and Sanger sequencing (26,27)
1977
Report of the first cloning vector (pBR322) (20)
1978
Nobel Prize awarded to Smith, Arber and Nathans for the discovery of restriction enzymes
1980

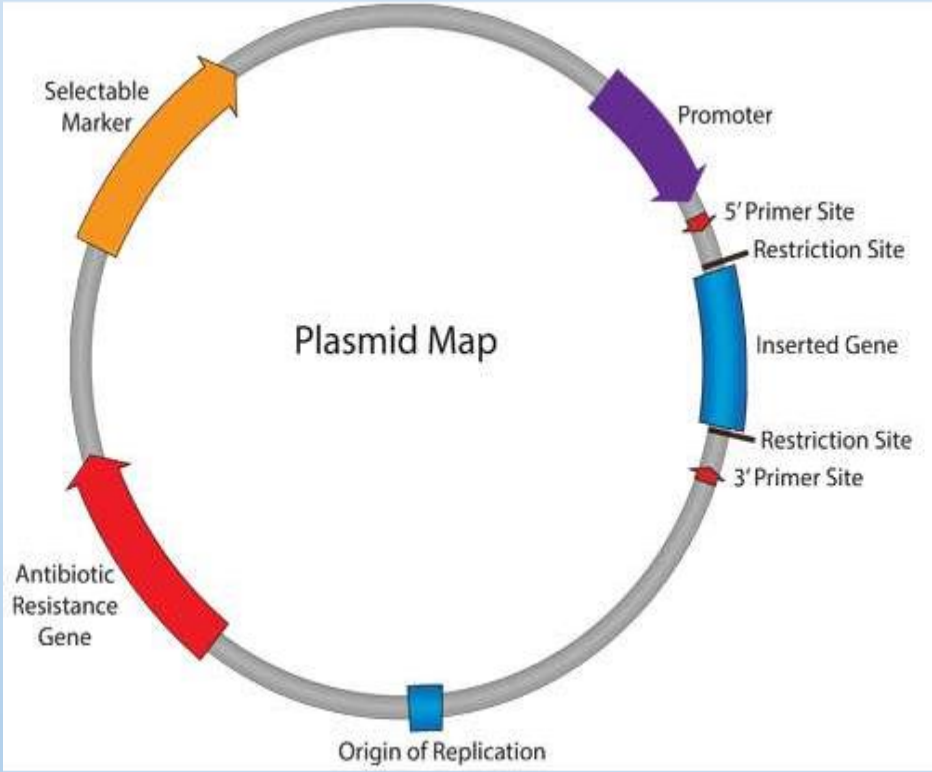
Features of A Cloning Vector

All commonly used cloning vectors have some essential features:

- **Origin of replication (ori):**
  - This makes **autonomous replication** in vector.
  - ori is a **specific sequence of nucleotide** from where replication starts.
  - When foreign DNA is linked to the sequence along with vector replication, foreign (desirable) DNA also starts replicating within host cell.
- **Cloning Site:**
  - Cloning site is a place where the vector DNA can be **digested** and desired DNA can be inserted by the same restriction enzyme.
  - It is a **point of entry** or analysis for genetic engineering work.
  - Recently recombinant plasmids contain a **multiple cloning site** (**MCS**) which have many (up to ~20) restriction sites.



- **Selectable Marker**
  - Selectable marker is a gene that confers **resistance to particular antibiotics or selective agent** that would normally kill the host cell or prevent its growth.
  - A cloning vector contains a selectable marker, which confer on the host cell an ability to **survive and proliferate** in a selective growth medium containing the particular antibiotics.

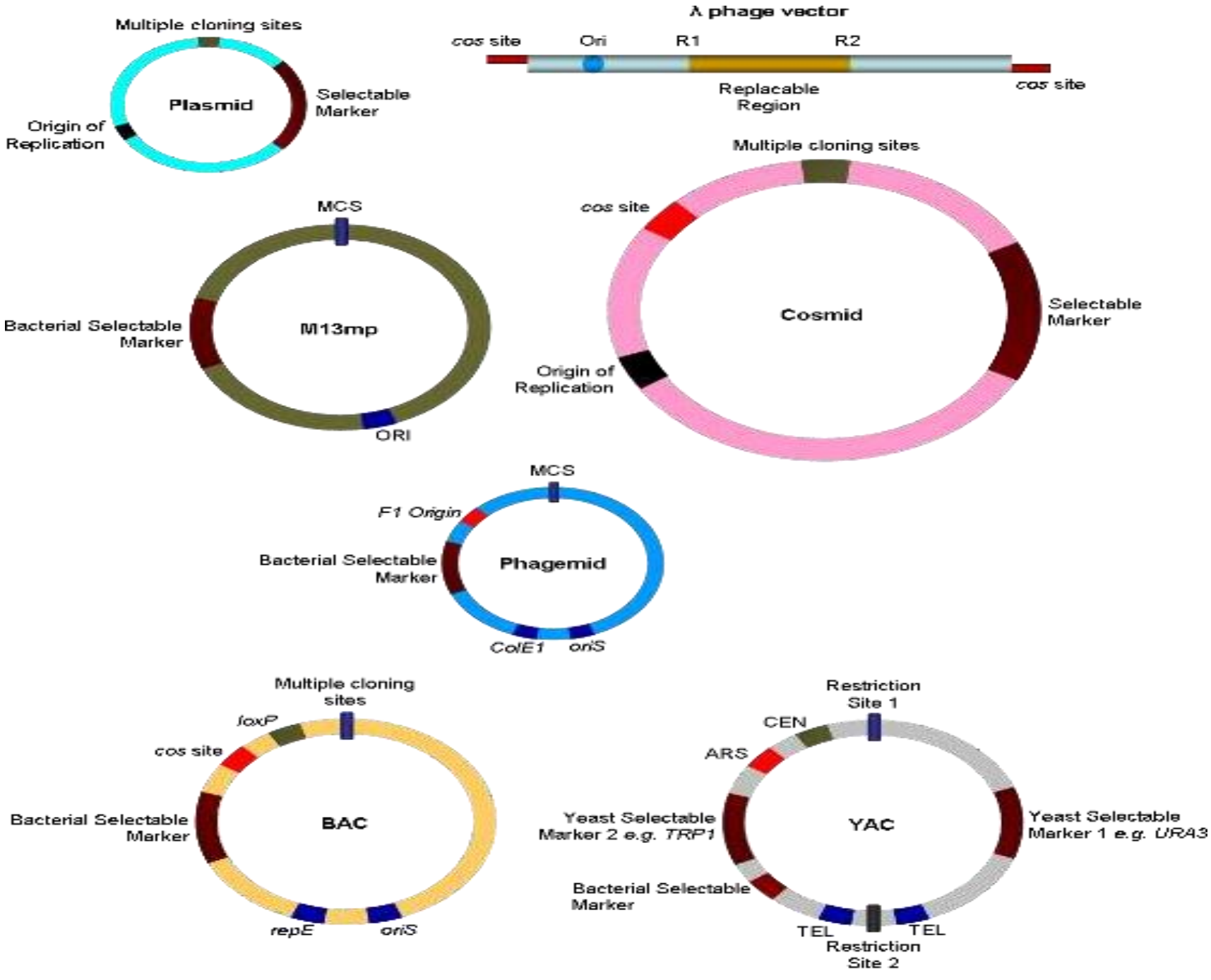


- **Reporter Gene or Marker Gene**
  - Reporter genes are used in cloning vectors to **facilitate the screening** of successful clones by using features of these genes that allow successful clone to be easily identified.
  - Such feature present in cloning vectors is used in blue-white selection.

- **Additional Properties of Vectors:**
  - It should be short, small.
  - Compatible with host cell.
  - Incompatible with other vector.
  - Should become high in copy number.
  - It should able to express itself utilizing the host machinery.
  - It should be able to move under two system (Prokaryote and Eukaryote system).

Types of Cloning Vectors

- Plasmid
- Bacteriophage
- Cosmid
- Bacterial Artificial Chromosome (BAC)
- Yeast Artificial Chromosome (BAC)
- Human Artificial Chromosome (HAC)
- Retroviral Vectors



Types of Vectors

Plasmid

- Plasmid is an **autonomously replicating circular double stranded extra-chromosomal DNA** which is physically separated from a chromosomal DNA and can replicate independently.
- They are most commonly found in **bacteria,** sometimes they are present in archaea and eukaryotic organisms.
- The size of the plasmid varies from **1 to over 200 kb**.
- Most general plasmids may be used to clone DNA insert of **up to 10 kb in size.**
- Many plasmids have **high copy number** and high copy number is useful as it produces greater yield of recombinant plasmid for subsequent manipulation
- However **low copy number** plasmids may be preferably used in certain circumstances, for example, when the protein from the cloned gene is toxic to the cells.
- **Example: pBR322, pUC18, F plasmid, Col Plasmid etc.**

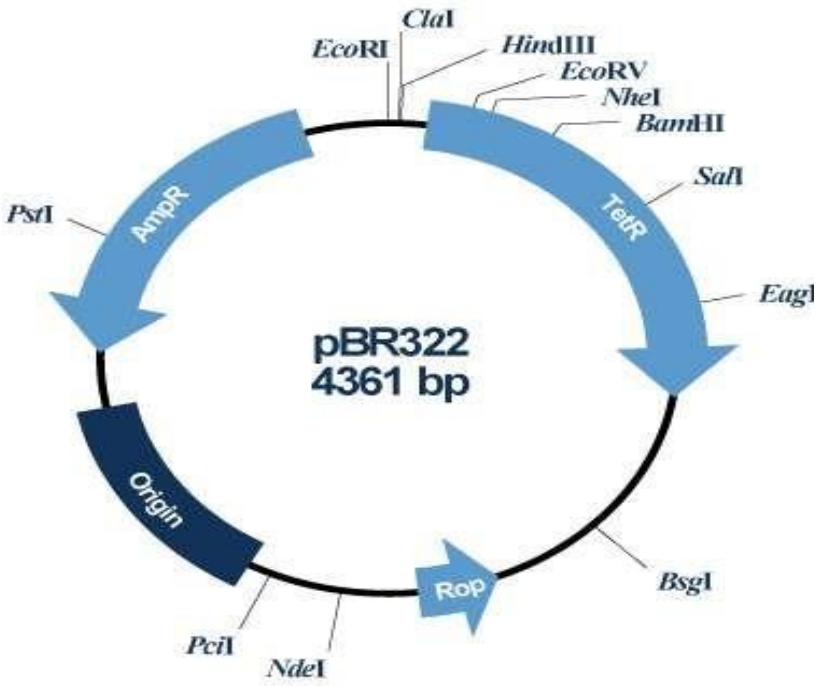


Table 2.1 Sizes of representative plasmids

Plasmid	Size		Organism
	Nucleotide length (kb)	Molecular wt (MDa)	
pBR345	0.7	0.46	*E. coli*
pBR322	4.362	2.9	*E. coli*
ColEl	6.36	4.2	*E. coli*
RP4	54	36	*Pseudomonas* + others
F	95	63	*E. coli*
TOL	117	78	*Pseudomonas putida*
pTiAch5	213	142	*Agrobacterium tumefaciens*

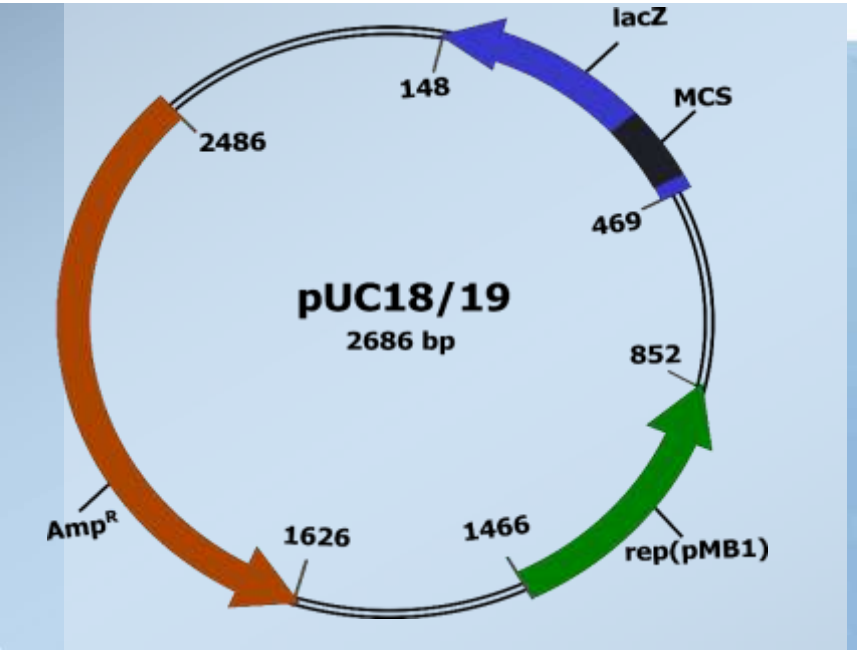
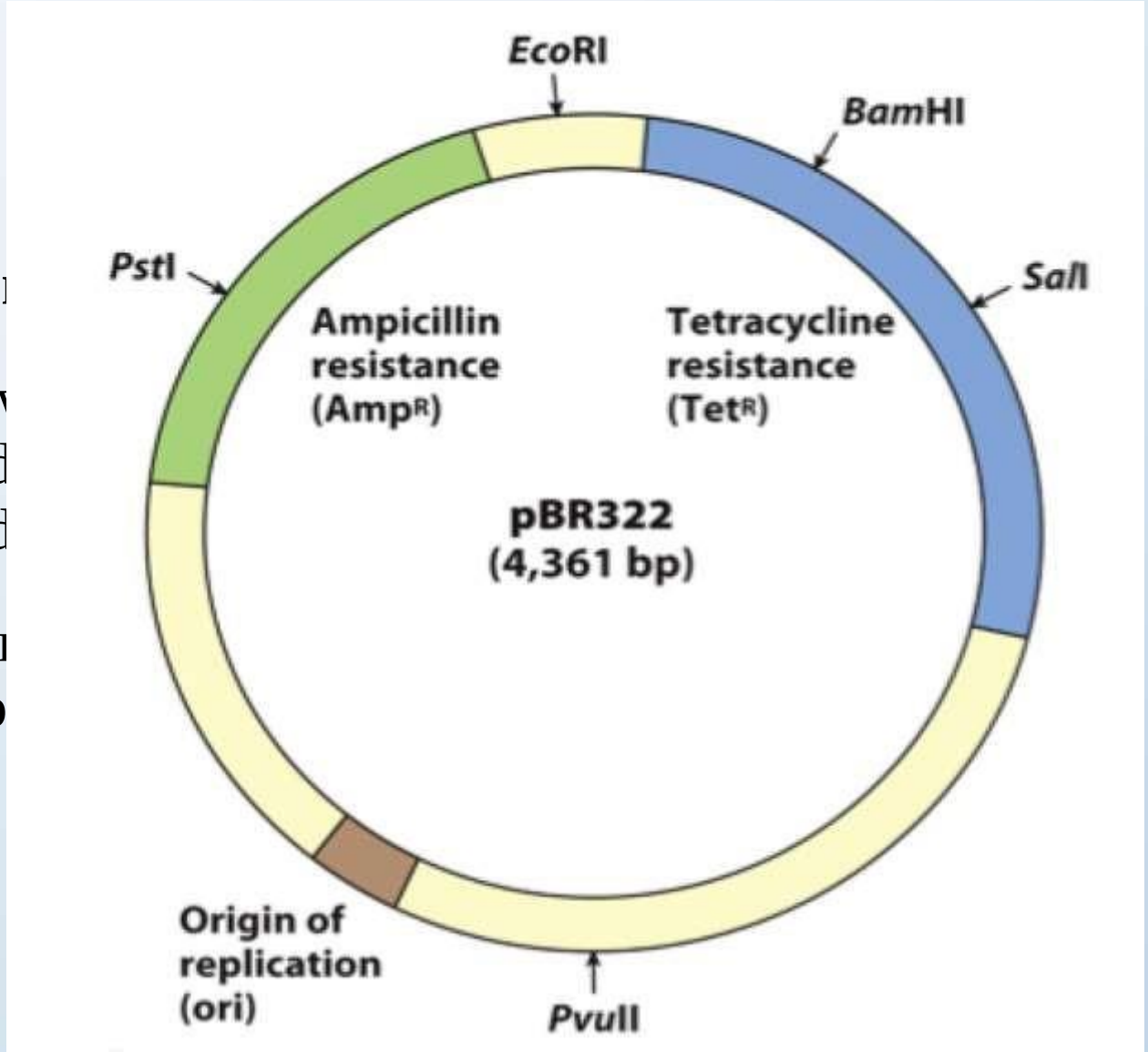


TABLE 4.2 Copy numbers of some plasmids

Plasmid	Approximate copy number
F	1
P1 prophage	1
RK2	4–7 (in *E. coli*)
pBR322	16
pUC18	~30–50
plJ101	40–300

The Nomenclature of Plasmid Cloning Vector

- The name ‘pBR322’ conforms with vector nomenclature.
- **‘p’** indicates that this is indeed **a plas**
- **‘BR’** identified the laboratory in v originally constructed (BR stand **Rodriguez** the two researchers who d
- **‘322’** distinguishes this plasmid fro the same laboratory (there are also p pBR327 etc.)



Why Plasmids are Good Cloning Vectors:

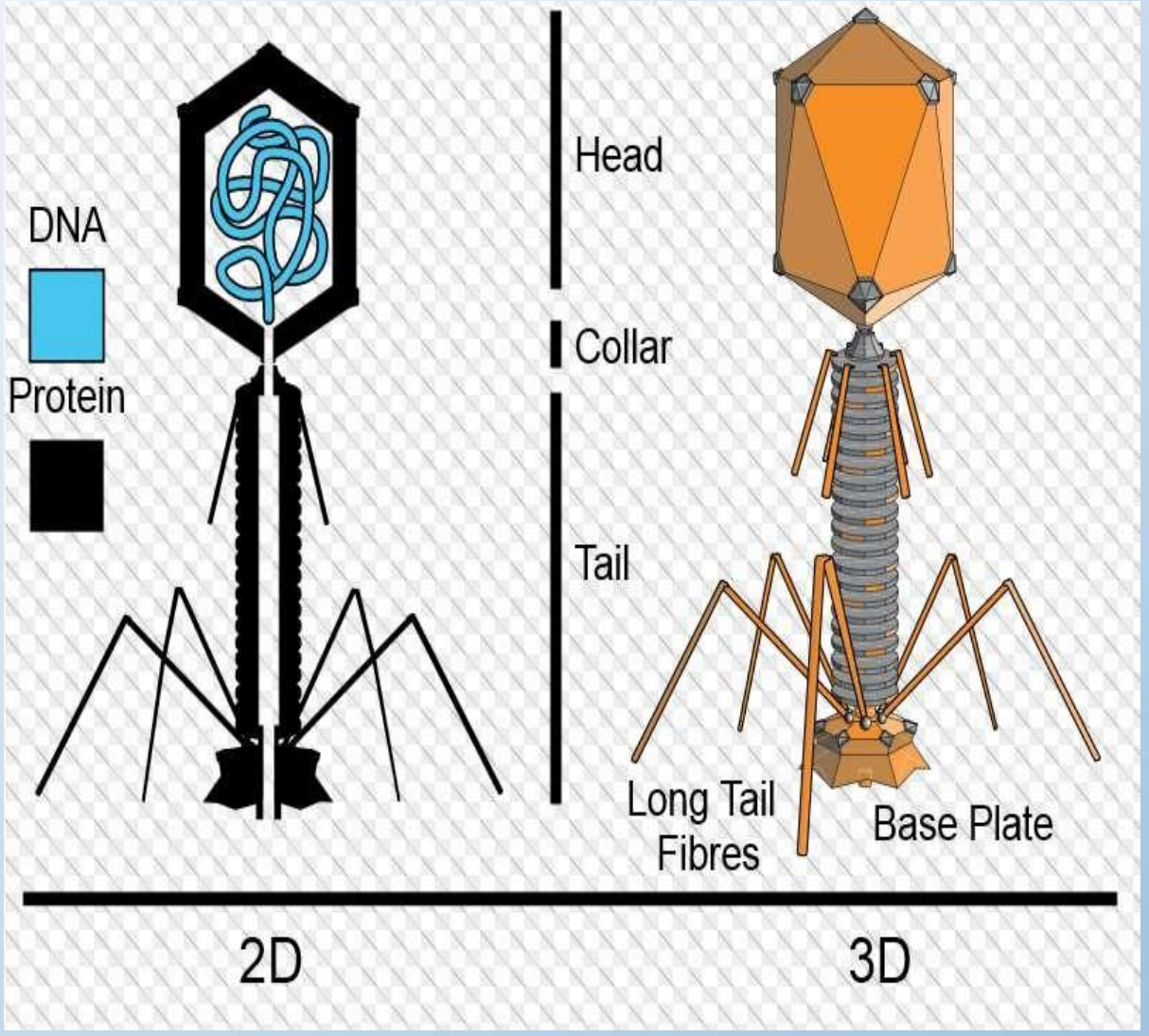
- Small size (easy to manipulate and isolate).
- Circular (more stable).
- Replication independent of host cell.
- Several copies may be present (facilitates replication).
- Frequently have antibiotic resistance (detection easy).

Disadvantages Using Plasmids:

- Cannot accept large fragments.
- Sizes range from 0 – 10kb.
- Standard methods of transformation are inefficient.

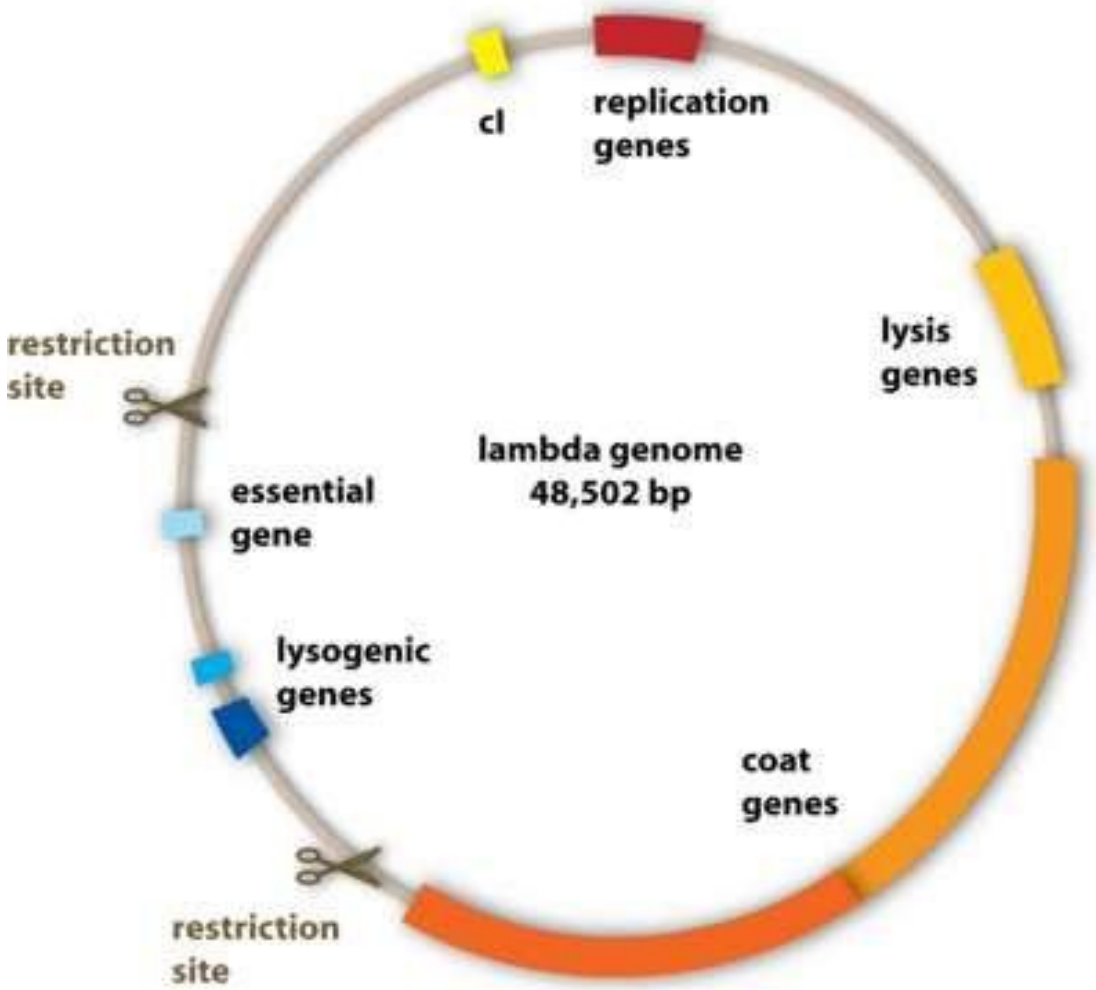
Bacteriophage

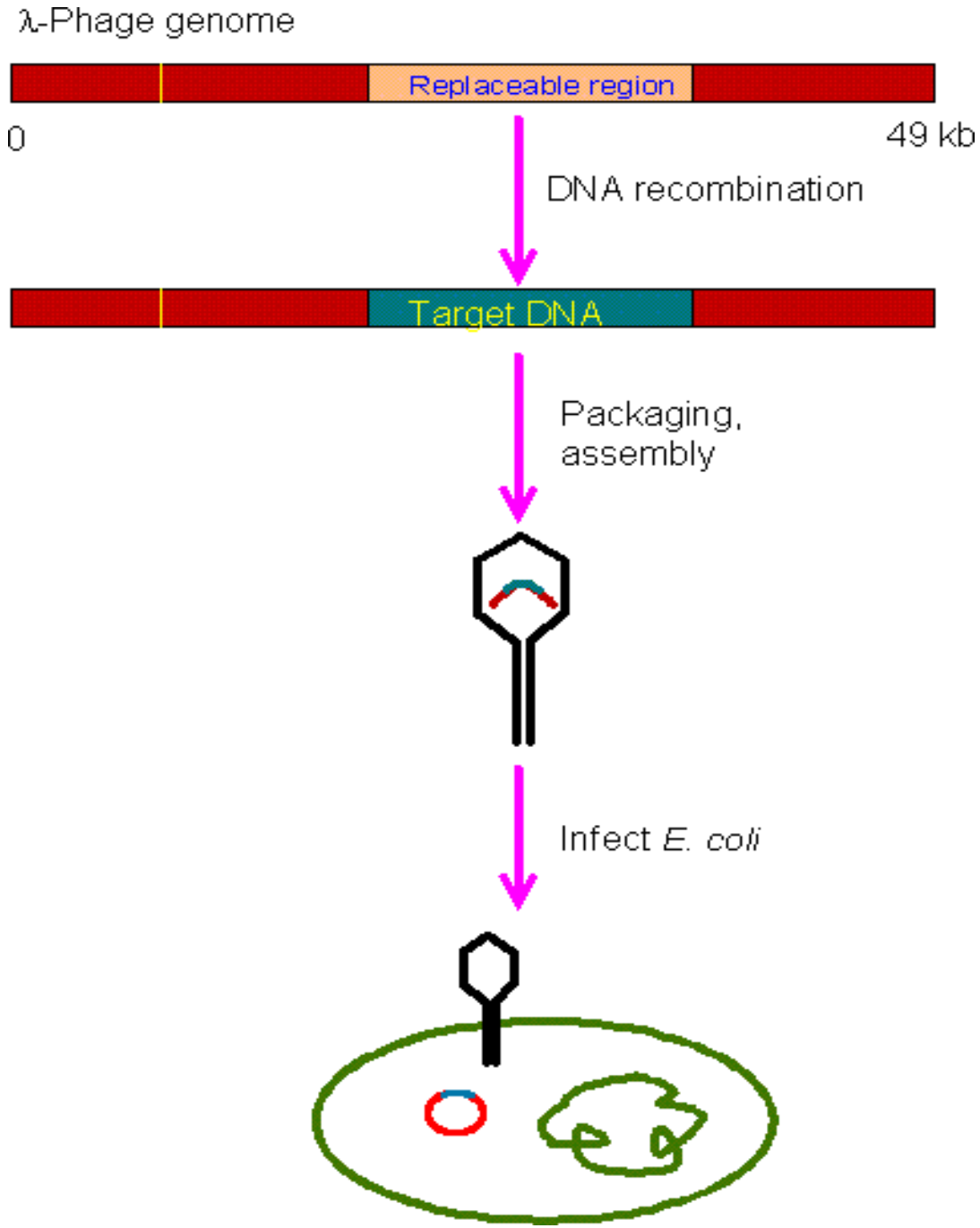
- The bacteriophages used for cloning are the **phage λ and M13 phage.**
- There is an **upper limit** on the amount of DNA that can be packed into a phage (a maximum of 53 kb).
- There is also a **lower size limit** for DNA that can be packed into a phage, and vector DNA that is too small cannot be properly packaged into the phage.



Phage Lambda

- Phage lambda is a **bacteriophage or phage**, i.e. bacterial virus, that uses *E. coli* as host.
- Its structure is that of a typical phage: **head, tail, tail fibres.**
- Lambda viral genome: **48.5 kb DNA** with a **12 base ssDNA "sticky end"** at both ends; these ends are complementary in sequence and can hybridize to each other (this is the **cos site**: cohesive ends).
- **Infection:** lambda tail fibres adsorb to a cell surface receptor, the tail contracts, and the DNA is injected.
- The DNA circularizes and lambda begins its life cycle in the *E. coli* host.
- There are two kinds of λ phage vectors **- insertion vector and replacement vector**.
  - Insertion vectors contain a unique cleavage site whereby foreign DNA with size of 5–11 kb may be inserted.
  - In replacement vectors, the cleavage sites flank a region containing genes not essential for the lytic cycle may be deleted and replaced by the DNA insert in the cloning process.


cI
replication genes
lysis genes
restriction site
lambda genome
48,502 bp
essential gene
lysogenic genes
coat genes
restriction site


λ-Phage genome
Replaceable region
0
49 kb
DNA recombination
Target DNA
Packaging, assembly
Infect E. coli

M13 Phage Vector

- M13 ve
  cloned
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- They a
  filamer
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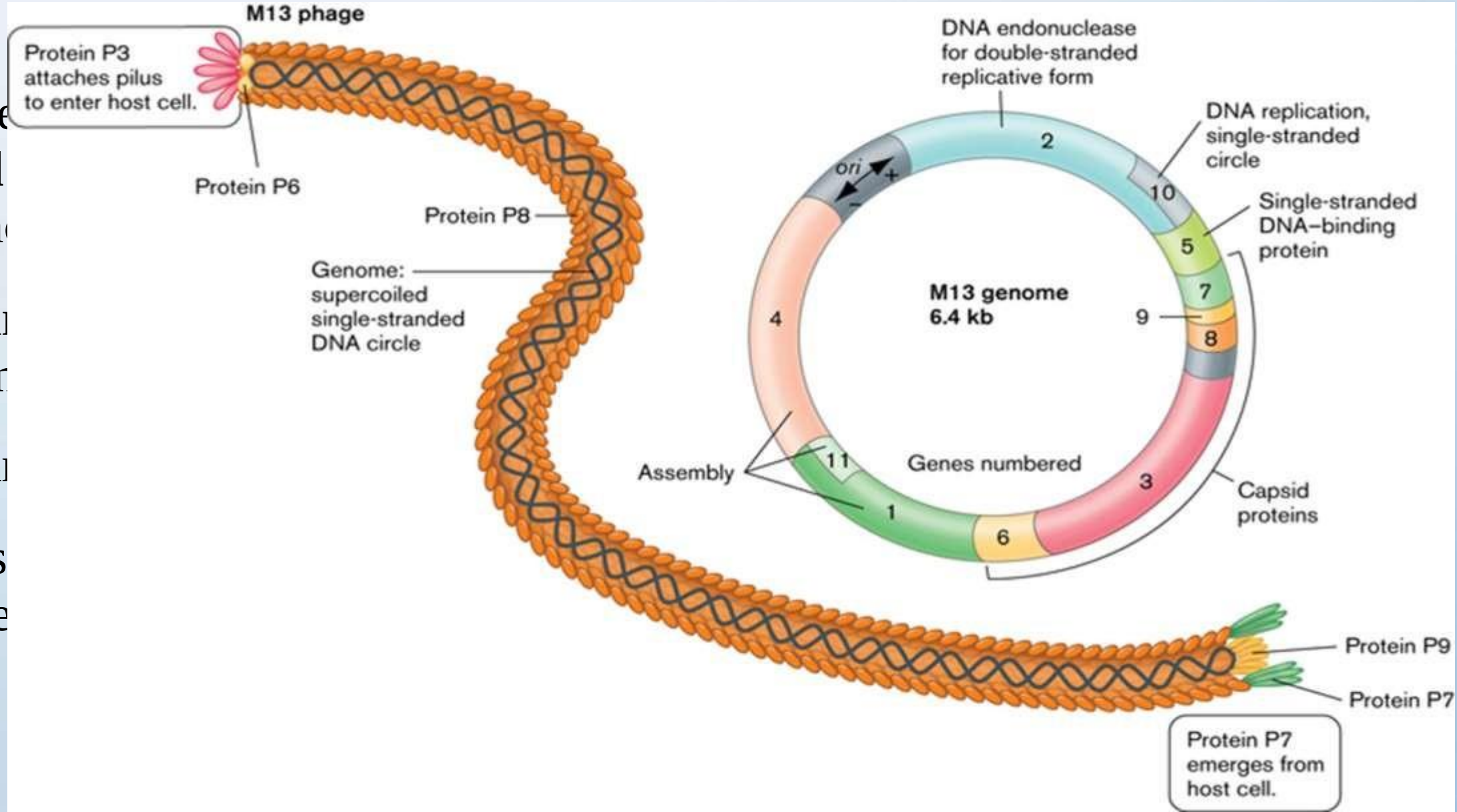
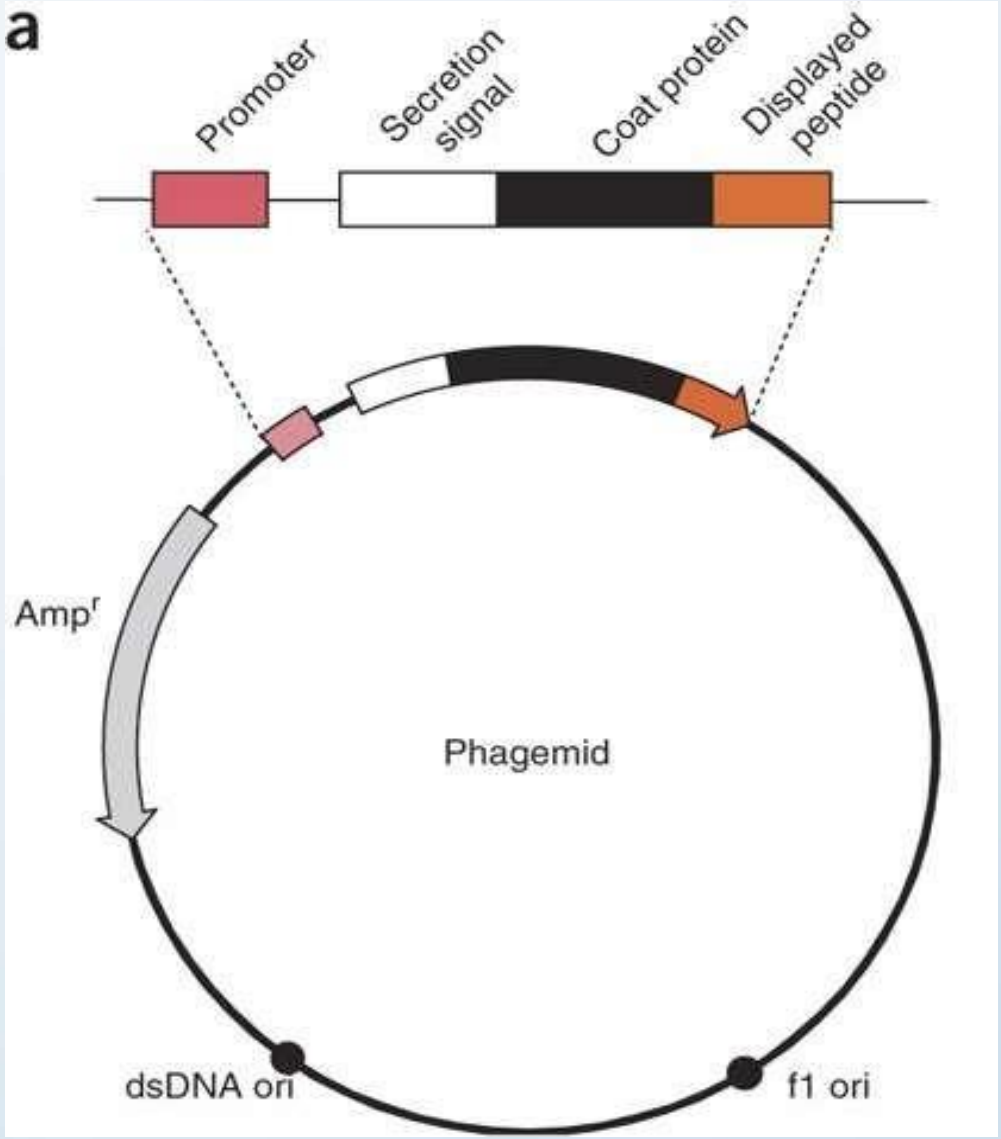
- Pure ss
  obtaine



Phagemid

- A **phagemid** or **phasmid** is a plasmid that contains an f1 origin of replication from an f1 phage.
- It can be used as a type of cloning vector in combination with filamentous phage M13.
- A **phagemid** can be replicated as a plasmid, and also be packaged as single stranded DNA in viral particles.

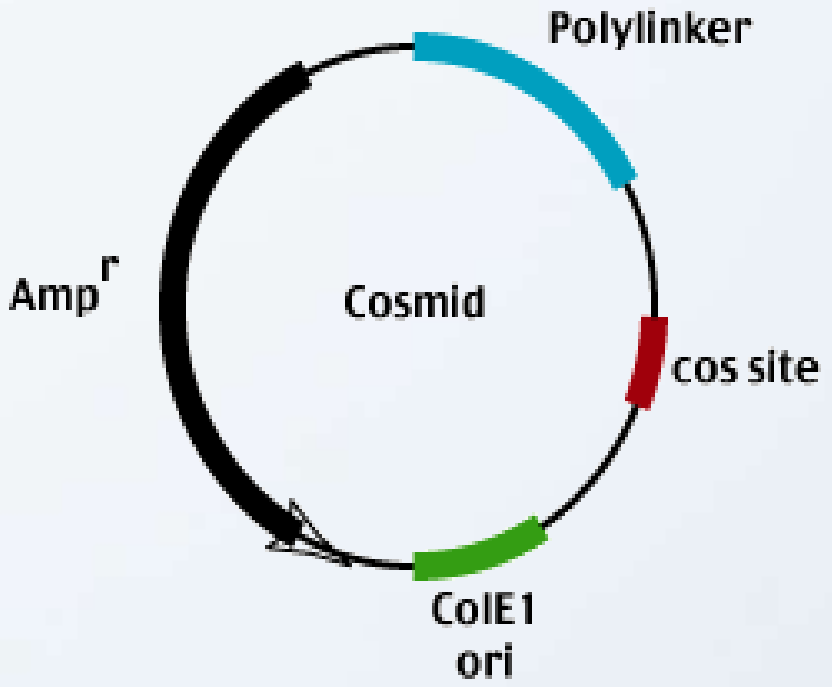


Phage Vectors Present Two Advantages Over Plasmid Vectors-

1. They are more efficient than plasmids for cloning of large DNA fragments; the largest cloned insert in lambda phage is 24 kb, while for plasmid vector it is less than 15 kb.
2. It is easier to screen a large number of phage plaques than bacterial colonies for identification of recombinant vectors.

Cosmid

- Cosmids are plasmids that incorporate a segment of **bacteriophage λ DNA** that has the **cohesive end site (cos)** which contains elements required for packaging DNA into λ particles.
- It is normally used to clone large DNA fragments between **25 and 45 Kb.**
- They can replicate as plasmids if they have a suitable origin of replication.
- They can also be packaged in phage capsids, which allows the foreign genes to be transferred into cells by **transduction.**

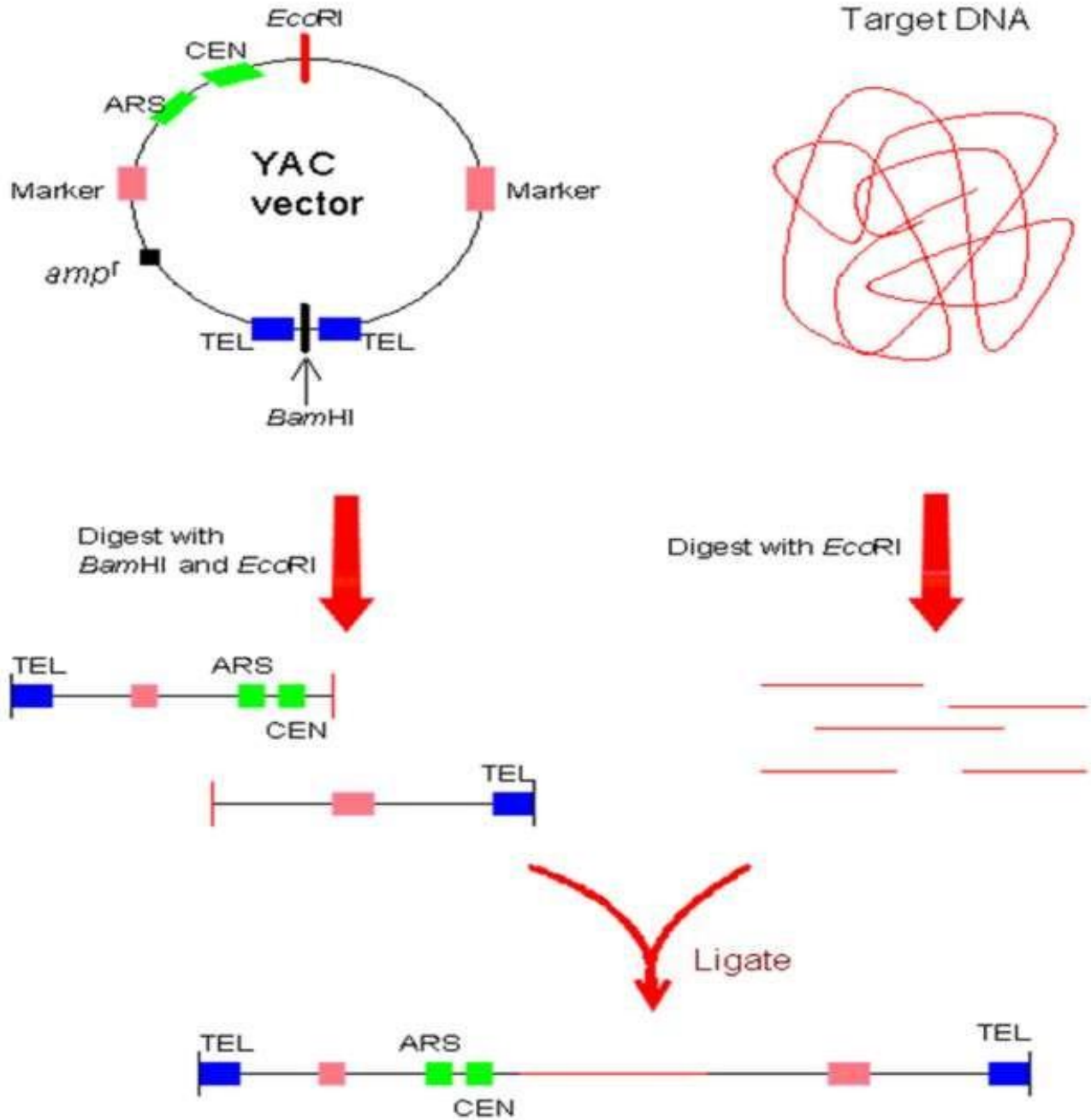


Advantages :

- High transformation efficiency.
- The cosmid vector can carry up to 45 kb whereas plasmid and Lambda phage vectors are limited to 25 kb.

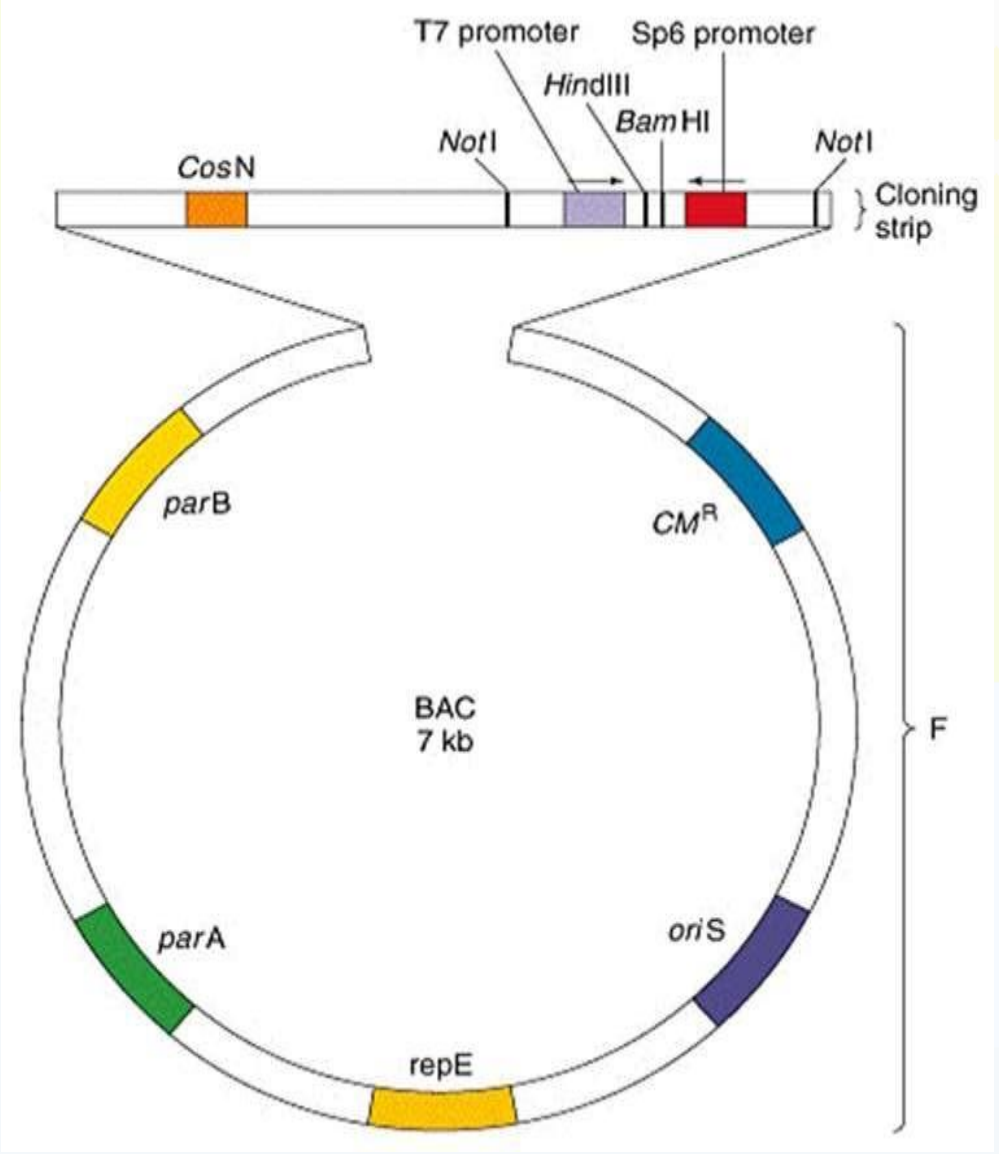
Yeast Artificial Chromosome (YAC)

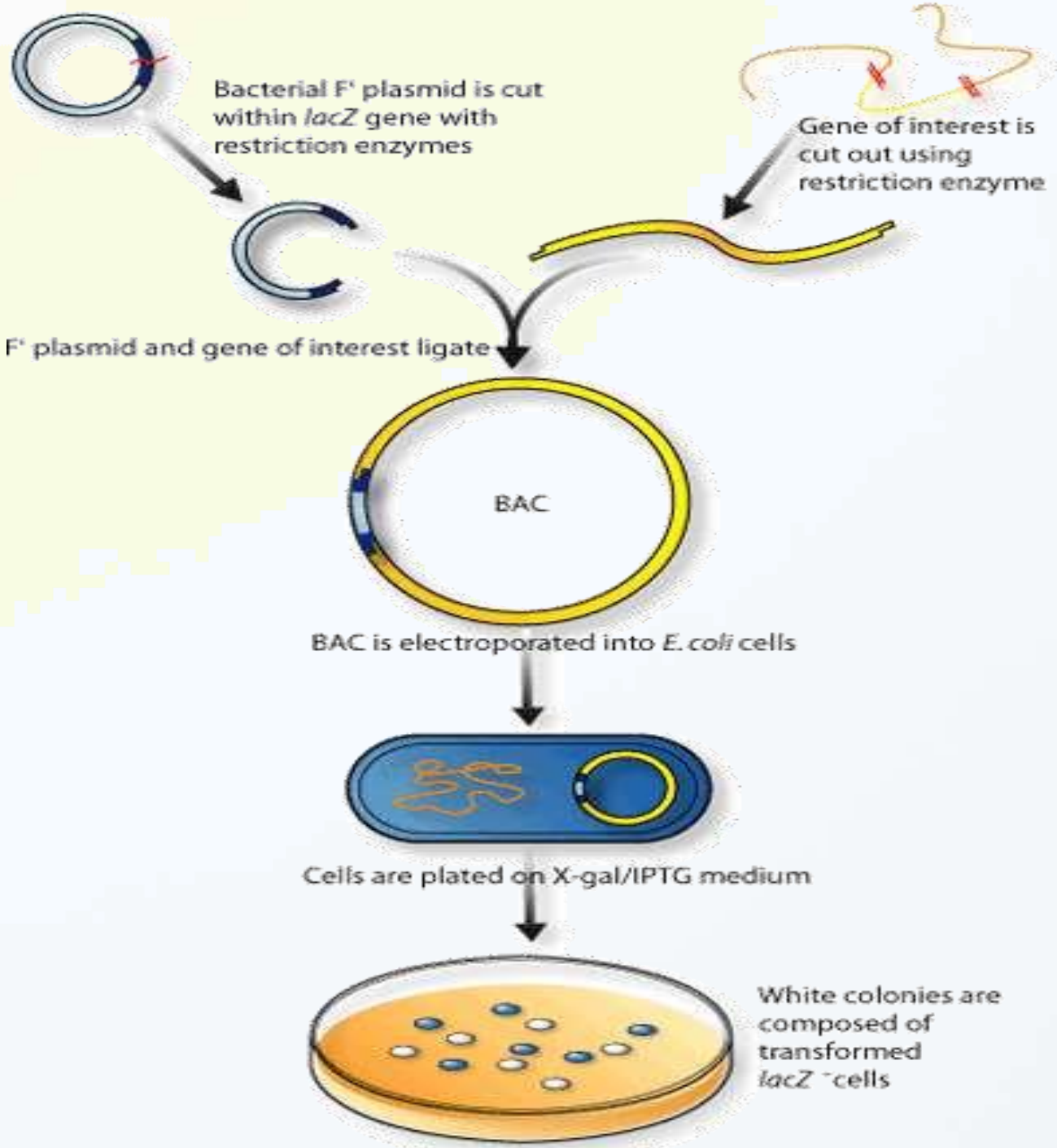
- The yeast artificial chromosome (YAC) vector is capable of carrying a large DNA fragment (up to 200 Kb), but its **transformation efficiency is very low**.
- Cloning vehicles that propogate in eukaryotic cell hosts as eukaryotic chromosomes.
- Final chimeric DNA is a linear DNA molecule with telomeric ends: **Artificial Chromosome.**
- YAC cloning vehicles often have a bacterial origin of DNA replication (**ori**) and a selection marker for propogation of the YAC through bacteria.
- The YAC can use both yeast and bacteria as a host.


EcoRI
CEN
ARS
YAC
vector
Marker
Marker
ampr
TEL
TEL
BamHI
Target DNA
Digest with
BamHI and EcoRI
Digest with EcoRI
TEL
ARS
CEN
TEL
Ligate
TEL
ARS
TEL
CEN

Bacterial Artificial Chromosome (BAC)

- BAC vectors are similar to standard *E. coli* plasmid vectors.
- Contain the origin and genes encoding the ori binding proteins required for plasmid replication.
- Derived from a naturally occurring large plasmid, **the F′ plasmid.**
- **Low copy number** (1-2 copies per cell)
- The bacterial artificial chromosome's usual insert size is **150-350 kb**.
- BACs are preferred for different kind of genetic studies of inherited or infectious diseases because **they accommodate much larger sequences without the risk of rearrangement**, and are therefore more stable than other types of cloning vectors.


T7 promoter
Sp6 promoter
HindIII
BamHI
NotI
NotI
CosN
Cloning strip
parB
CM^R
BAC
7 kb
F
parA
oriS
repE


Bacterial F' plasmid is cut within lacZ gene with restriction enzymes
Gene of interest is cut out using restriction enzyme
F' plasmid and gene of interest ligate
BAC
BAC is electroporated into E. coli cells
Cells are plated on X-gal/IPTG medium
White colonies are composed of transformed lacZ⁻ cells

P1-Derived Artificial Chromosome (PAC)

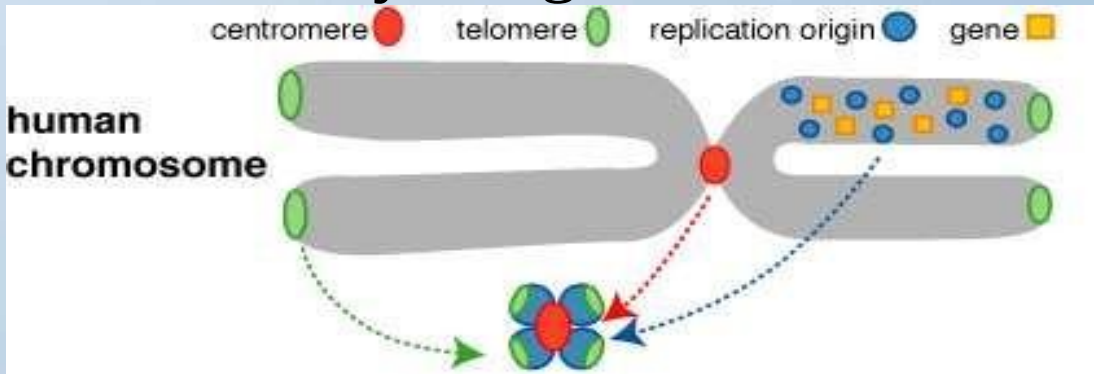
- PAC was developed by Loannou *et al.* (1994). The constructed vector incorporates features of both P1 and F′ and can be transformed into *E.coli* by electroporation. In a PAC vector, inserts of size 100-300 kb can be cloned. It is devoid of problem such as instability of cloned DNA.

Advantages of BACs compared to YACs

- Stable
- Ease to transformation
- Speed of growth of E. coli host
- Simpler to purify
- More user friendly
- They are helpful in the development of vaccines

Human Artificial Chromosome (HAC)

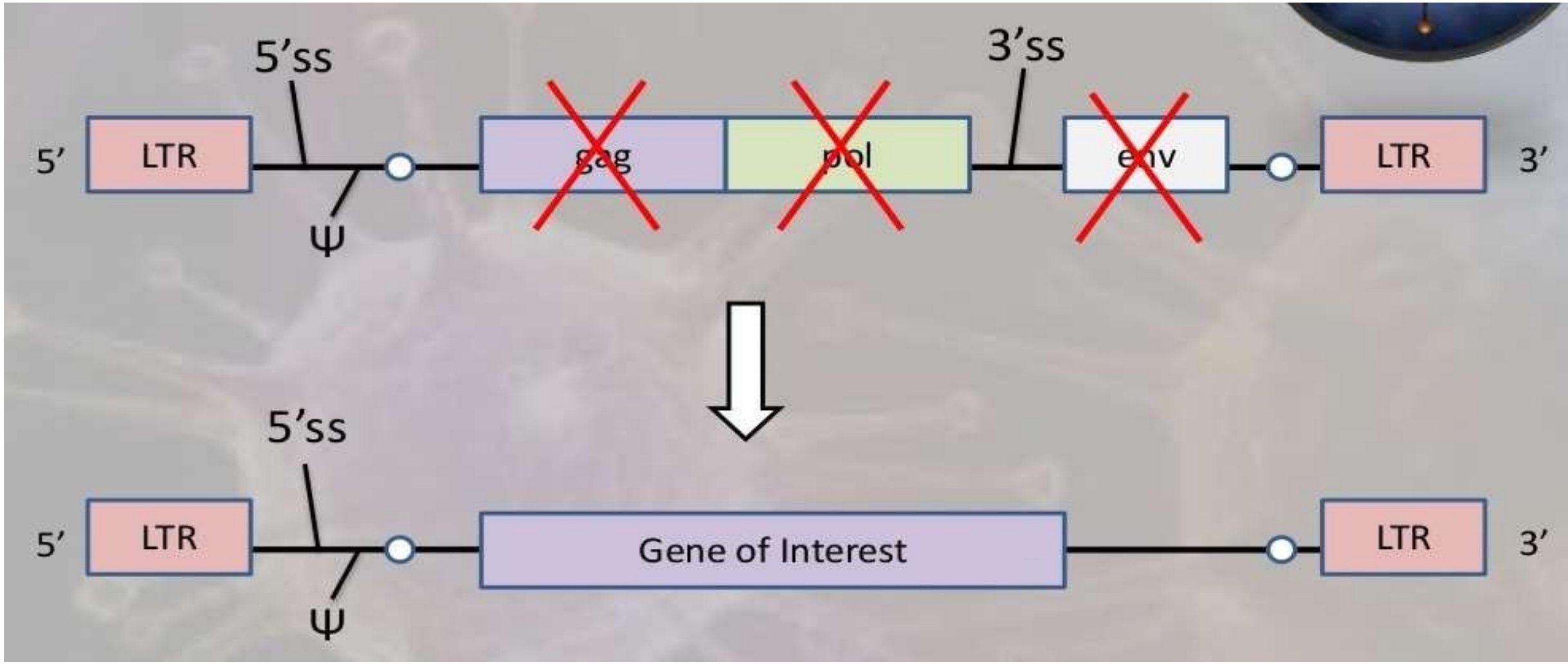
- Human artificial chromosome may be potentially useful as a gene transfer vectors for gene delivery into human cells.
- It is a tool for expression studies and determining human chromosome function.
- It can carry very large DNA fragment (there is no upper limit on size for practical purposes), therefore it does not have the problem of limited cloning capacity of other vectors.
- It also avoids possible insertional mutagenesis caused by integration into host chromosomes by viral vector.



Retroviral Vectors

- Retroviral vectors are used to introduce new or altered genes into the genomes of human and animal cells.
- Retroviruses are RNA viruses.
- The viral RNA is converted into DNA by the viral reverse transcriptase and then is efficiently integrated into the host genome
- Any foreign or mutated host gene introduced into the retroviral genome will be integrated into the host chromosome and can reside there practically indefinitely.
- Retroviral vectors are widely used to study oncogenes and other human genes.

RETROVIRAL VECTORS



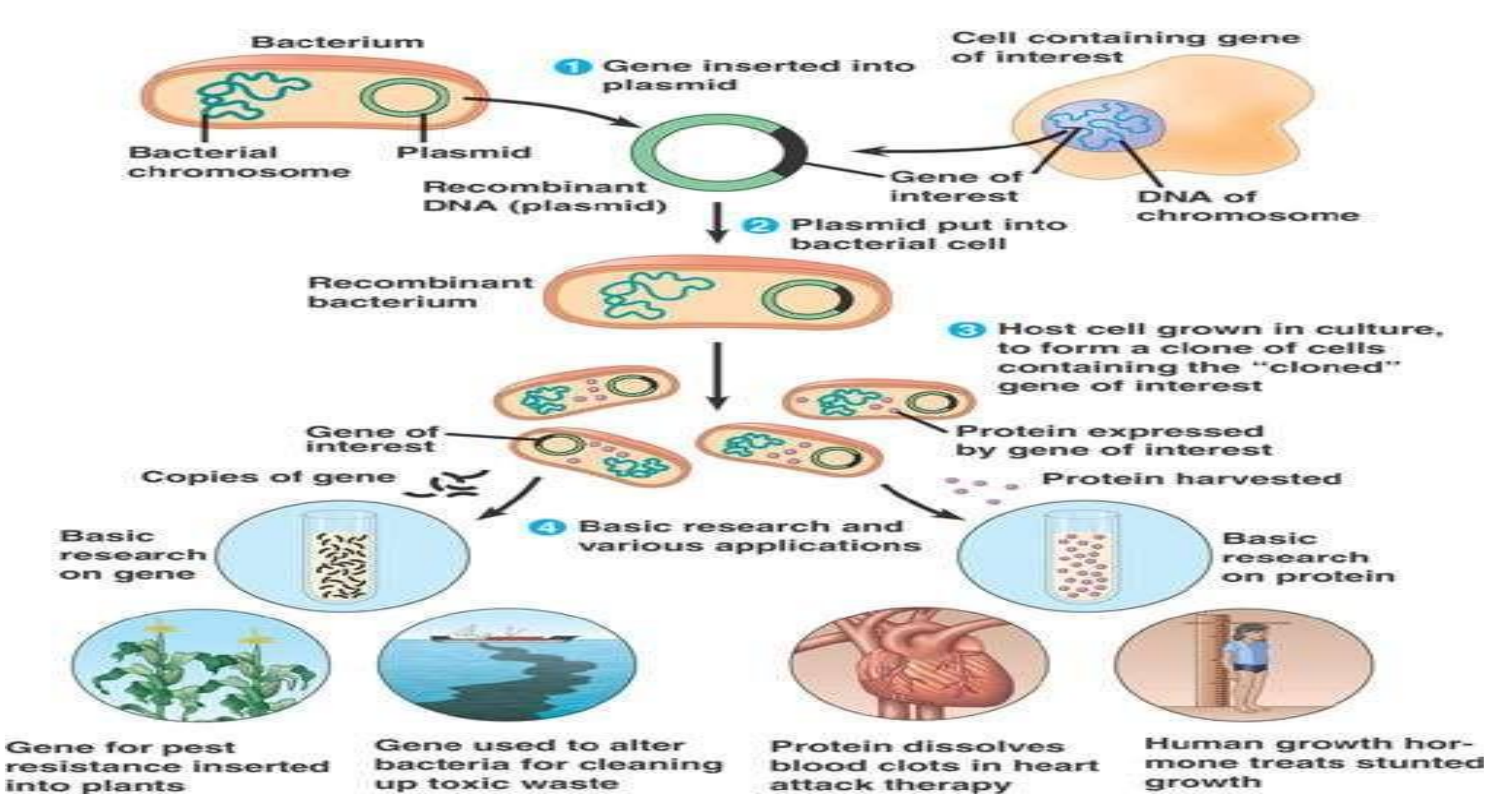
What Determines Choice of Vector?

- Insert size
- Vector size
- Restriction size
- Cloning efficiency

Vector	Insert size (kb)
Plasmid	<10 kb
Bacteriophage	9 – 15 kb
Cosmids	23 – 45 kb
BACs	≤ 300 kb
PACs	100 – 300 kb
YACs	100 – 3000 kb

Vector in Molecular Gene Cloning

- **Prepare the vector** and DNA to be cloned by digestion with restriction enzymes to generate complementary ends.
- Ligate the foreign DNA **into the vector** with the enzyme DNA ligase
- Introduce the DNA into bacterial cells (or yeast cells for YACs) by transformation
- Select cells containing foreign DNA by screening for selectable markers (usually drug resistance).


Bacterium
Bacterial chromosome
Plasmid
1 Gene inserted into plasmid
Recombinant DNA (plasmid)
Cell containing gene of interest
Gene of interest
DNA of chromosome
2 Plasmid put into bacterial cell
Recombinant bacterium
3 Host cell grown in culture, to form a clone of cells containing the "cloned" gene of interest
Gene of interest
Protein expressed by gene of interest
Copies of gene
Protein harvested
4 Basic research and various applications
Basic research on gene
Basic research on protein
Gene for pest resistance inserted into plants
Gene used to alter bacteria for cleaning up toxic waste
Protein dissolves blood clots in heart attack therapy
Human growth hormone treats stunted growth

References

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- https://www.neb.com/tools-and-resources/feature-articles/foundations-of-molecular-cloning-past-present-and-future
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- *www.uenf.br/cbb/lbt/files/.../Cloning-vectors.pdf*
- *http://yoanx7.blogspot.com/2013/05/dna-cloning-and-its-applications-preview.html*
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- *https://www.ndsu.edu/pubweb/~mcclean/plsc431/cloning/clone3.htm*
- *http://www.chemistrylearning.com/cloning-vector/*

Thank You!!!


ANY
questions?