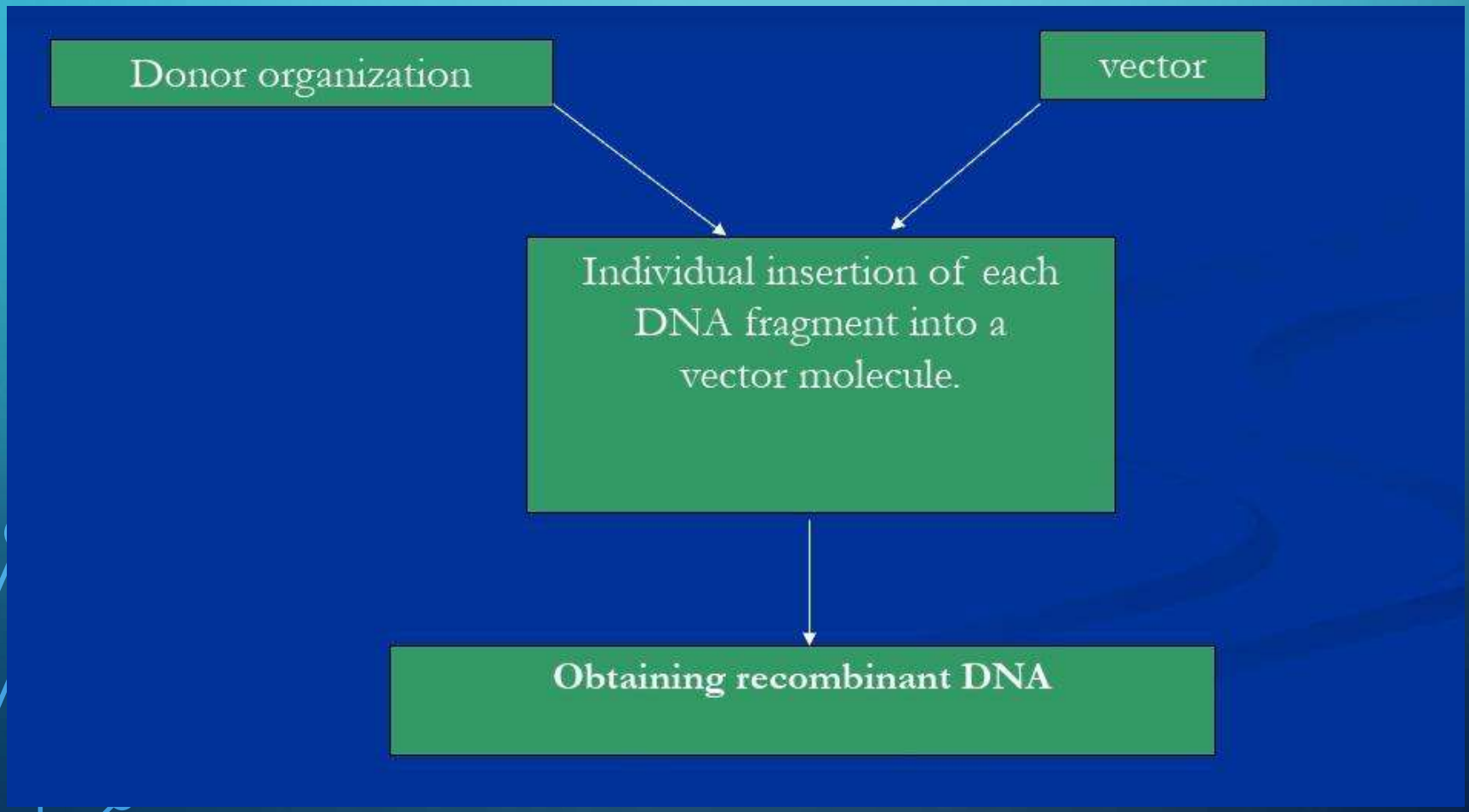
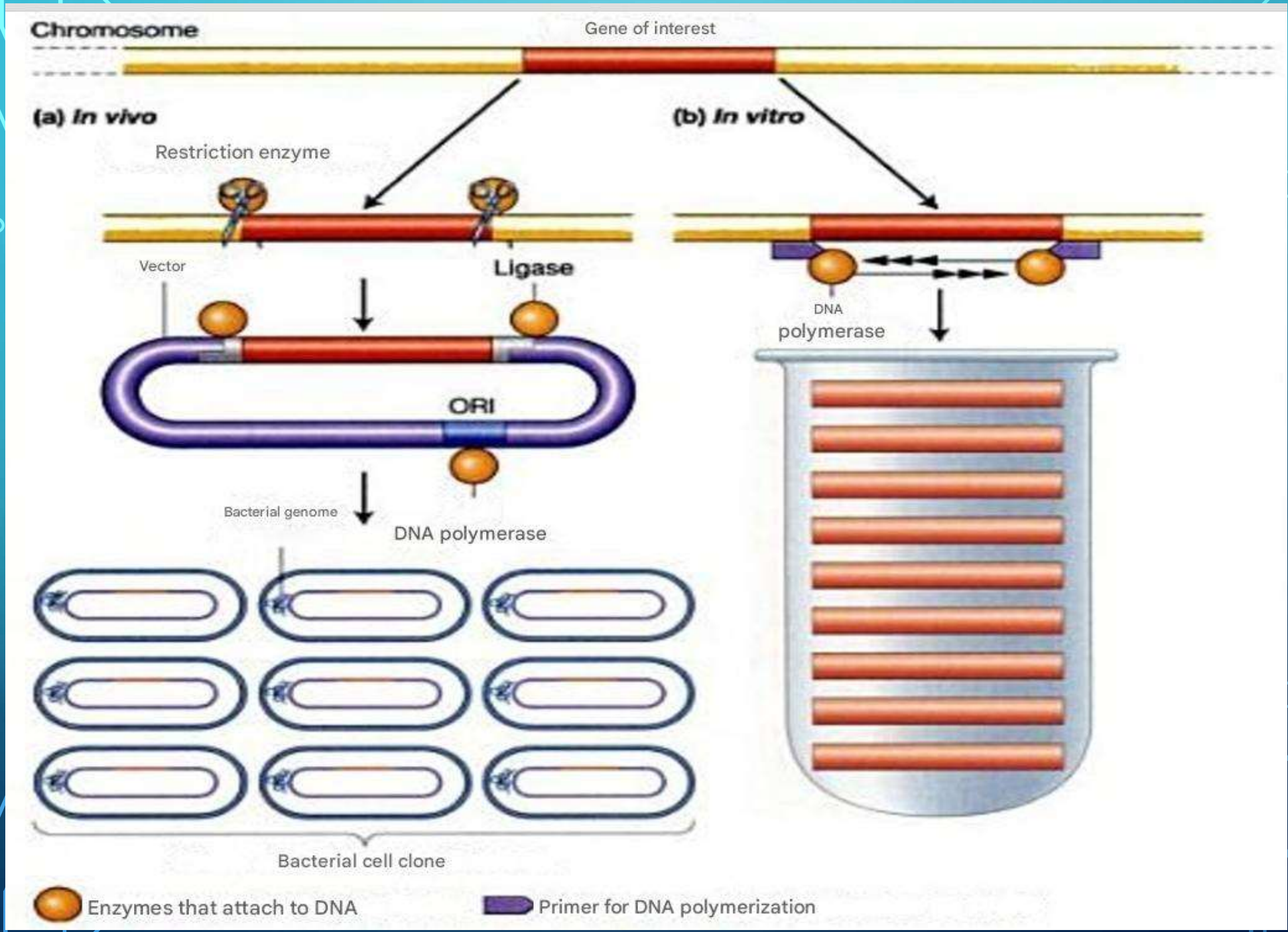
IV. GENETIC ENGINEERING

1. Cloning: Cloning is the action of isolating and inserting into a vector a fragment of DNA of interest to multiply it identically.




Chromosome
Gene of interest
(a) In vivo
(b) In vitro
Restriction enzyme
Vector
Ligase
ORI
Bacterial genome
DNA polymerase
DNA
polymerase
Bacterial cell clone
Enzymes that attach to DNA
Primer for DNA polymerization

- **Donor DNA Types:**

-Genomic DNA: chromosomal (cutting of gene of interest);

- cDNA

-DNA obtained by chemical synthesis

- **Restriction enzymes:**

- Restriction enzymes are of bacterial origin. They have the particularity of cutting double-stranded DNA molecules at specific sites in the sequence: they are endonucleases .

-The name of restriction enzymes comes from the genus and species name of the bacteria from which they were isolated.

There are three types of restriction enzymes:

-Types I and III: complex proteins that cut double-stranded DNA outside their recognition site.

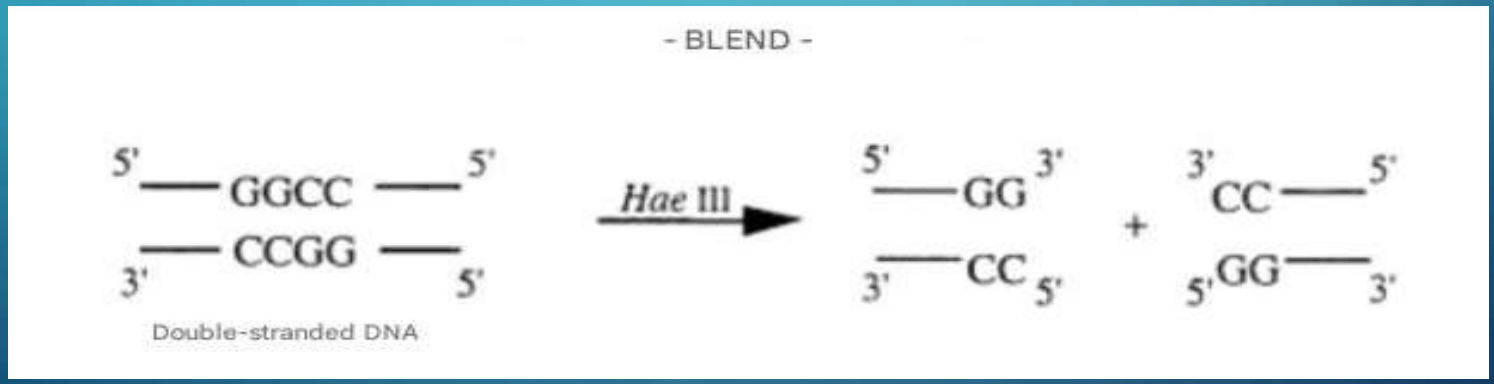
-Type II: are the essential tools for genetic engineering. They recognize a specific sequence of 4, 6 or 8 bp and cut within this sequence, called a restriction site.

Restriction enzymes cut in the sequence in two ways:

-clean cut: clean ends are obtained by cutting at the same place on both strands.

Example:

Hae III enzyme isolated from *Haemophilus aegyptius* cuts double-stranded DNA at the GG/CC sequence

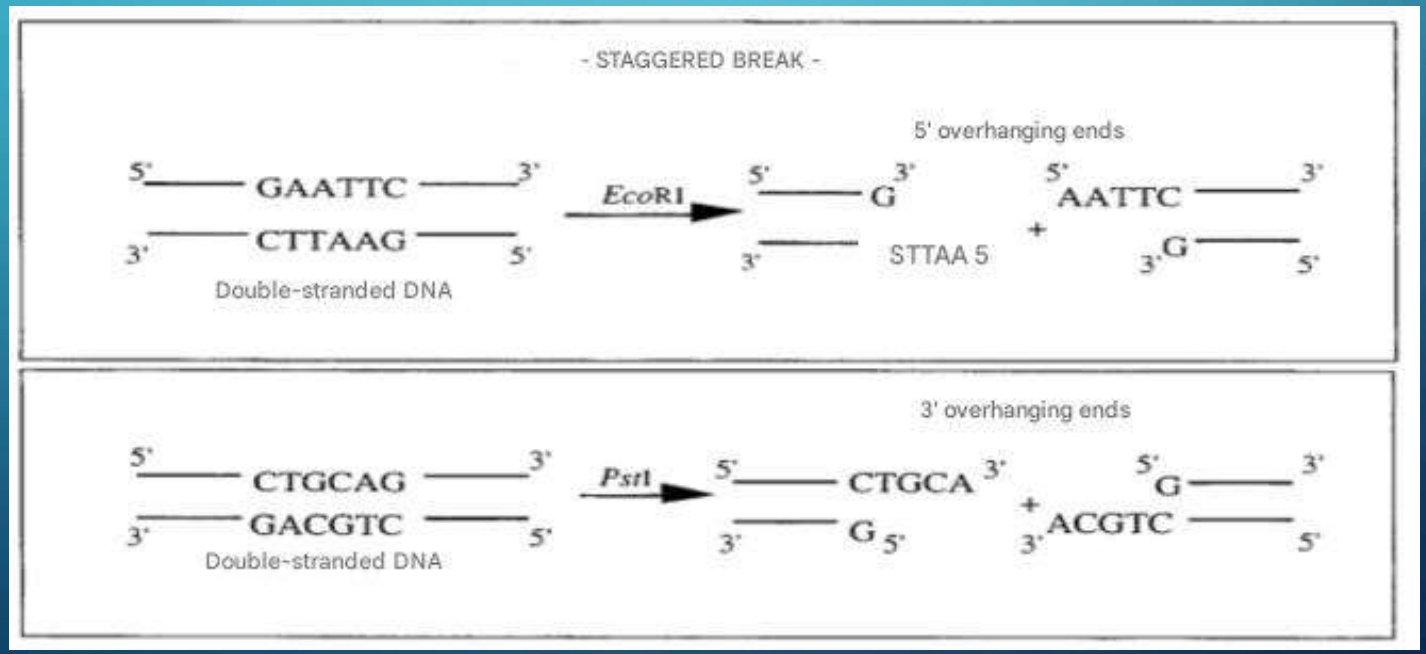


-Staggered cutting on both strands: generates single-stranded ends with complementary sequences called cohesive ends or sticky ends.

Example:

-*EcoR* I enzyme isolated from *Escherichia coli:* cuts double-stranded DNA in the palindromic sequence G/AATTC.

Pst I enzyme isolated from *Providencia stuarti* cuts double-stranded DNA in the palindromic sequence CTGCA/G



- **DNA ligases:**

-Catalyze the formation of a phosphodiester bond between two DNA segments.

-The formation of the phosphodiester bond requires a cofactor: ATP.

-DNA ligase is used in particular to suture DNA fragments produced by restriction enzymes.

Preparation of plasmids

-Bacteria containing plasmids are cultured (up to 2 liters).

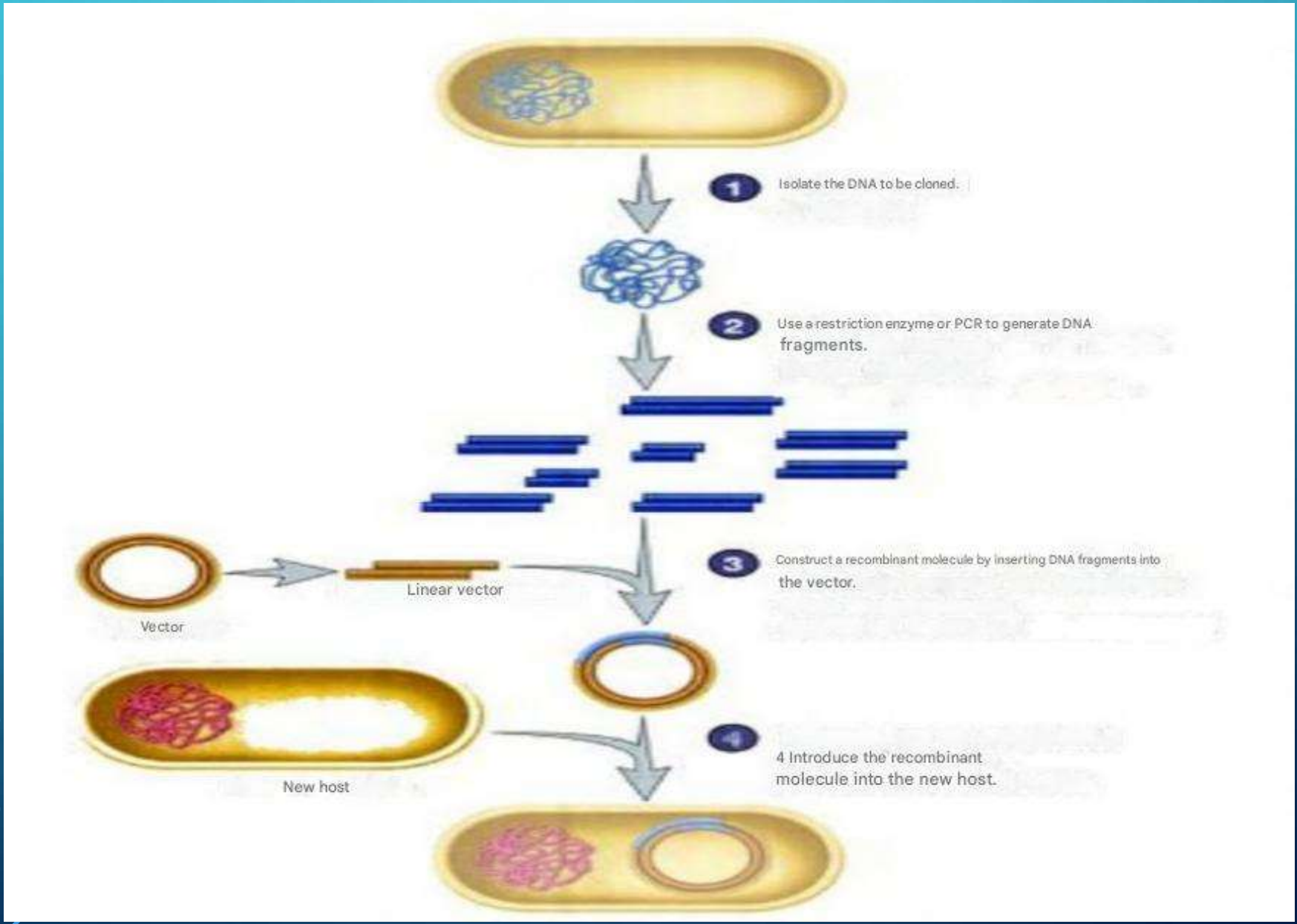
-When the bacterial concentration reaches a sufficient value, treatment is carried out with an antibiotic.

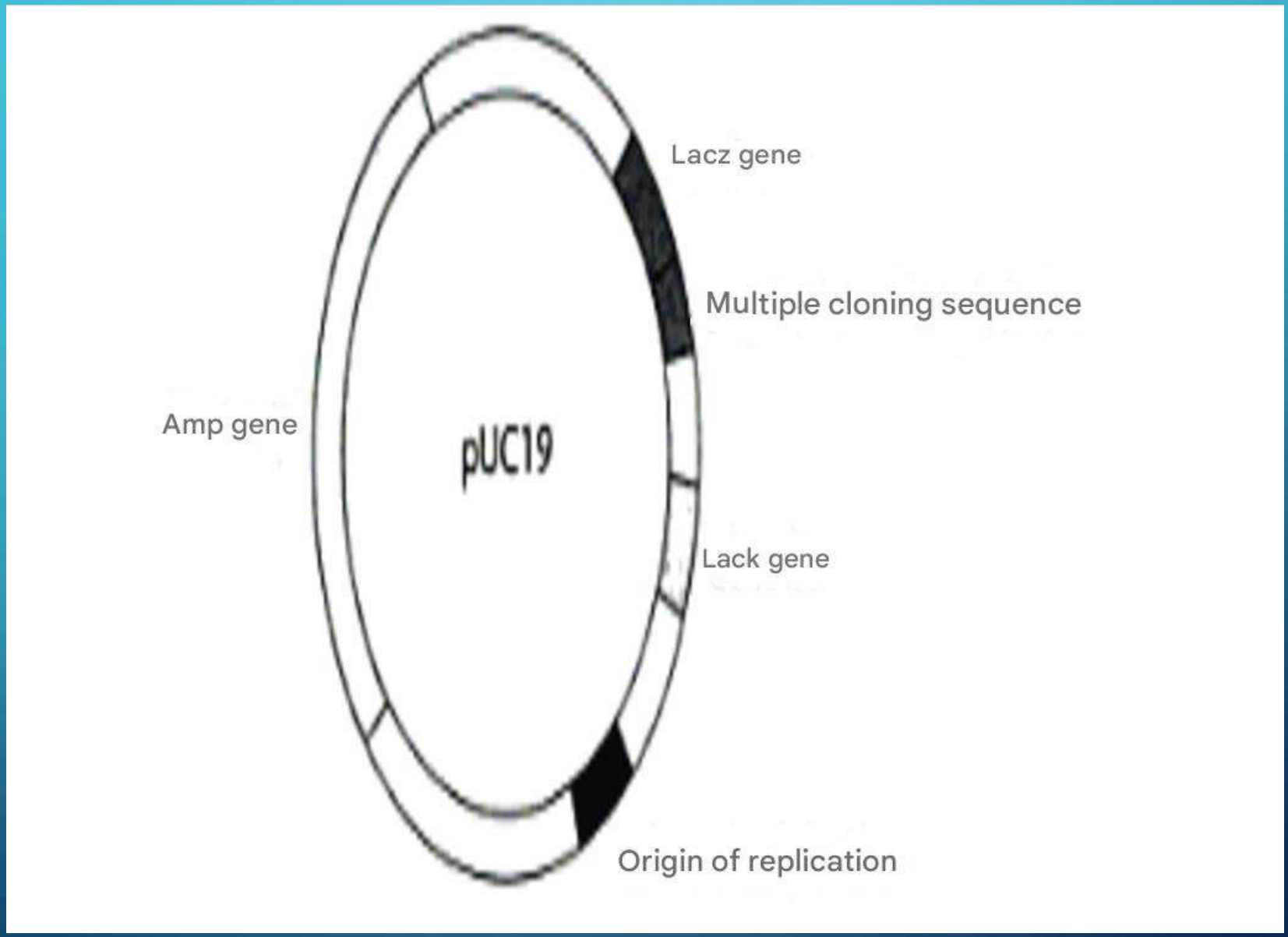
-The bacteria are recovered by centrifugation.

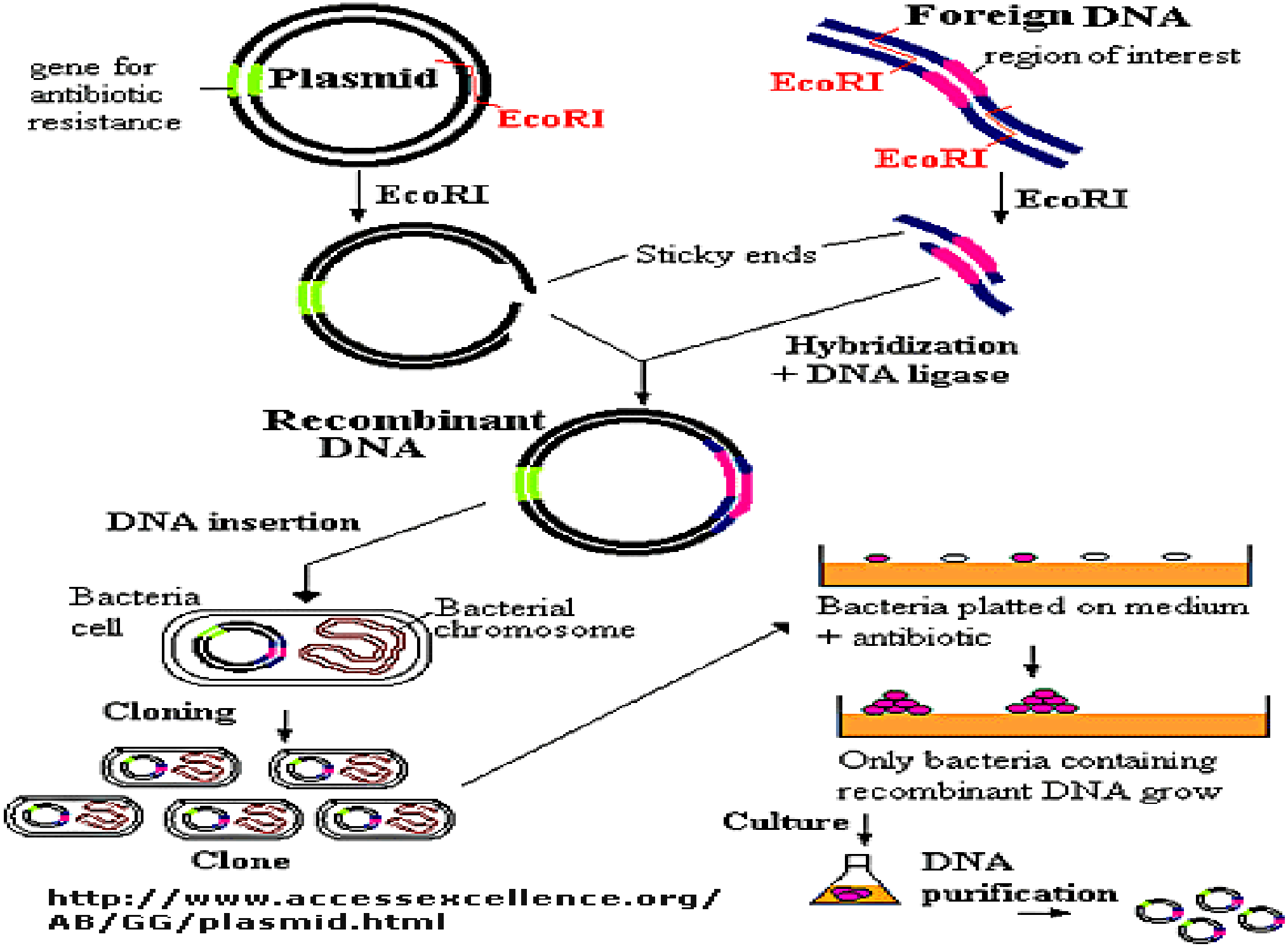
-The bacterial wall is permeabilized by a gentle treatment allowing the plasmids to pass into solution.

-Plasmid purification can then be carried out by HPLC or density gradient ultracentrifugation.

Recombination, Hybrid Constitution and Bacterial Transformation:




Lacz gene
Multiple cloning sequence
Amp gene
pUC19
Lack gene
Origin of replication



http://www.accessexcellence.org/AB/GG/plasmid.html

Cloning into a plasmid

Bacteriophages:

-bacteriophage λ : linear double-stranded DNA (50kb), has cohesive ends (cos sequence) allowing it to circularize in the infected bacterium.

-In practice, phages are used as vectors for constructing cDNA or genomic libraries: the lambda -GEM®-11 phage proposed by the biotechnology company Promega .

-This phage accepts DNA fragments from 9 kb to 23 kb. It has promoters for T7 RNA polymerase (extracted from *E. coli*) and SP6 RNA polymerase (extracted from *Salmonella typhimurium)* . Which allow the synthesis of RNA probes from one end of the cloned fragment.

- **Benefits:**
- The size of insertable DNA fragments is larger than that of plasmids (40-50 kb).
- Transformation of bacteria is more efficient than for plasmids.
- **Disadvantages:**
- Number of restricted restriction sites in the phage genome.
- Obligation to package DNA.
- Size constraints for DNA to be inserted

➢ Cosmids:

-hybrid artificial vectors: lambda phage-plasmids.

-They contain a gene for resistance to antibiotics (ampicillin).

-Additionally, a cos site from a lambda virus was included in their circular DNA which will allow the cosmid to be packaged into the head of a lambda virus.

Advantages and disadvantages of cosmids

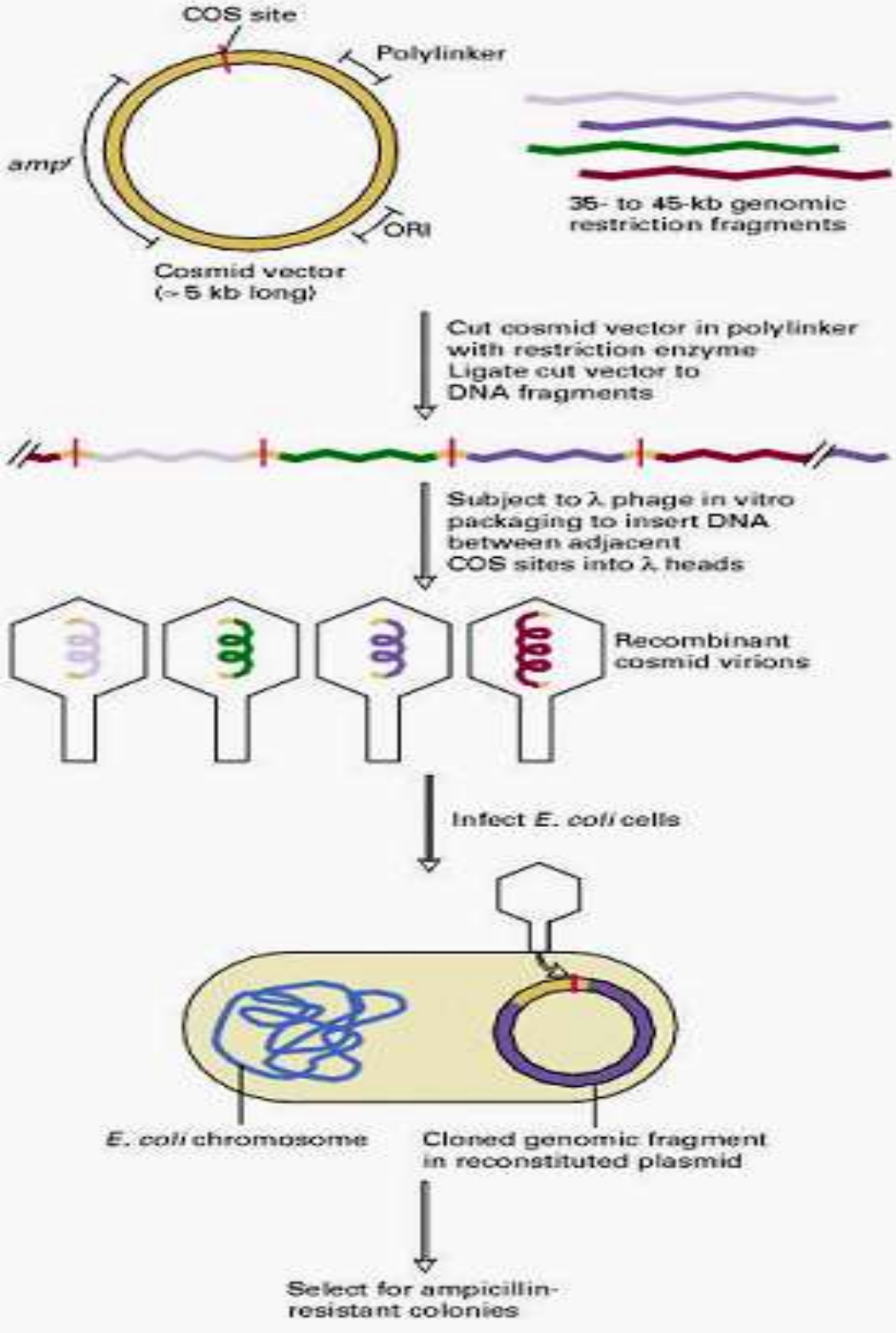
Benefits:

The size of insertable fragments can reach 50 kb.

Their incorporation into bacteria (transformation) is more efficient than for plasmids.

Disadvantages:

Obligation to package DNA.


COS site
Polylinker
amp^r
ORI
Cosmid vector
(~ 5 kb long)
35- to 45-kb genomic
restriction fragments
Cut cosmid vector in polylinker
with restriction enzyme
Ligate cut vector to
DNA fragments
Subject to λ phage in vitro
packaging to insert DNA
between adjacent
COS sites into λ heads
Recombinant
cosmid virions
Infect E. coli cells
E. coli chromosome
Cloned genomic fragment
in reconstituted plasmid
Select for ampicillin-
resistant colonies

- Example of protein production by *E. coli* and *Saccharomyces cerevisiae* :
- *Escherichia coli* : It was and still is the first host used for the production of recombinant proteins. Examples of recombinant proteins produced: human growth hormone, insulin, interferon α and interleukin-2.
- *Saccharomyces cerevisiae* : This is baker's yeast, which has been used for thousands of years in human nutrition. Examples of recombinant proteins produced: hepatitis B virus surface antigen, insulin, etc.

A genomic DNA library is a set of recombinant vectors, each containing a different piece of the genome of the species being studied.

A cDNA library is considered a snapshot of the mRNA populations represented in an organ

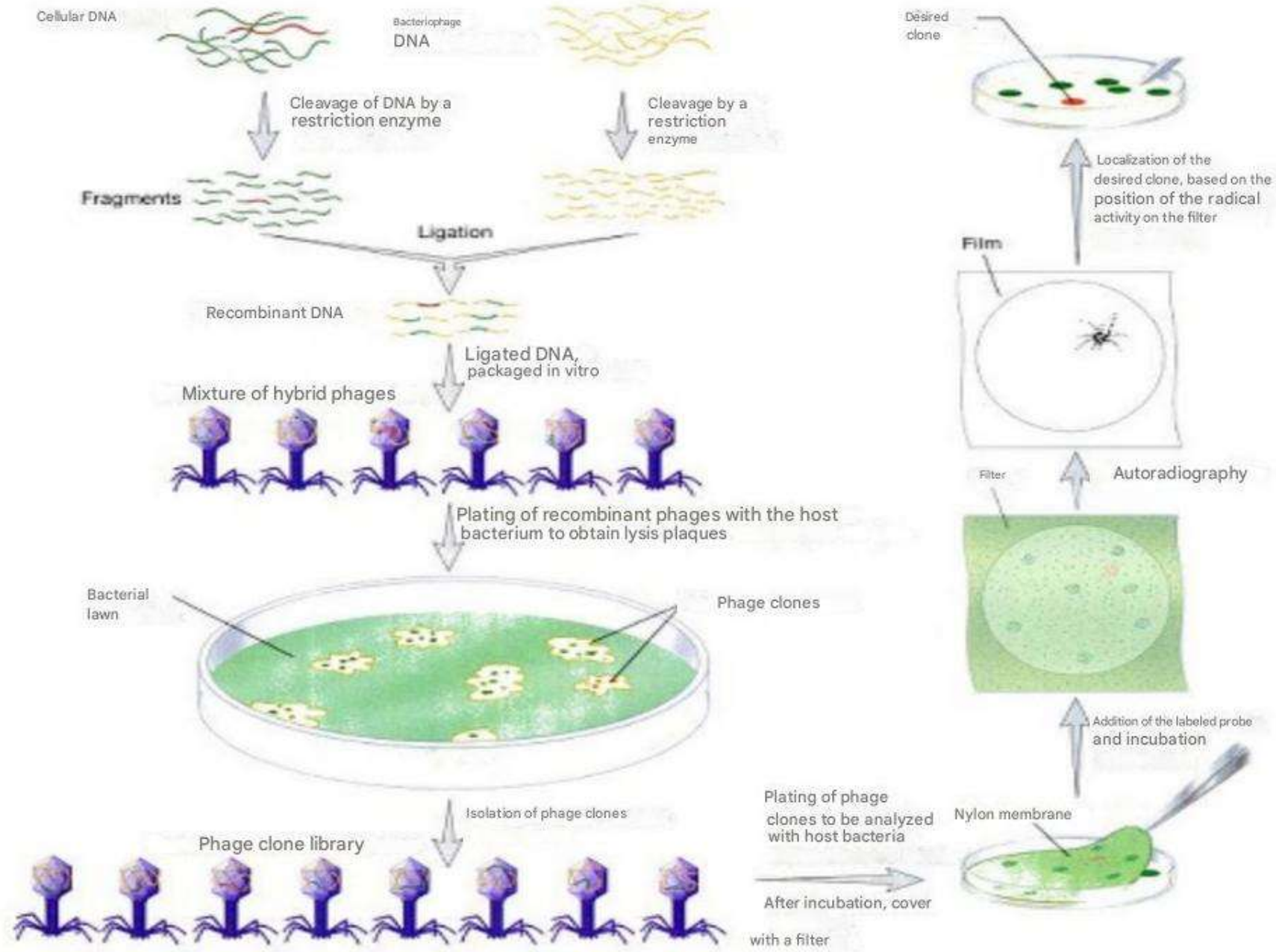


Figure Use of lambda phage as a vector. (a) Preparation of a genomic library. Each lysis plaque in the bacterium contains a recombinant clone carrying a different DNA fragment. (b) Detection and cloning of the desired recombinant phage.