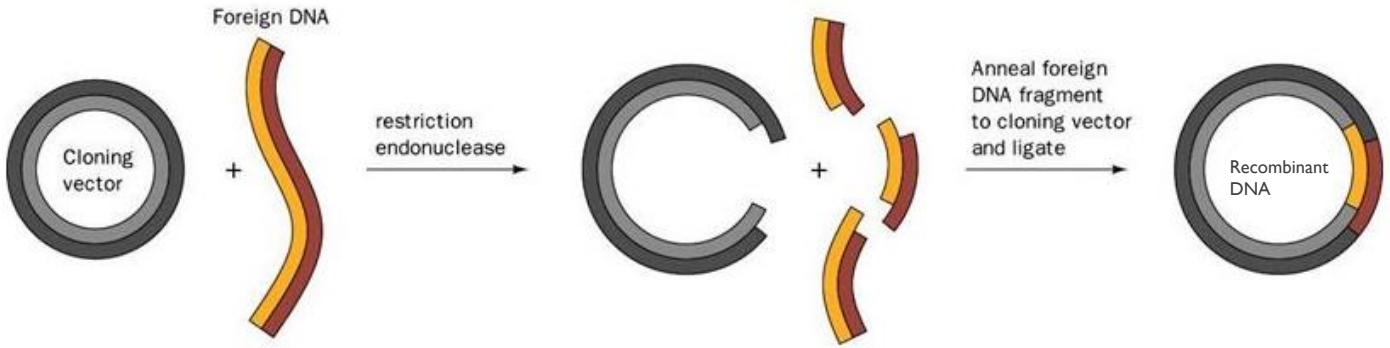
BCH 462- Biotechnology & Genetic engineering [Practical]

Lab (2) Competent Cells Formation and Transformation

Molecular cloning

An important tool to understand the **structure**, **function** and **regulation** of individual genes and their products.

- It is a cell-based technique.
- A clone is an identical copy, the term originally was applied to cells produced when a cell of a single type was isolated and allowed to reproduce to create a population of identical cells.
- Used to create copies of certain DNA fragments using a vector carrying the DNA of interest.
- DNA cloning involves separating a specific gene or DNA segment from a larger chromosome, attaching it to a small molecule of carrier DNA, which eventually inserted to a host cell (usually bacteria) then self-replicate.



DNA cloning steps

1- Selecting a small molecule of DNA capable of self-replication.

2- Cutting DNA at precise locations.

3- Joining two DNA fragments covalently.

4- Transformation

5- Selecting or identifying host cells that contain recombinant DNA.

DNA cloning steps cont.

1- ***Selecting a small molecule of DNA capable of self-replication.***

2- ***Cutting DNA at precise locations.***

3- ***Joining two DNA fragments covalently.***

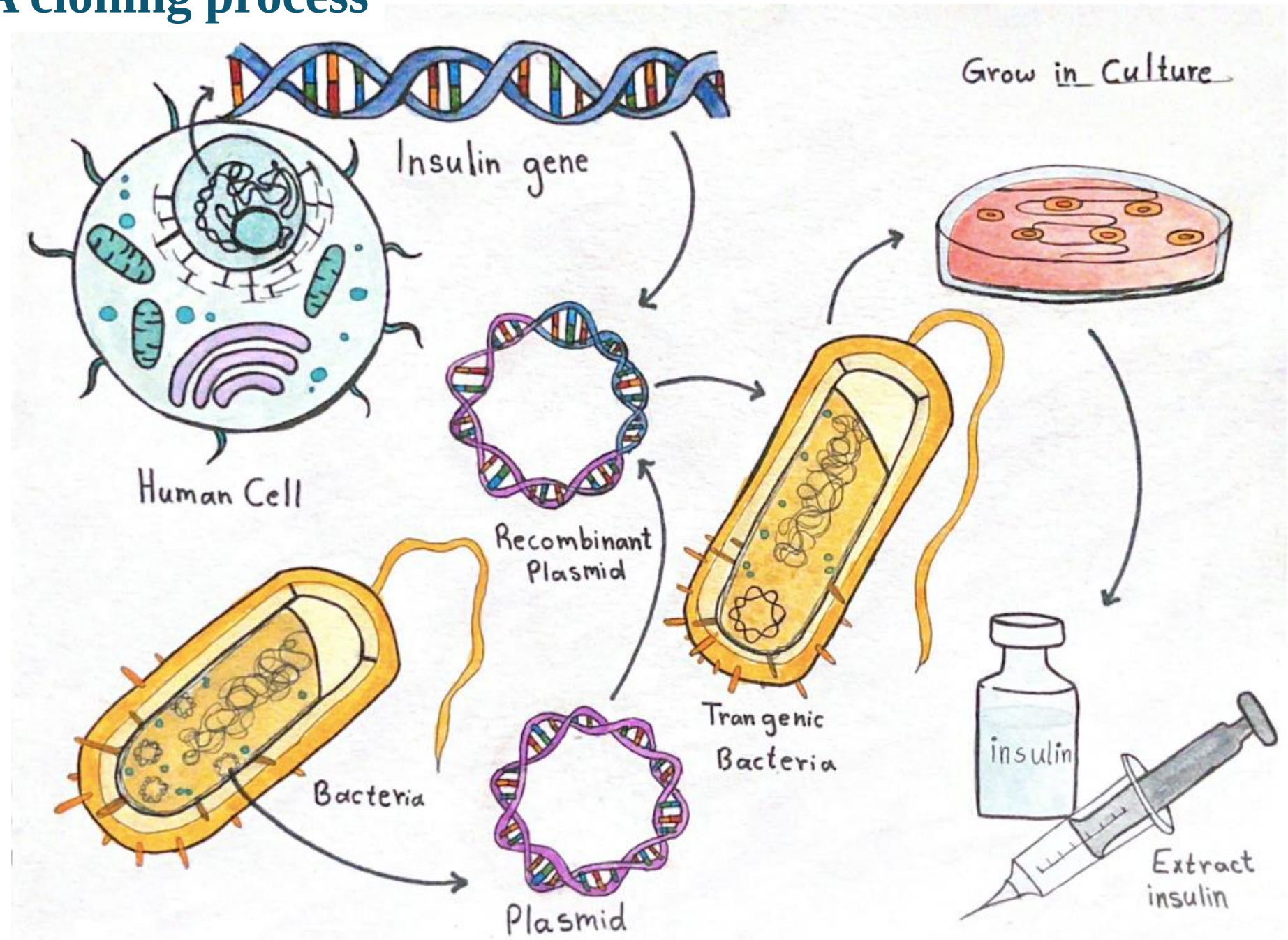
4- ***Transformation***

- Introducing of the recombinant DNA into bacterial cells (the host)
- Recombinant DNA amplified using bacterial DNA replication machinery.

5- ***Selecting or identifying host cells that contain recombinant DNA.***

- The cloning vector generally has features that allow the host cells to **survive** in an environment where cells lacking the vector would die.
- Cells containing the vector are thus "**selectable**" in that environment.

DNA cloning process



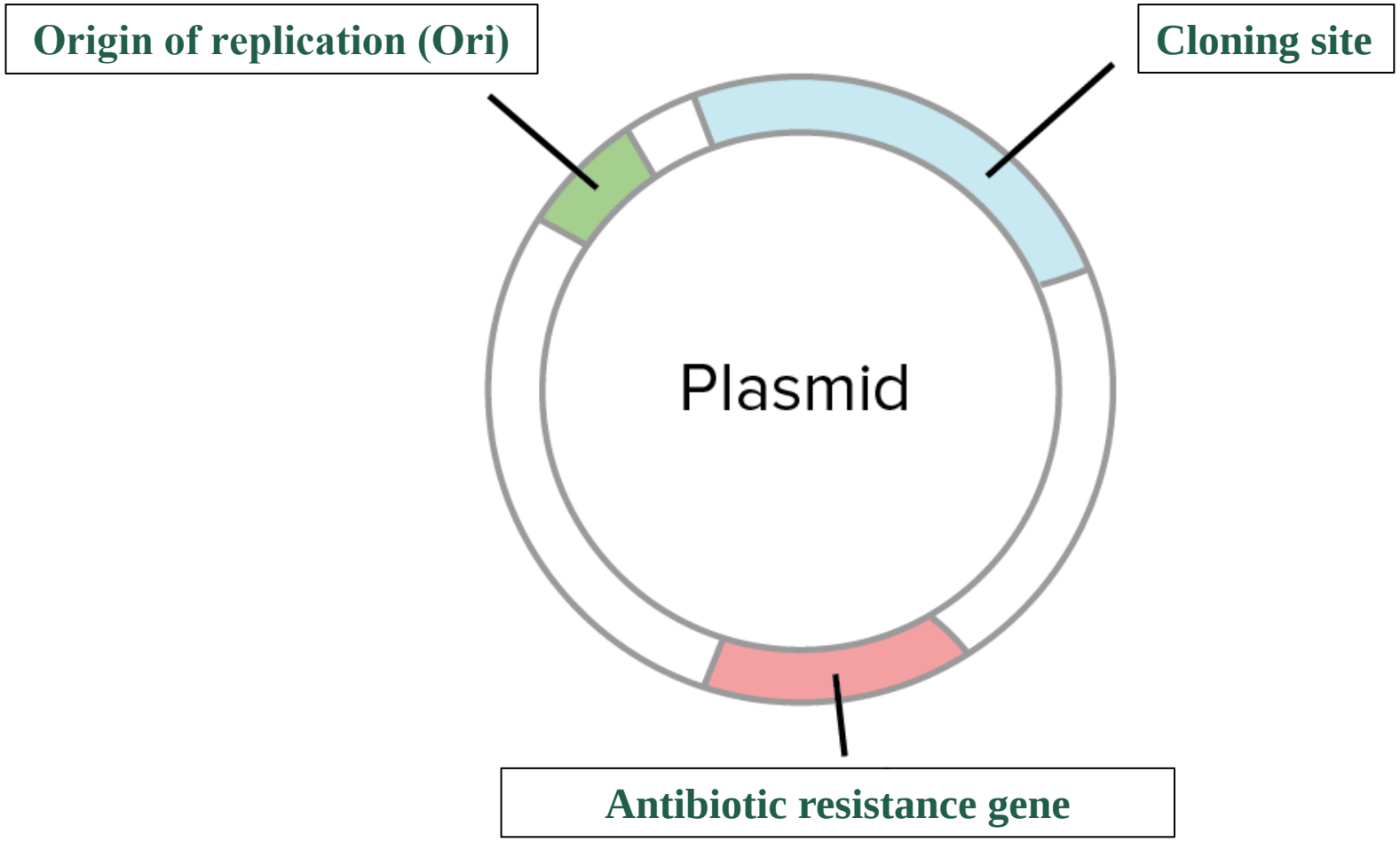
Done by:
مريم الكلثم ❤

Cloning vector

The DNA into which a foreign piece of DNA is cloned is called a "vector" (a vector is a carrier or delivery agent).

- **Vectors** are those small DNA molecules that carry a foreign DNA fragment when inserted into it.
- Most cloning vectors used in the laboratory are modified versions of naturally occurring small DNA molecules found in bacteria (plasmid).
- Based on the nature and sources the vectors are grouped into different classes, including **bacteriophages** and **plasmids**.
- The cloning vector is chosen according to the **size** and **type** of DNA to be cloned.

Plasmid vectors should contain three important parts:

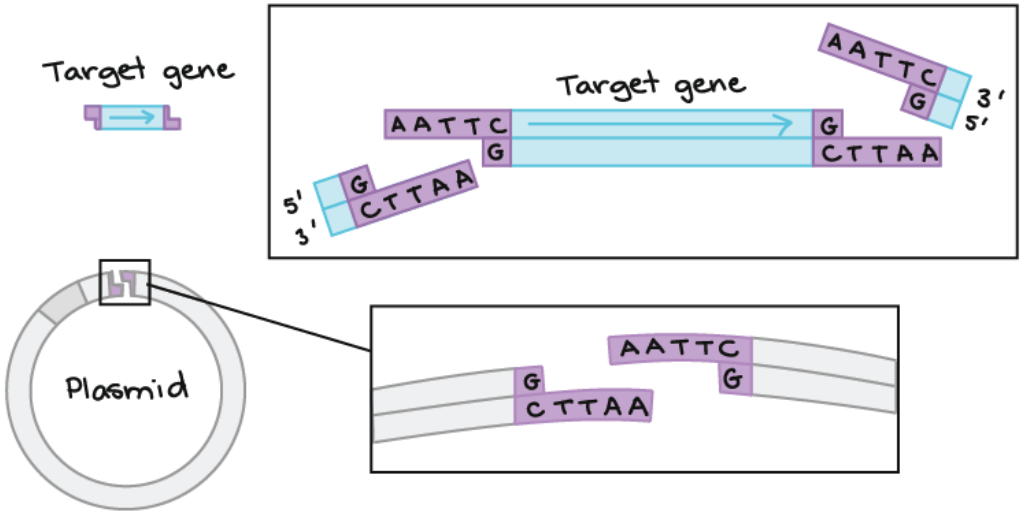


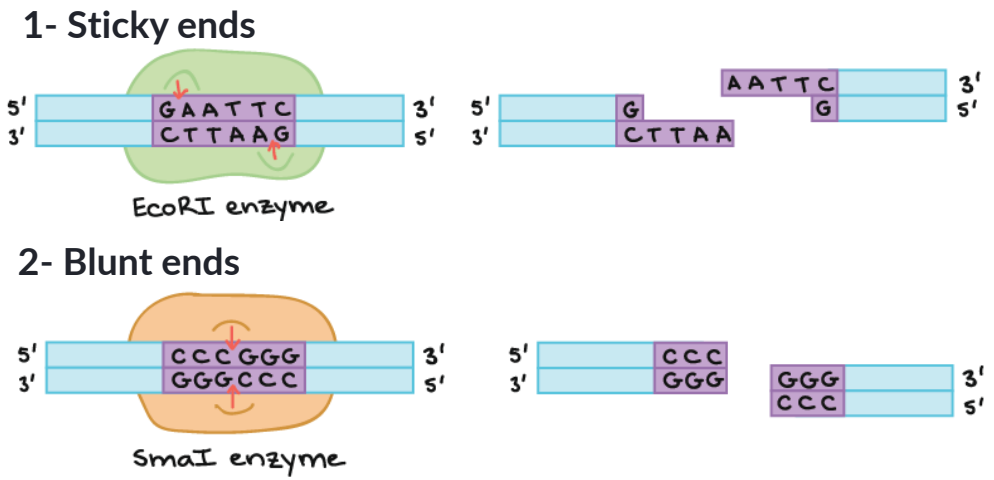
Restriction enzymes [R.E]

- A **restriction enzyme** is a DNA-cutting enzyme that recognizes specific sites in DNA.
- Are found in **bacteria** (and other prokaryotes).

> Pause and Think: Why restriction enzymes were originated in bacteria? And how would the bacteria protect its DNA from digestion?

- They recognize and bind to specific sequences of DNA, called **restriction sites** and cleave the DNA into smaller fragments.
- Each restriction enzyme recognizes just one or a few restriction sites.
- Because they cut within the molecule, they are often called **endonucleases**.
- A restriction enzyme will make a **double-stranded** cut in the DNA molecule.

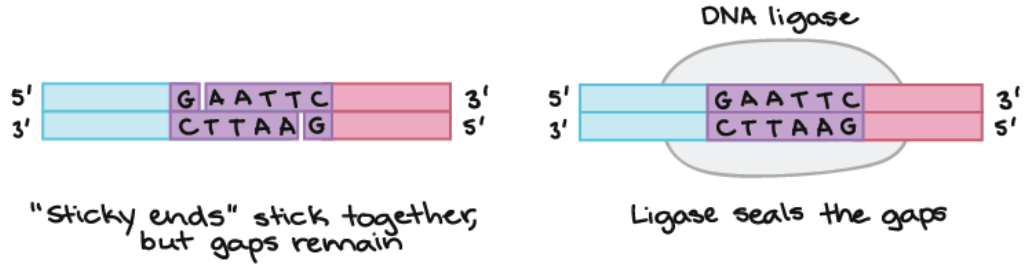




DNA ligase

- DNA ligase can join matching sticky ends of DNA pieces from different sources that have been cut by the same restriction enzyme. (Why ?)
- The mechanism of DNA ligase is to form two covalent phosphodiester bonds between 3’ ends of one nucleotide, (“acceptor”) with 5’ phosphate end of another (“donor”).
- ATP is required for the ligase to work.
- Composite DNA molecules of this type, comprising covalently linked segments from two or more sources, are called recombinant DNAs.

Pause and Think in which processes DNA ligase participate? And what its function?



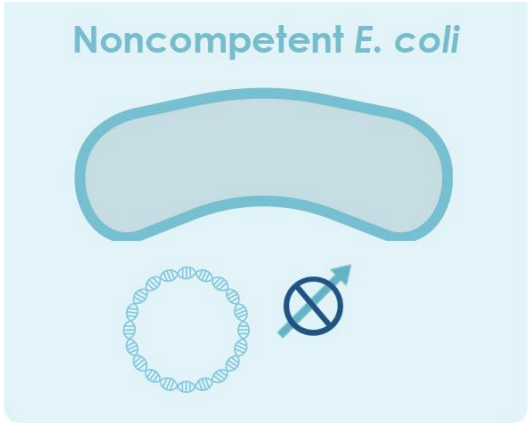
Competence

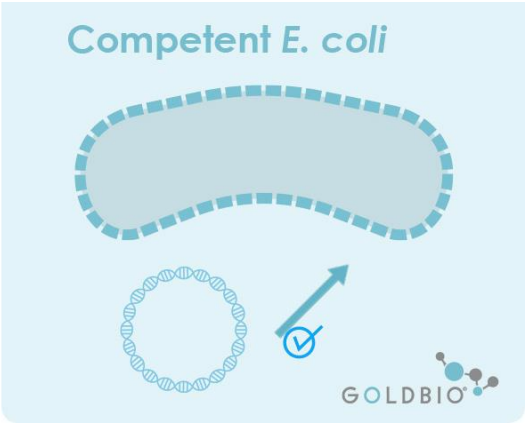
- For a bacterial cell to take up DNA from its surroundings, it must be in a special physiological state called **competence**.
- **Competence** is the ability of a cell to undergo transformation, which means the ability to take up extracellular DNA from its environment.
- Competence play role in **pathogenesis** and **survival**. How?

There are two classes of competent cells:

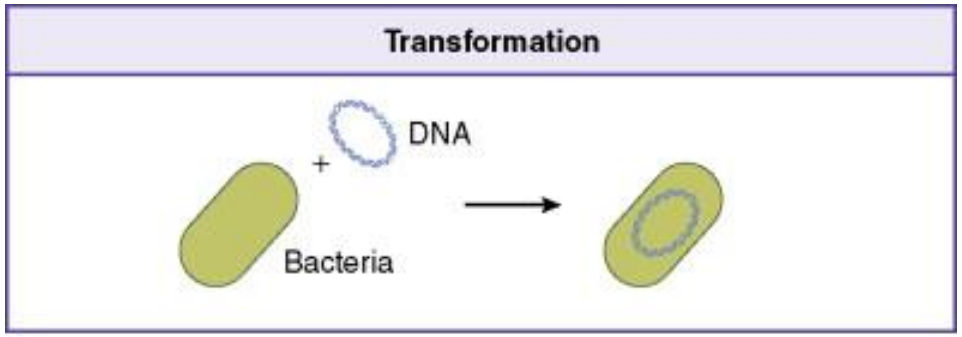
1- Natural competence: a genetically specified ability of bacteria that is occur under natural condition.

2- Artificial competence: when cells in laboratory cultures are treated to be permeable to DNA.



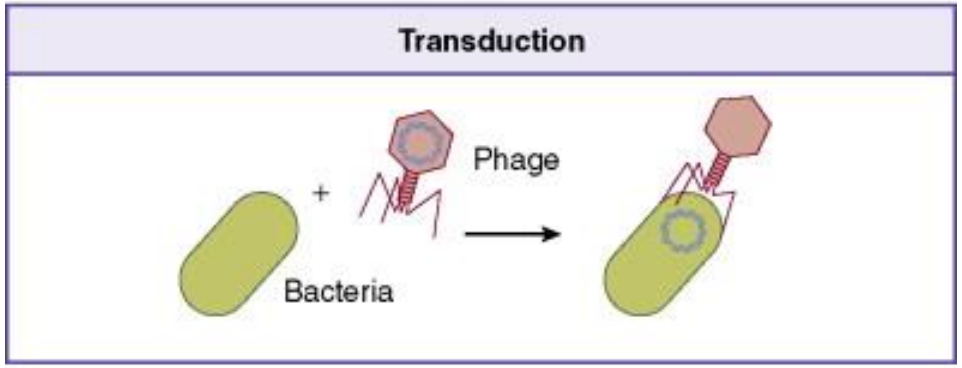


Natural competence



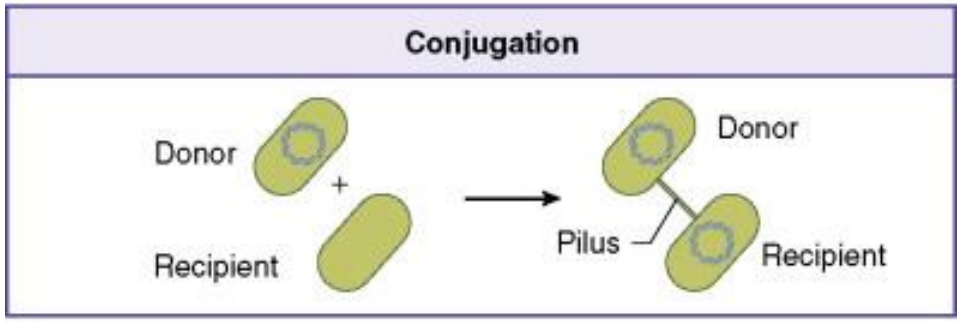
A

Transformation: acquisition of extracellular DNA from the environment, transformation is the only direct uptake of DNA.



B

Transduction: is the process by which DNA is transferred from one bacterium to another by a virus [**bacteriophages**].



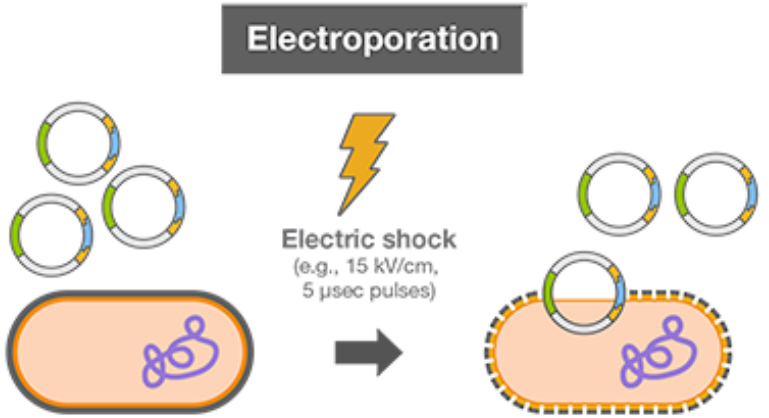
C

Conjugation: DNA is transferred directly from one organism to another and it requires direct cell-cell contact via a sex pilus.

Methods of artificial transformation

1. Electroporation or Electro-permeabilization

Electroporation is a physical method that uses an **electrical pulse** to create temporary pores in cell membranes through which substances like nucleic acids can pass into cells.



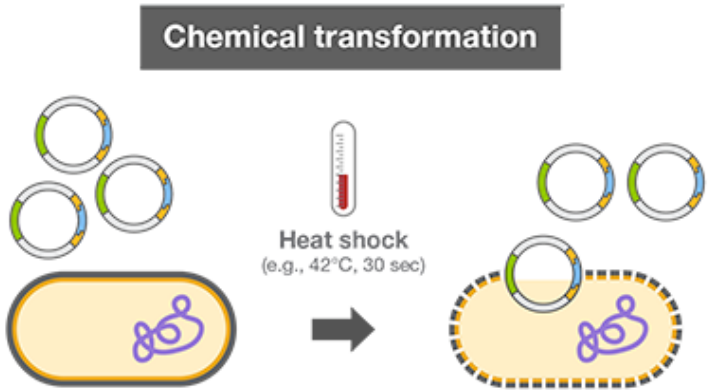
2. Chemical transformation

Less efficient than electroporation.

It involves **two** major steps:

1- **$CaCl_2$ treatment**, to permeabilize the bacterial cell membrane.

2- Brief **heat shock** to facilitate the DNA up take.



Practical Part

Practical part

- **Aims:**
  - Making a competent cells using **calcium chloride $CaCl_2$** method.
  - Transformation of the competent cells with recombinant plasmid DNA using **chemical transformation** method.

Practical part

- **Principle**

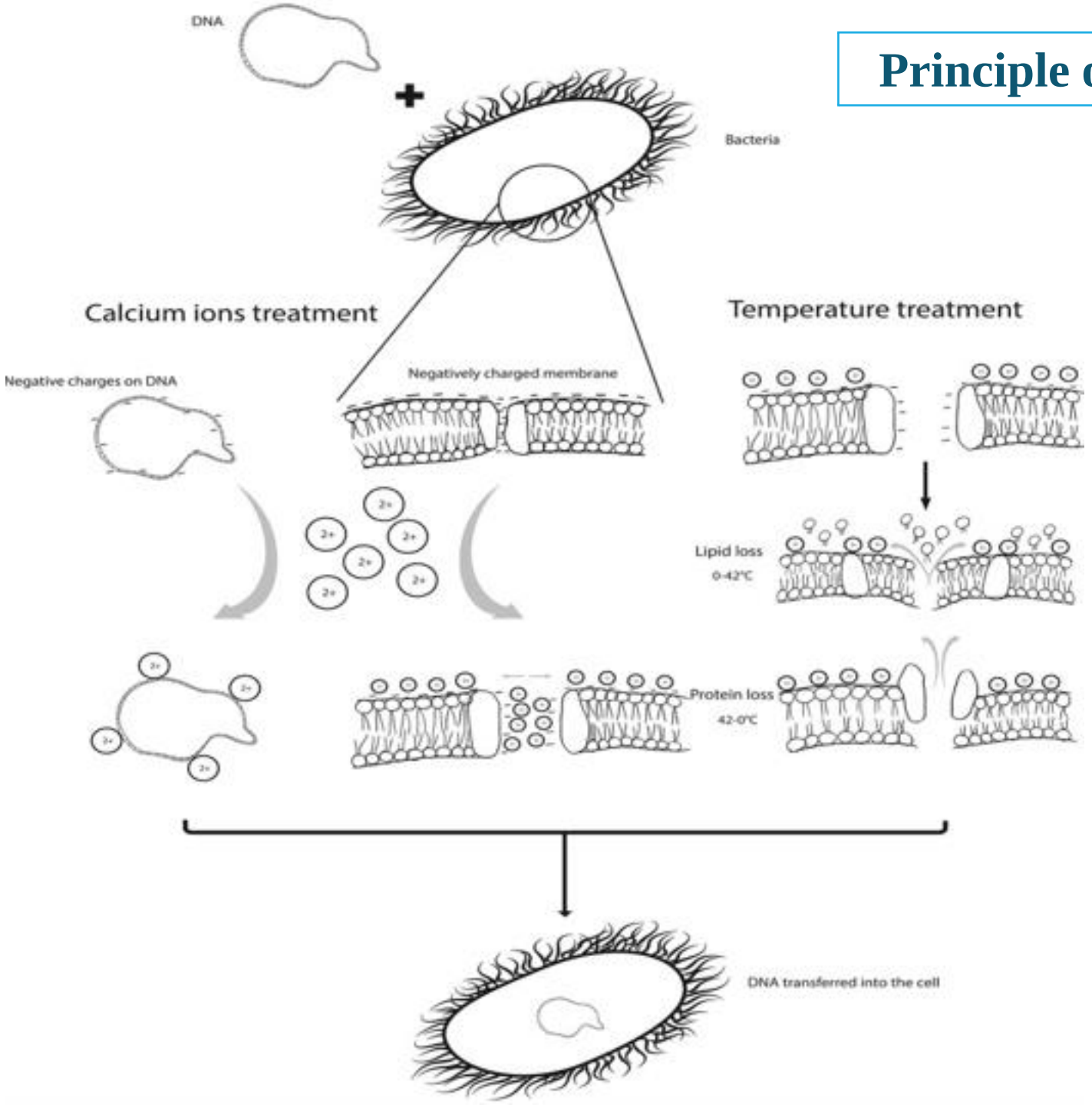
1- **Competent cell formation**

- By **Chemical Transformation** cells are incubated in **$CaCl_2$ solution** that help the cells to take up the DNA plasmid by increasing the bacterial cells membranes permeability [renders them competent to take up DNA].

2- **Transformation of competent cells with DNA**

- Once the cells are made competent, the plasmid DNA is mixed with the cells.
- The competent cells are then subjected to heat shock, which allows the DNA to enter the cells.

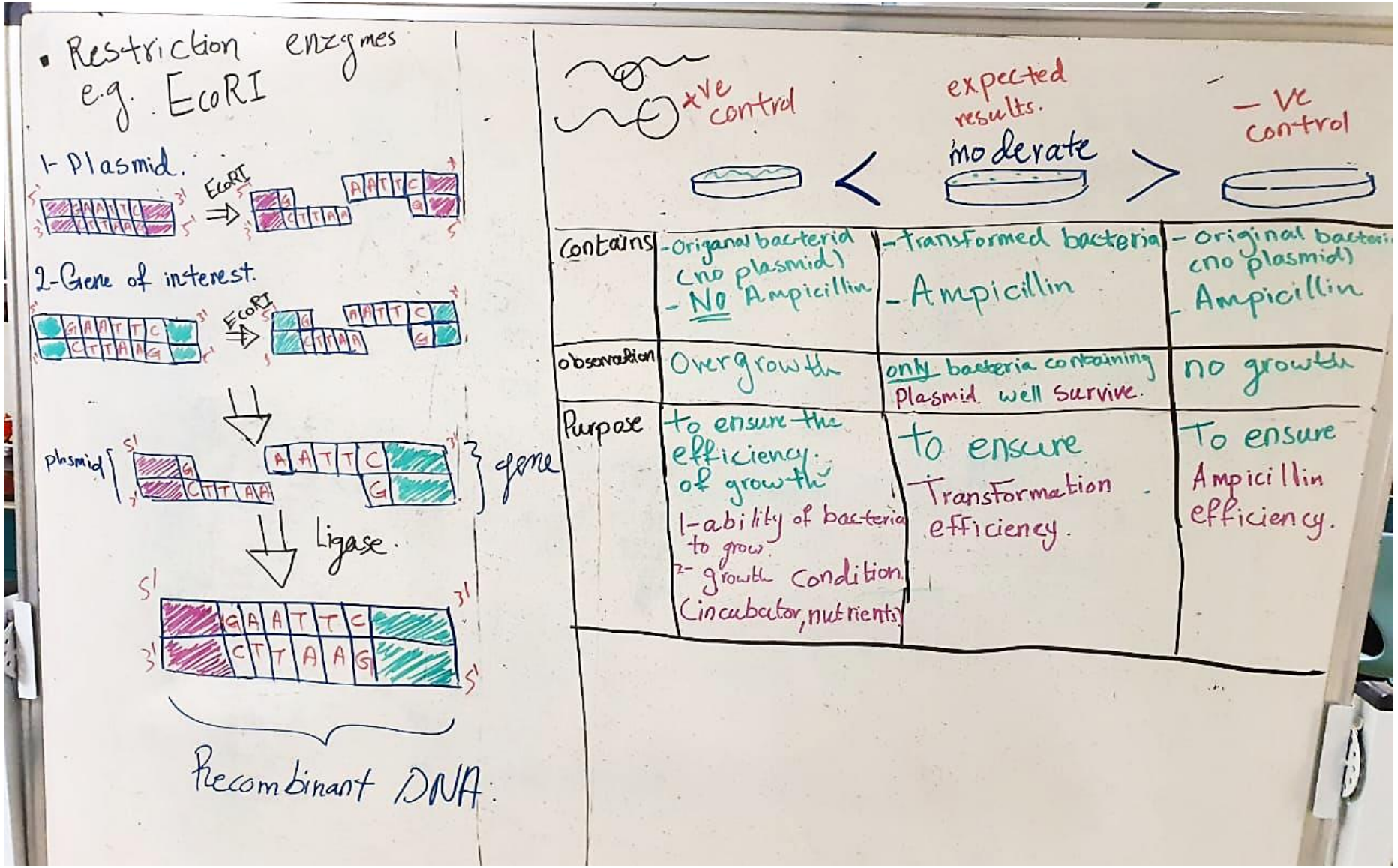
Principle of chemical transformation



Practical part

3-**Transformation efficiency**

- The transformed cells are then grown in LB agar plate containing appropriate antibiotic to be able to count the transformed colonies only, (which they are colonies containing transformed cells-containing the DNA plasmid), each colony on an antibiotic plate presents a single transformation event.
- Then calculations of the **transformation efficiency** will be done.


Restriction enzymes
e.g. EcoRI
1- Plasmid.
EcoRI
2- Gene of interest.
EcoRI
plasmid
gene
Ligase.
Recombinant DNA.
+ve control
expected results.
moderate
-Ve control
Contains
-Original bacteria (no plasmid)
- No Ampicillin
-Transformed bacteria
- Ampicillin
- original bacteria (no plasmid)
- Ampicillin
observation
Overgrowth
only bacteria containing plasmid well survive.
no growth
Purpose
to ensure the efficiency of growth
1-ability of bacteria to grow
2- growth condition (incubator, nutrients)
to ensure Transformation efficiency.
To ensure Ampicillin efficiency.

Practical part

- **Calculations**

- **Transformation efficiency** 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:**

1- The number of colonies that were successfully transformed.

2- The amount of plasmid DNA used for the transformation.

Transformation efficiencies generally range from $\mathbf{1x10^6 - 1x10^{10}}$ CFU/µg

Transformation efficiency= $\frac{\text{Total number of colonies}}{\text{Amount of DNA plated [µg]}} = \_\_\_\_\_$ CFU/µg

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

Transformation efficiency

$TE = \frac{\text{\# of colonies}}{\mu g \text{ of DNA plated (A)}}$ CFU/μg

D.F. = $\frac{\text{Final Vol.}}{\text{Initial Vol.}}$

B.6 = $\frac{1000}{(10-50)\ 20\mu l}$

(A) μg of DNA plated = $\frac{\text{DNA transformed}^{(1)}\ (\mu g) \times \text{Volume spread}^{(2)}\ (\mu l)}{\text{Total volume of transformation}^{(3)}\ (\mu l)}$

(1) DNA transformed (μg) = 100 ng ⇒ 0.1 μg plasmid Step B.1

(2) volume spread (μl) = 5 μl Step B.8

(3) Total volume of transformation (μl) =

25 μl of plasmid (B.1) + 100 μl of cells (B.2) + 1000 μl LB (B.5) = 1125 μl

(A) $\frac{0.1\ (\mu g) \times 5\ \mu l}{1125\ \mu l} =$ ____ μg

Homework

- Draw a flowchart to show the molecular cloning steps. Indicate by arrow the step that performed in the lab today.

Supporting materials

- **The Mechanism of Transformation with Competent Cells:**

  https://www.youtube.com/watch?v=7Ul9RVYG5CM

- **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