COMPETENT CELLS FORMATION & TRANSFORMATION OF COMPETENT CELLS WITH RECOMBINANT PLASMID DNA

BCH462- Practical

Molecular cloning:

- Cell-based technique used to create copies of certain DNA fragments using a vector carrying the DNA.
- Another method ?
- It is an important tool to understand the structure, function and regulation of individual genes and their products.

Isolated and amplified specific DNA fragment
"e.g.: gene A"

The cell-based DNA cloning steps involve:

1

DNA fragment Cloning vector Recombinant DNA

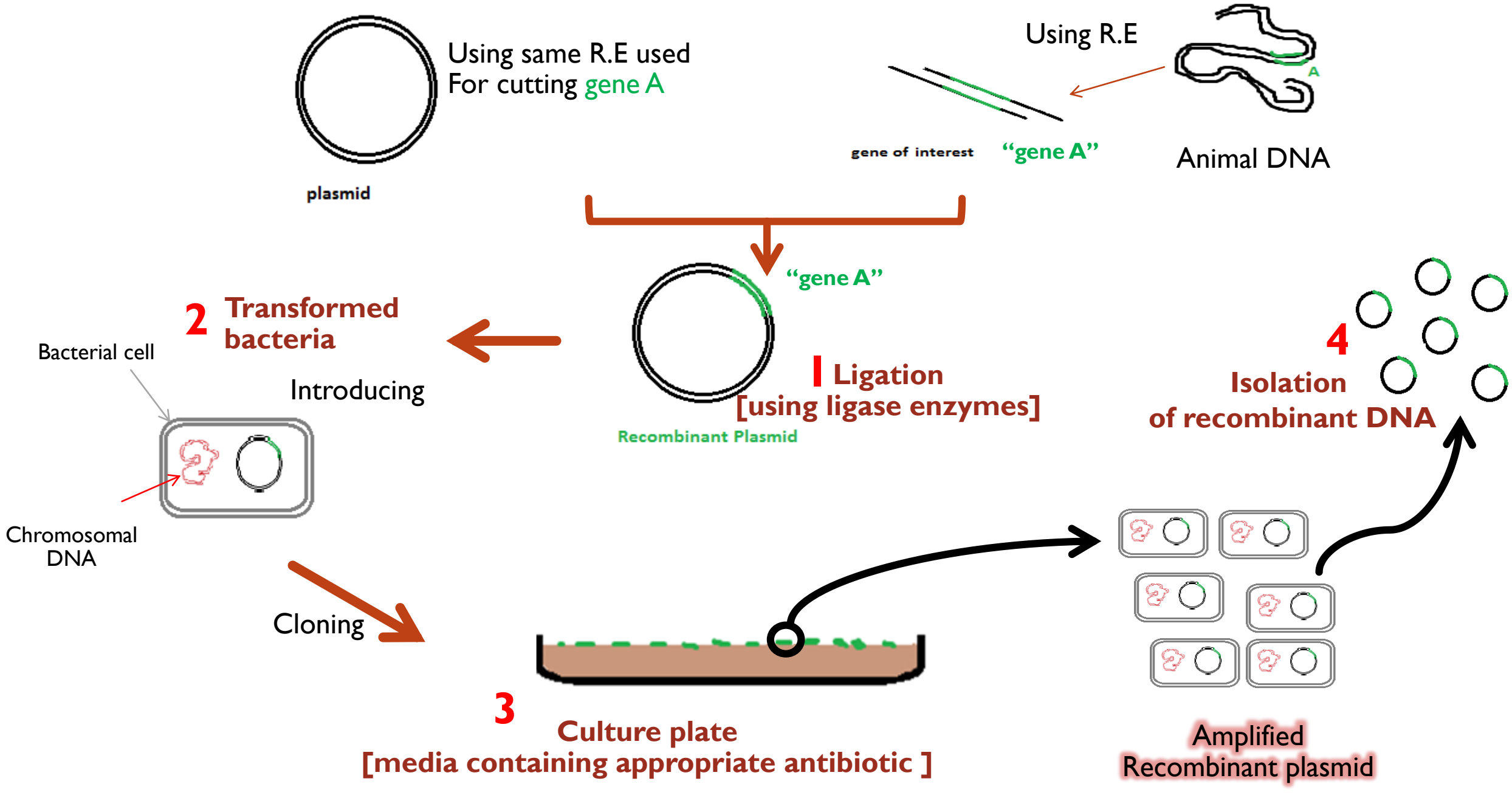
2

Recombinant DNA the host **Transformed** bacteria

3

Self-replicate

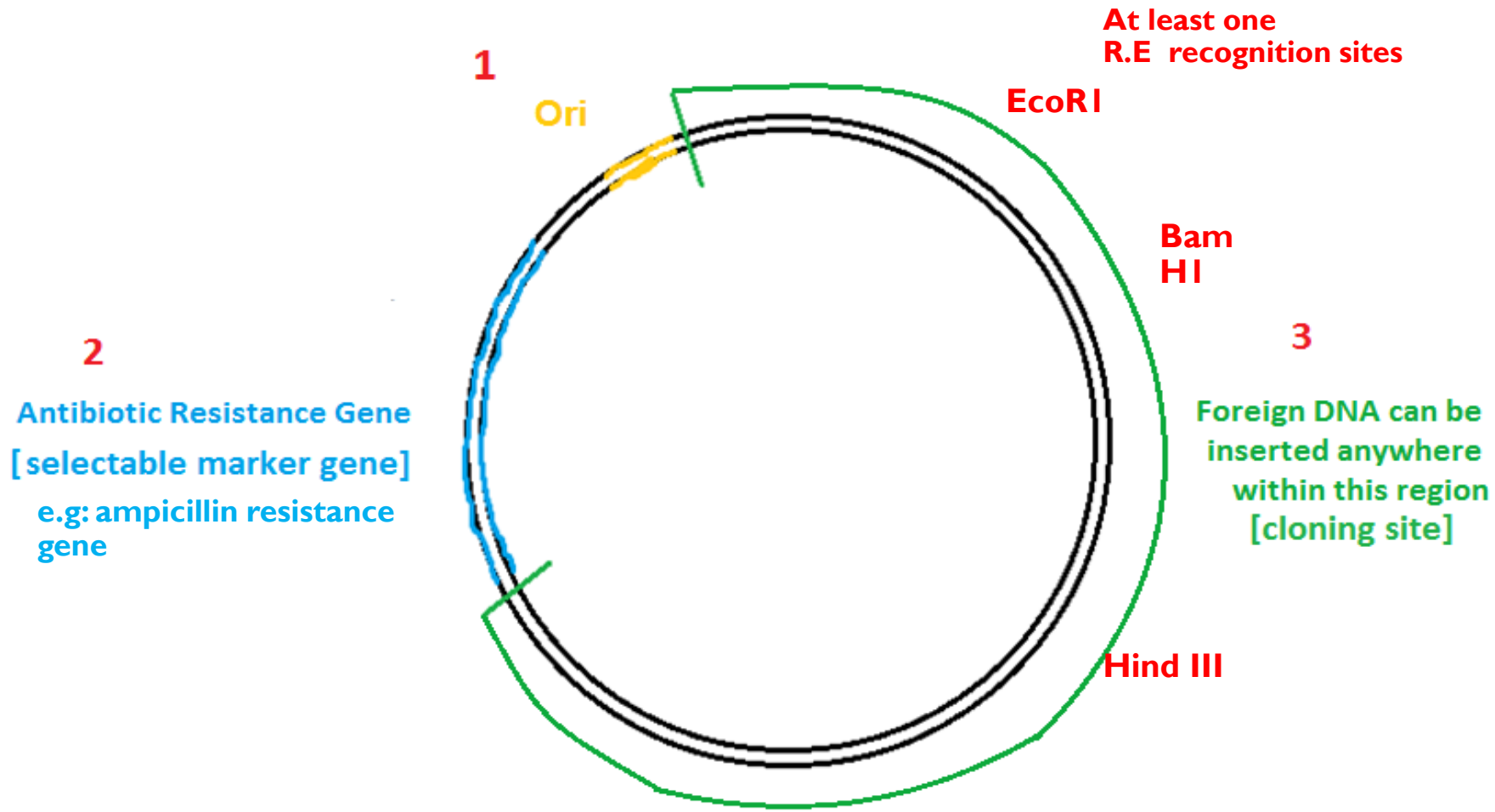
DNA cloning using plasmid



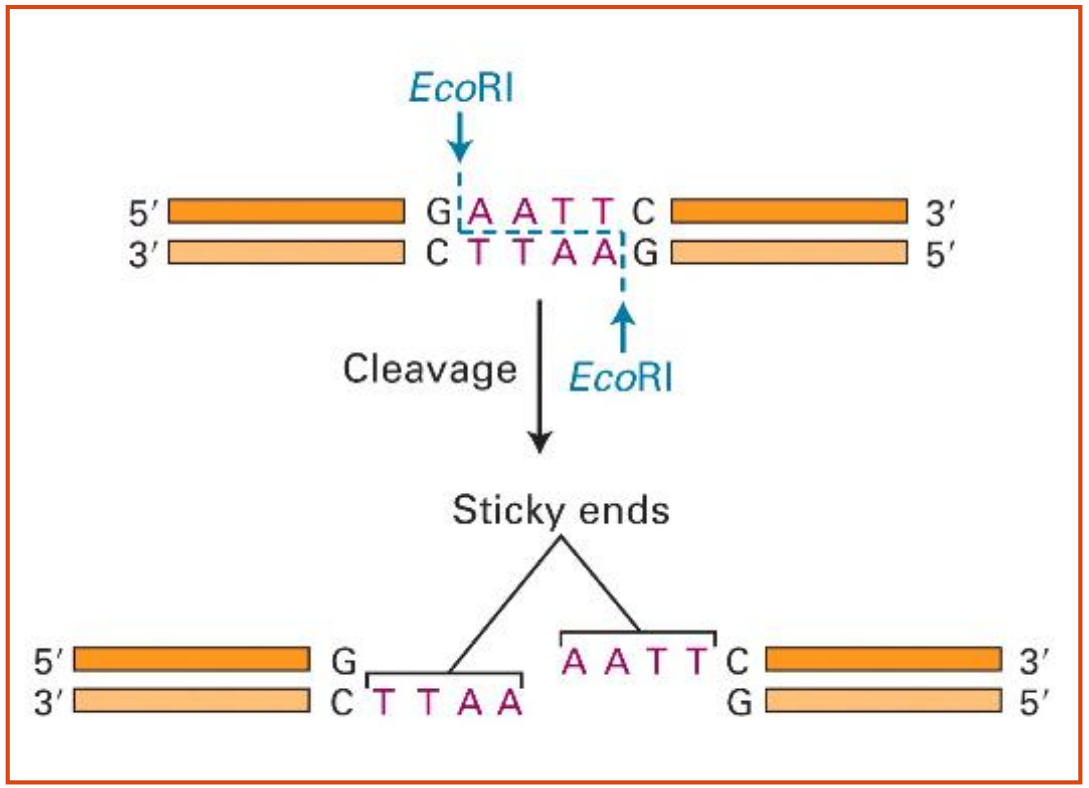
Cloning vectors:

- What is it ?
- Features.
- Types?

Plasmids vectors contain three important parts:

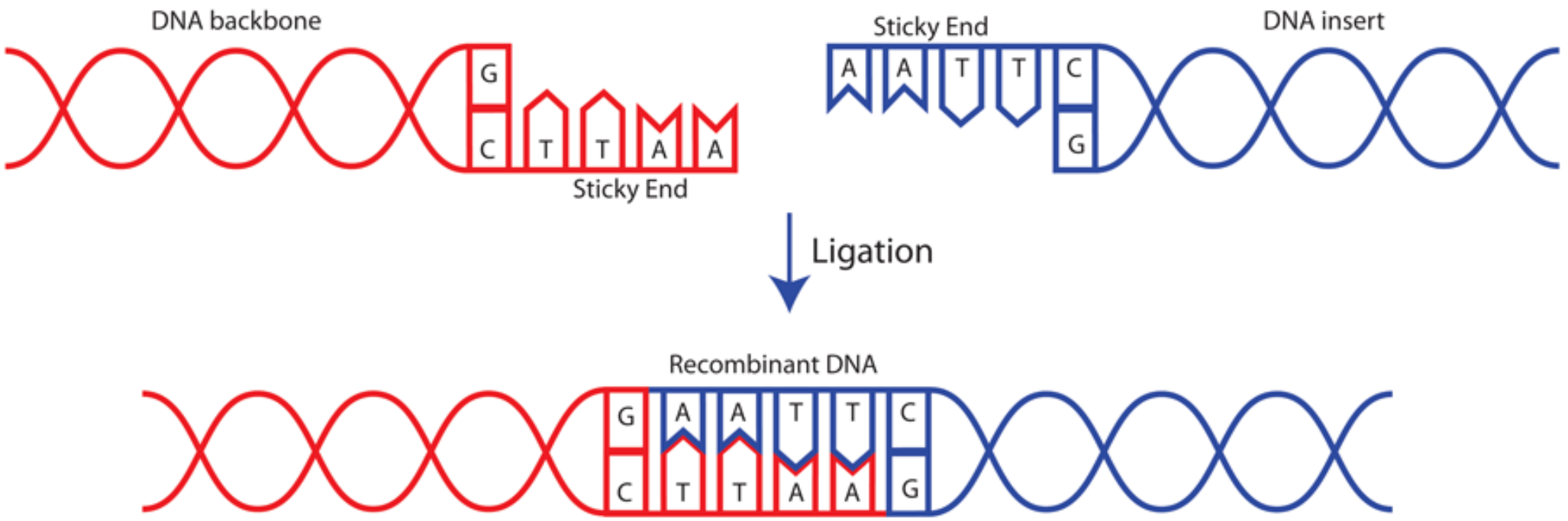


Restriction enzymes:

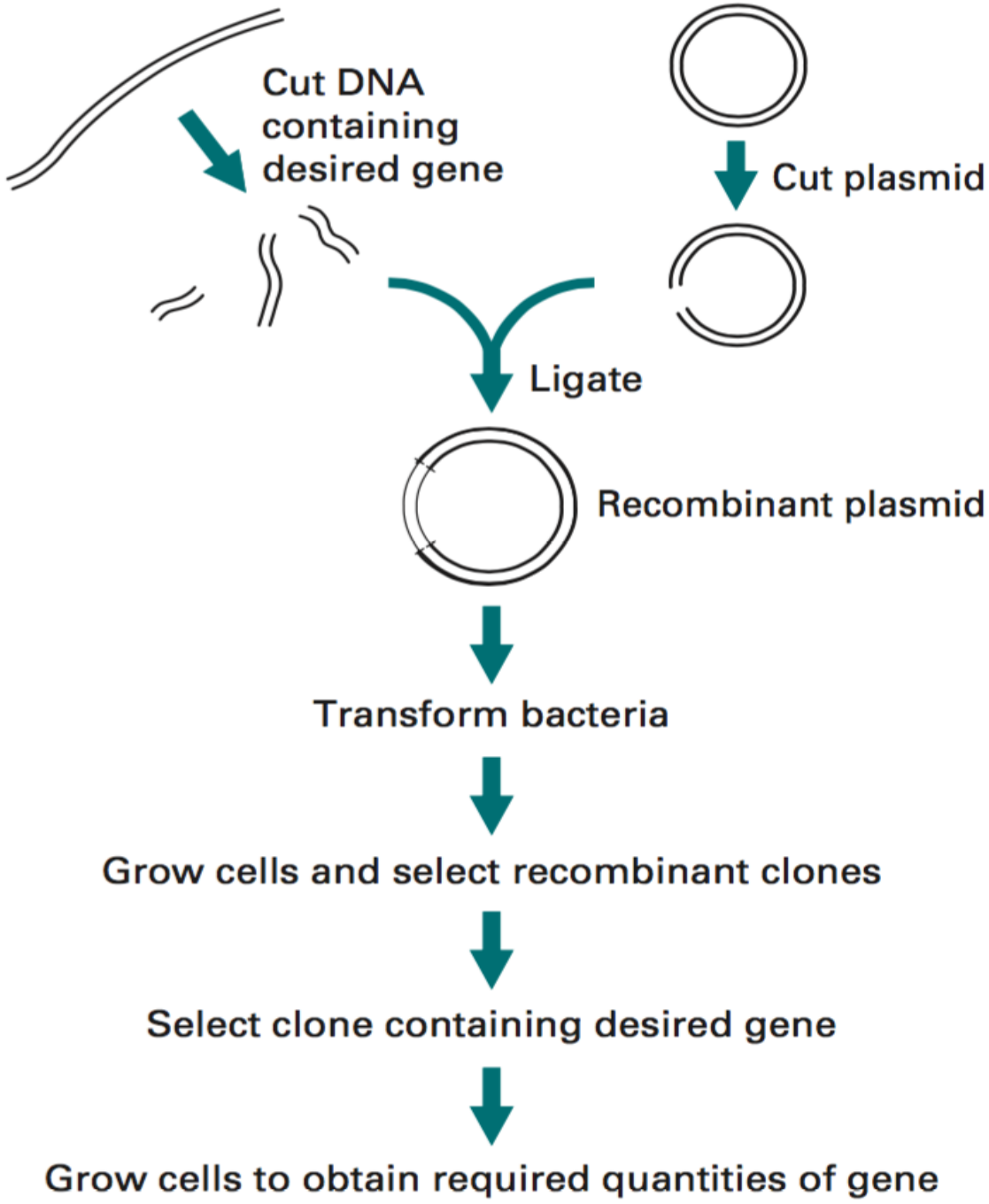


Restriction endonucleases.

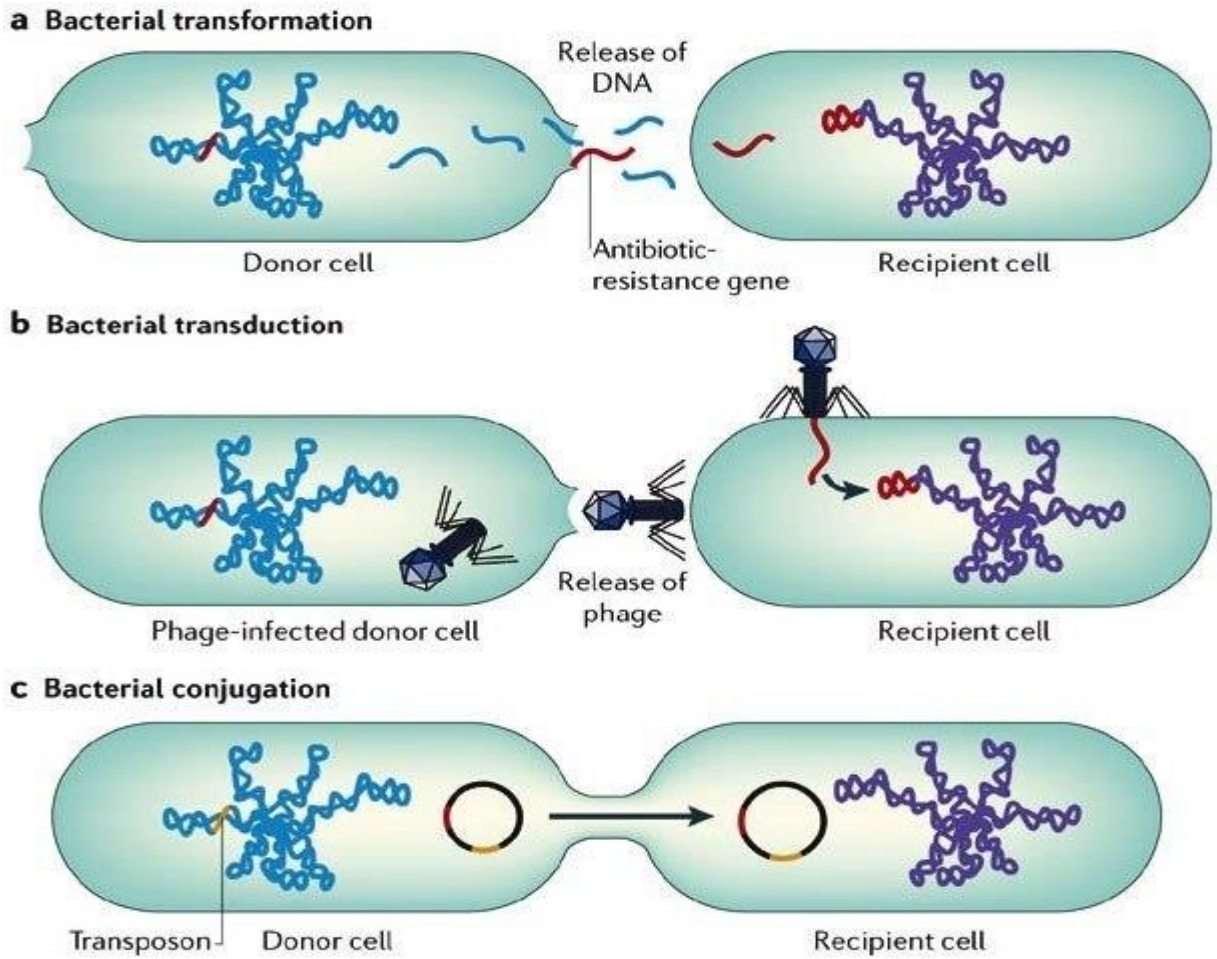
DNA ligase:


DNA backbone
G
C
T
T
A
A
Sticky End
Sticky End
DNA insert
A
A
T
T
C
G
Ligation
Recombinant DNA
G
A
A
T
T
C
C
T
T
A
A
G

Outline of gene cloning:



Bacteria in general can acquire new genetic information by:



Competence:

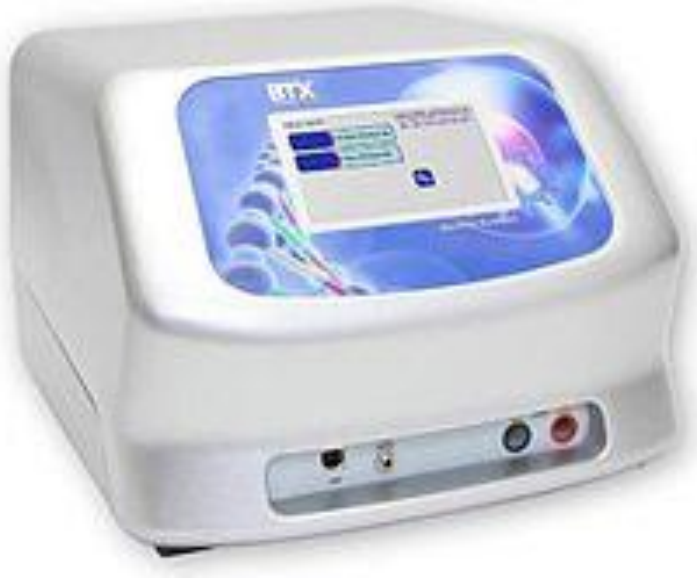
- For a bacterial cell to take up DNA from its surroundings, it must be in a special physiological state called competence.
- It defines as the ability of the cell to undergo transformation (the ability of a cell to take the DNA from the environment).
- Two classes of competence.

Transformation:

- It is the introducing of the recombinant DNA into bacterial cells.
- Recombinant DNA technology was developed as gene cloning to be its major application.

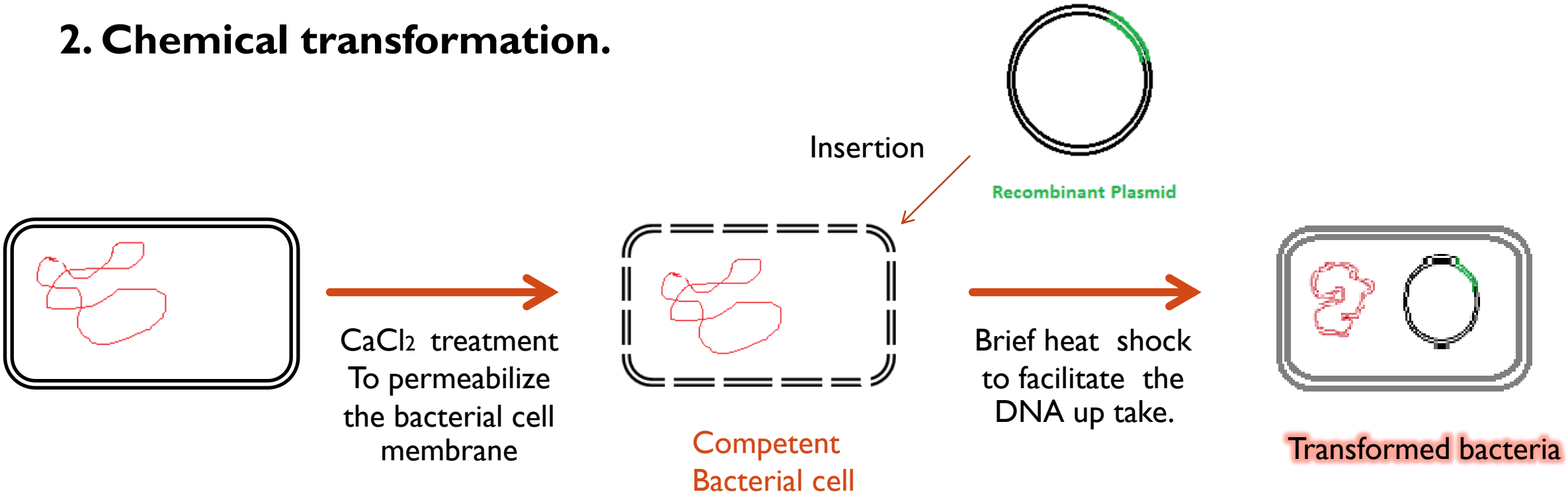
Method of transformation:

1. Electroporation, or Electropermeabilization.



Method of transformation cont':

2. Chemical transformation.



Transformation efficiency:

- It is a quantitative value that describes how effective you were at getting plasmid DNA into your competent cells.
- The number represents how many cells were transformed per microgram (µg) of plasmid DNA used.
- This calculation requires two values: the number of cells that were successfully transformed and the amount of plasmid DNA used for the transformation.

$$\text{Transformation efficiency} = \frac{\text{Total number of colonies on LB/Amp plate}}{\text{Amount of DNA plated [µg/ml]}}$$

$$= \_\_\_\_\_ \text{ CFU/µg}$$

* CFU/µg = colony-forming units per microgram of transforming DNA.

PRACTICAL PART

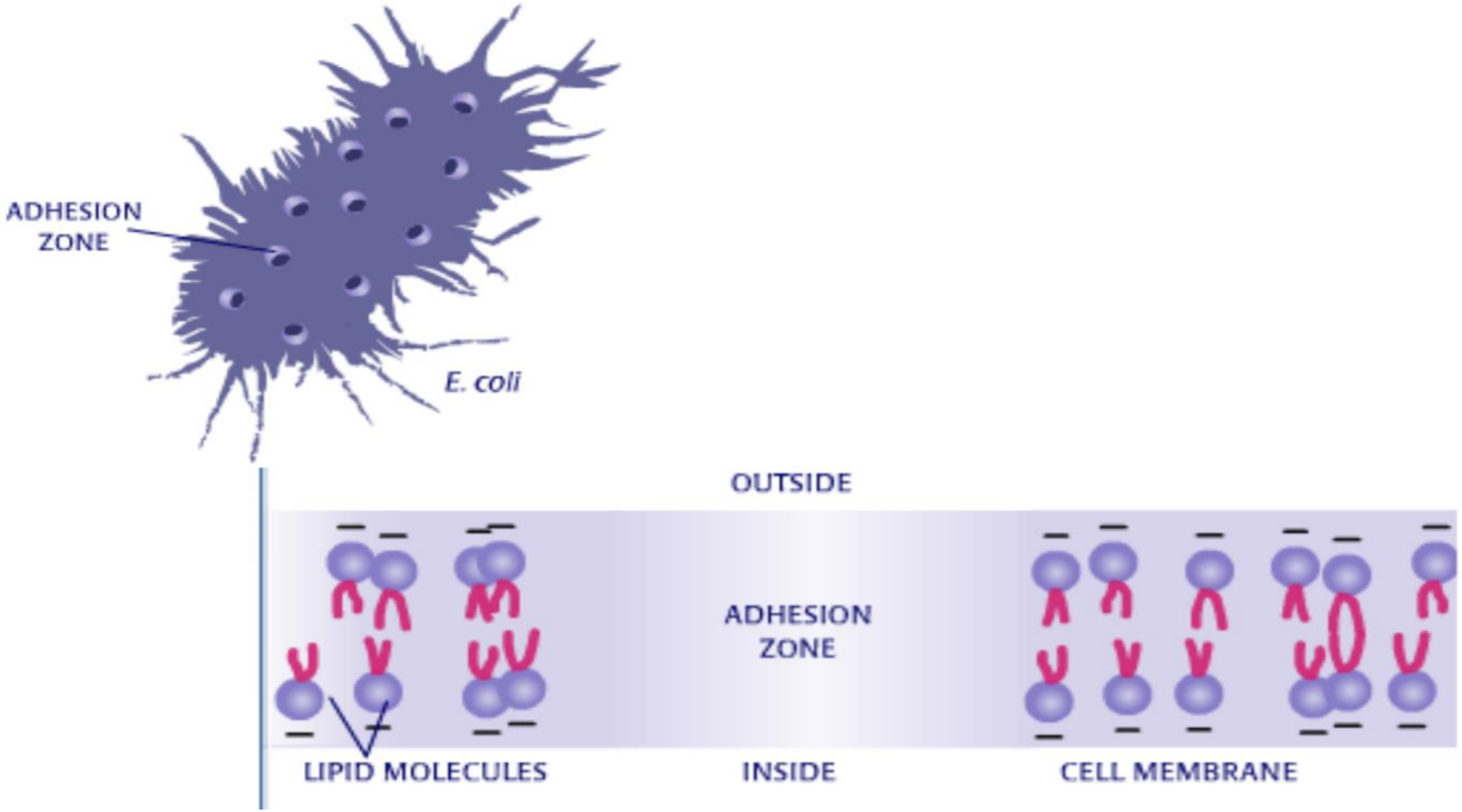
Aims:

- Making a competent cells using calcium chloride method.
- Transformation of the competent cells with recombinant plasmid DNA using chemical transformation method.

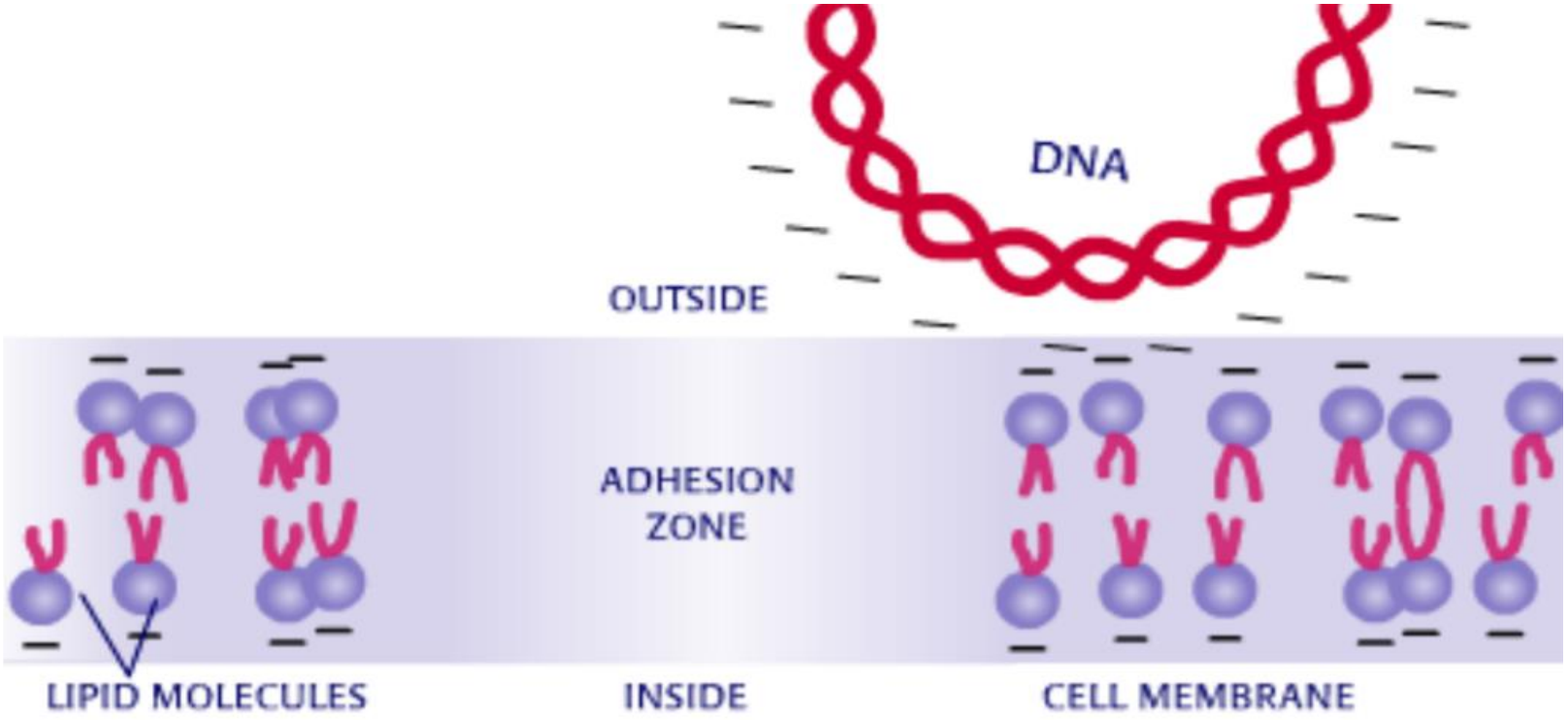
Principle:

- Introduction of recombinant plasmid into cells is achieved by the transformation of competent cells.
- In the chemical transformation method the competent cells are prepared by treating the cell with a divalent cation like calcium chloride solution ➔ Increasing the bacterial cells membranes permeability [renders them competent to take up DNA].
- Once the cells are made competent, the recombinant plasmid DNA is mixed with the cells.
- The competent cells are then subjected to heat shock, which allows the DNA to enter the cells.
- The cells are then plated onto a LB agar plate containing appropriate antibiotic to be able to count the transformed colonies only (which they are colonies containing transformed cells containing the recombinant DNA), each colony on an antibiotic plate presents a single transformation event.
- The recombinant plasmid can be amplified as well.

Chemical transformation principle:



Chemical transformation principle:



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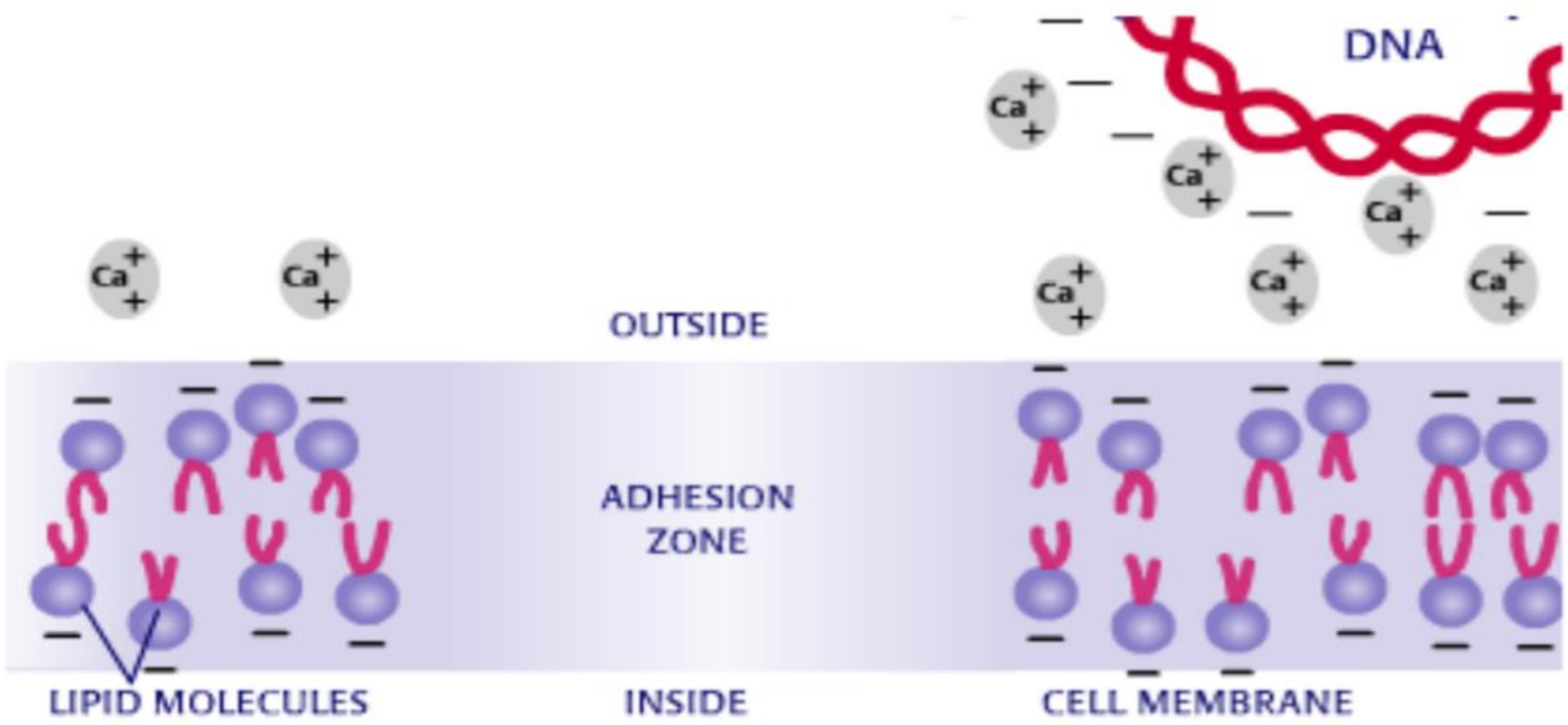
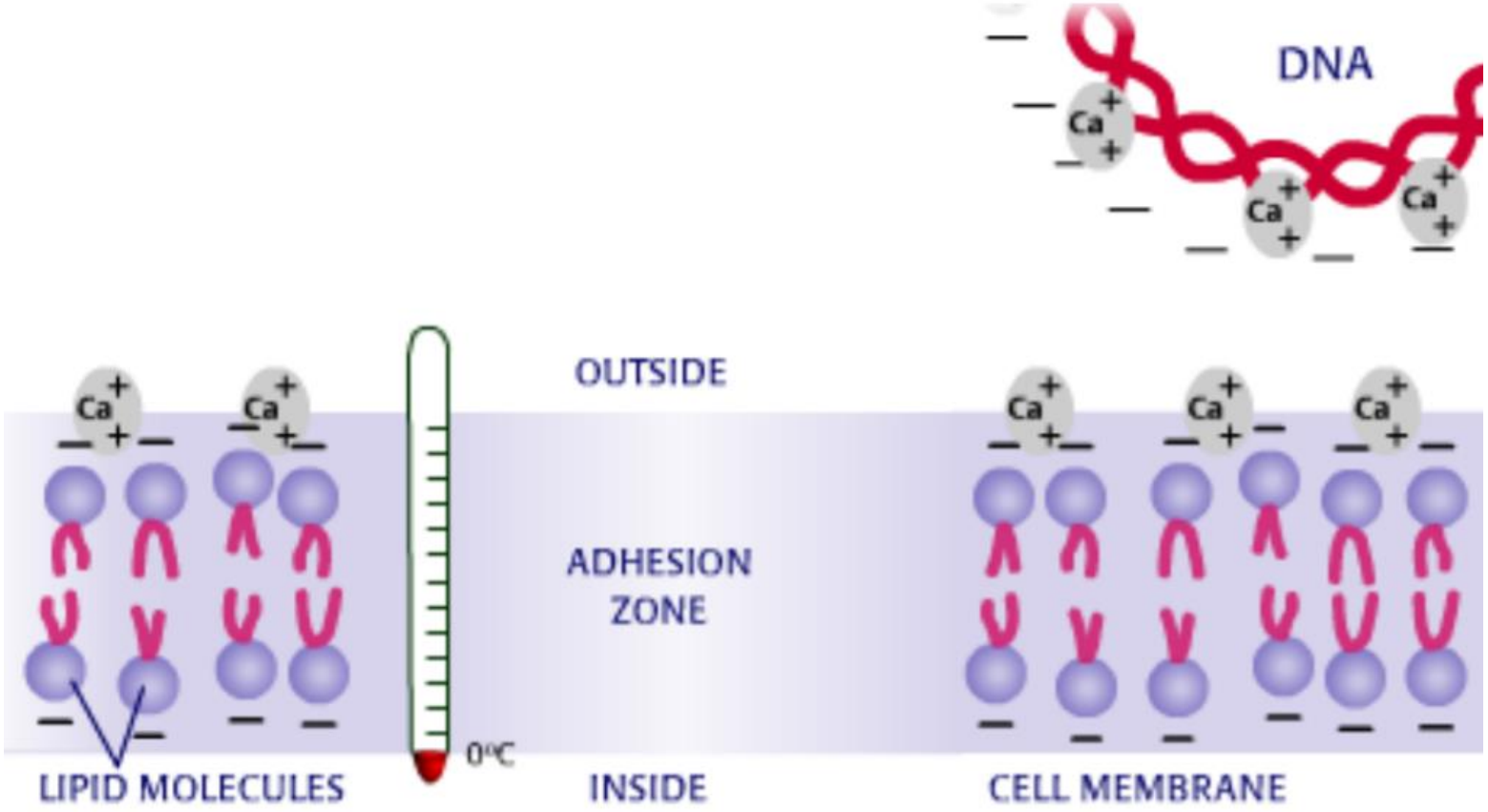
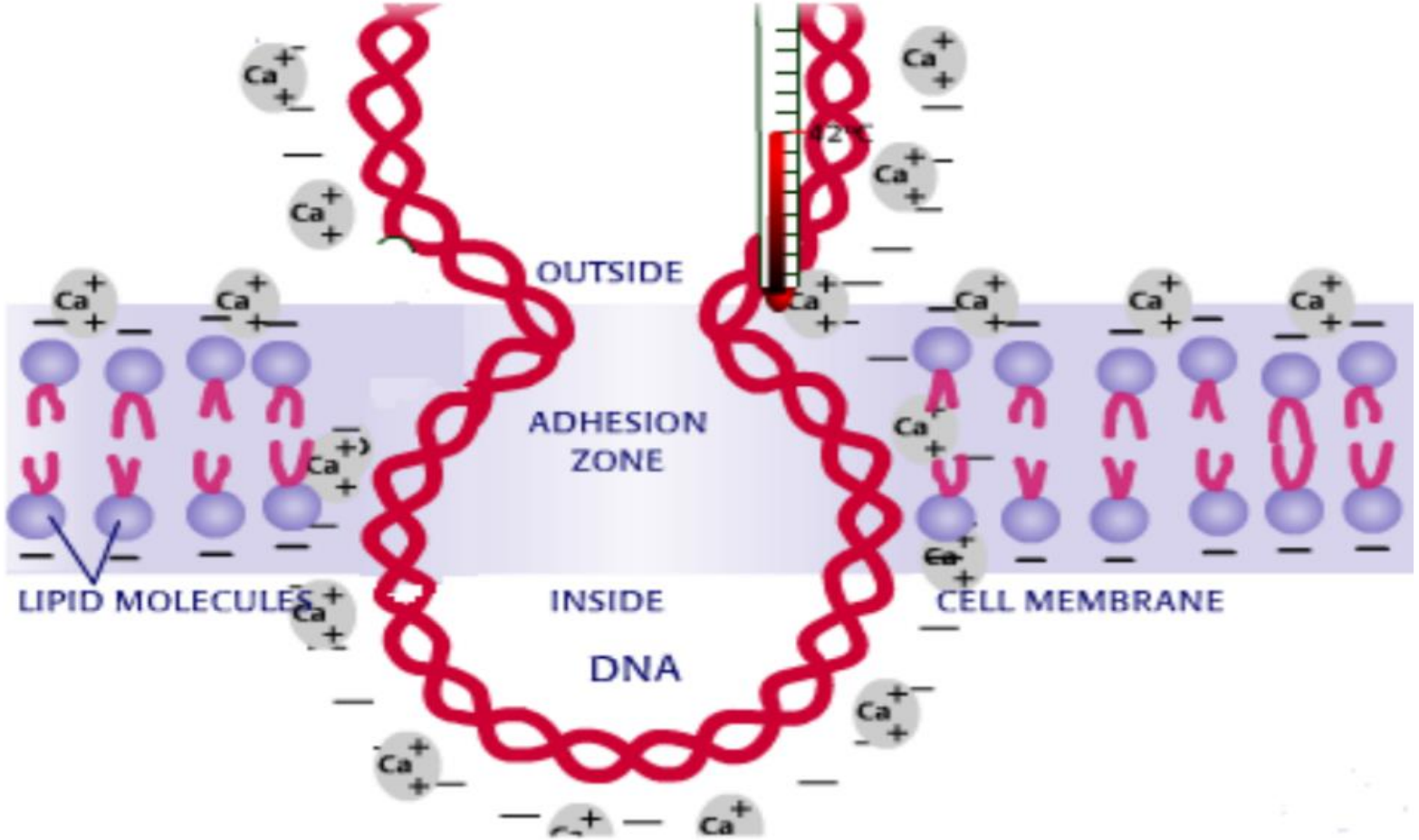
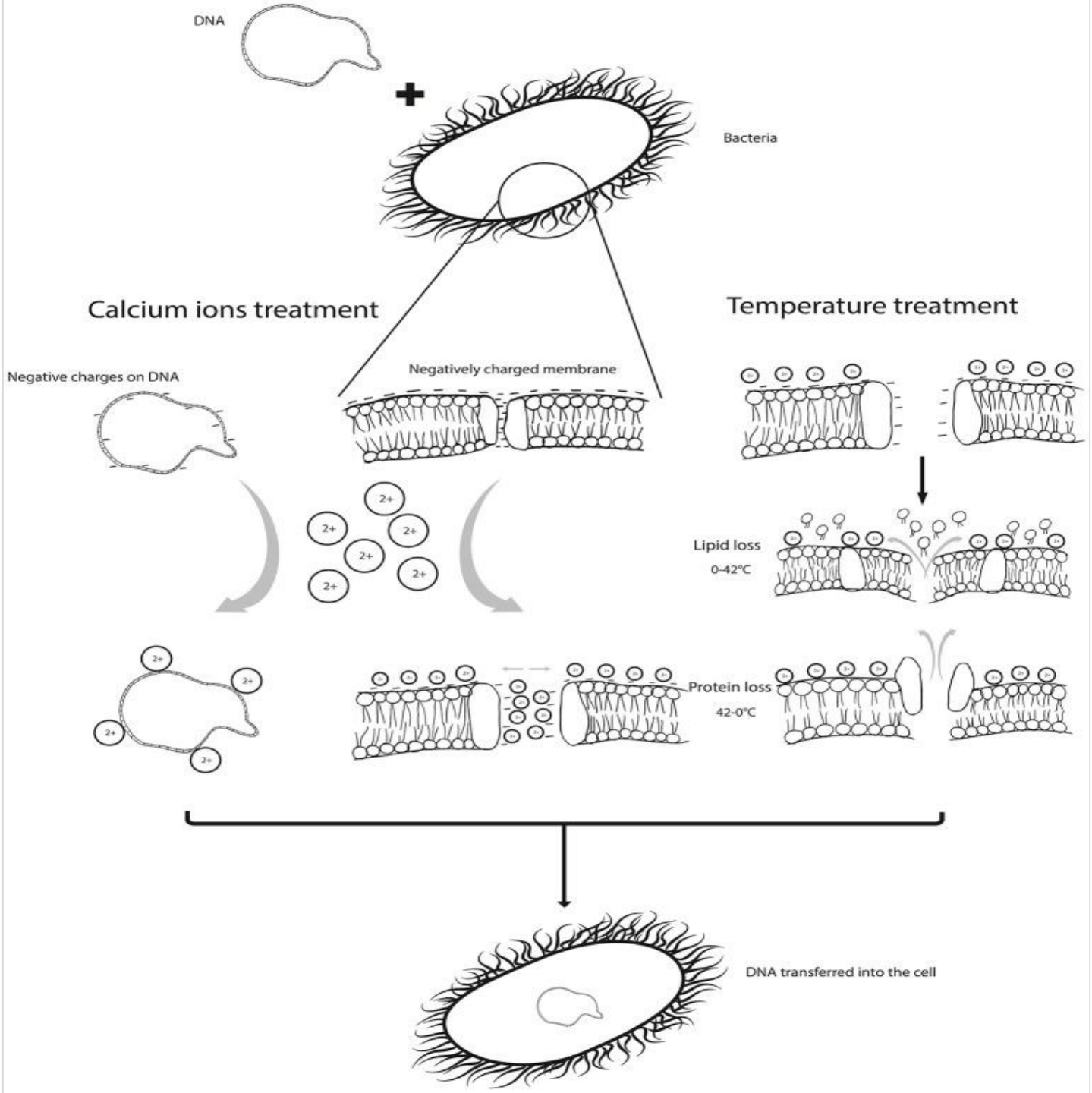


Chemical transformation principle:



Chemical transformation principle:





This figure shows the repulsion caused by negative charges on the cell membrane and DNA and the porosity of the membrane. These are manipulated by chemical treatment, such as calcium ions which neutralize negative charges. Physical parameters can be applied to improve porosity and permeability.

Results:

- Transformation efficiency = $\frac{\text{Total number of colonies on LB/Amp plate}}{\text{Amount of DNA plated [µg/ml]}}$

= _____ CFU/µg

* CFU/µg = colony-forming units per microgram of transforming DNA.

Supporting materials:

- **Principle of chemical transformation :**

http://www.dnalc.org/resources/animations/transformation2.html

- **Mechanism of Recombination:**

http://www.dnalc.org/resources/3d/20-mechanism-of-recombination.html

Home Work …

- Is there another type of cloning vectors? If yes, what are they?
- Draw a flowchart to show the molecular cloning steps. Indicate by arrow the step that performed in the lab today.
- What precautions would you take under considerations for performing this experiment?