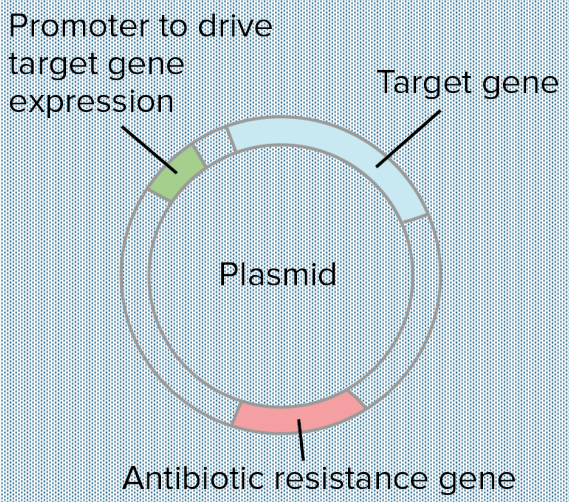
Lab#2

# BCH 462

# Competent Cells Formation and Transformation of Competent Cells with plasmid DNA.



# Outlines:

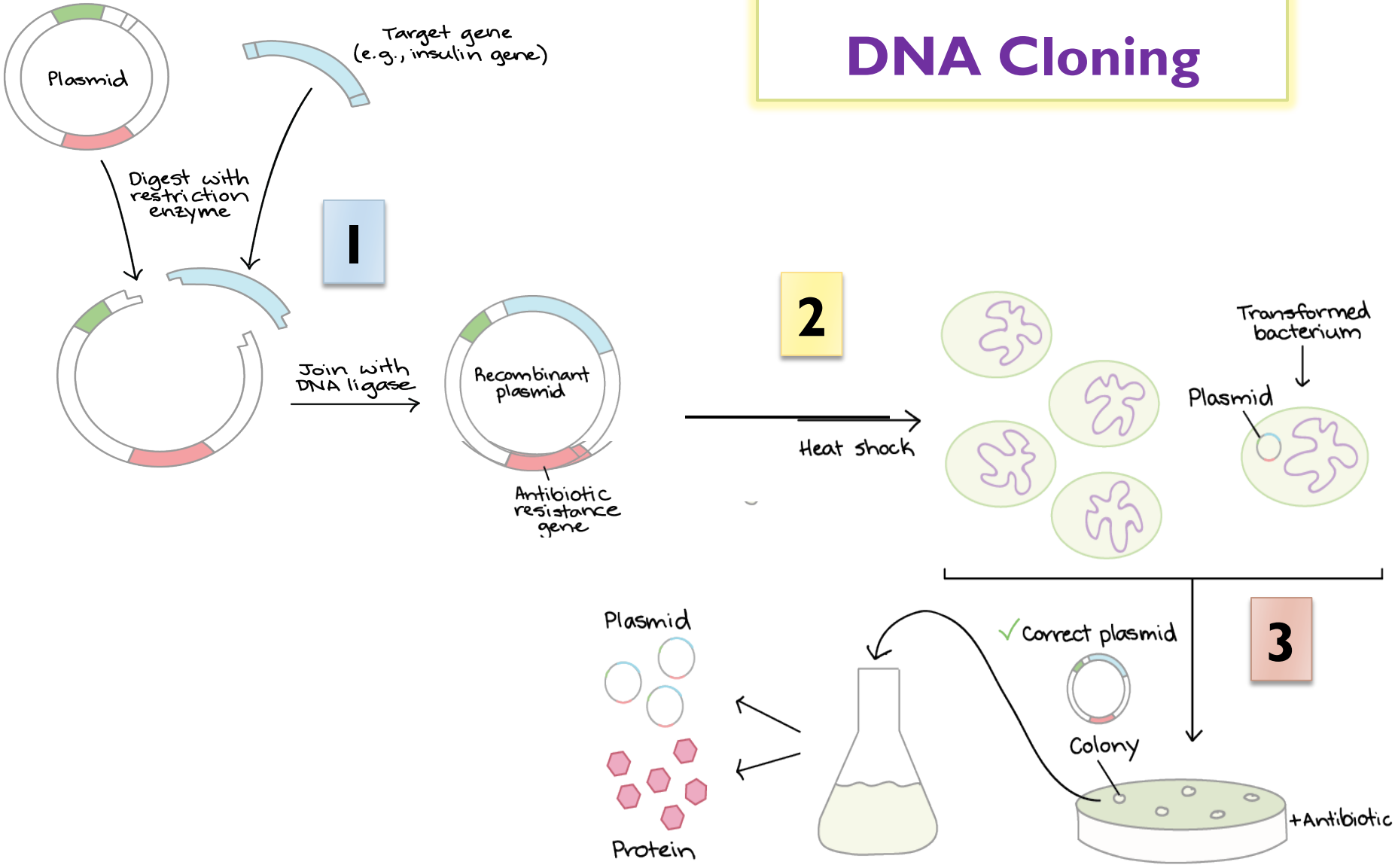
1-Insertion of foreign gene to the plasmid.

2-Competent cell.

3-Transformation of bacterial cell.

4-transformation efficiency.

# DNA Cloning


Plasmid
Target gene
(e.g., insulin gene)
Digest with restriction enzyme
1
Join with DNA ligase
Recombinant plasmid
Antibiotic resistance gene
2
Heat shock
Transformed bacterium
Plasmid
3
Correct plasmid
Colony
+Antibiotic
Plasmid
Protein

# Mechanism of Recombination:

https://www.youtube.com/watch?v=8rXizmLjegI

# What is cloning vector?

A DNA molecule that carries foreign DNA into a host cell, replicates inside a bacterial cell and produces many copies of itself and the foreign DNA.

**They must be:**

1. capable of independent replication within the host cells (e.g bacteria).
2. they most contain at least one specific nucleotide sequence recognized by a restriction endonuclease.

**Tow major types of cloning vector can be found in bacterial cells they are:**

-plasmid.

-bacteriophages.

# Insertion of foreign gene to the plasmid involves 2 main enzymes:

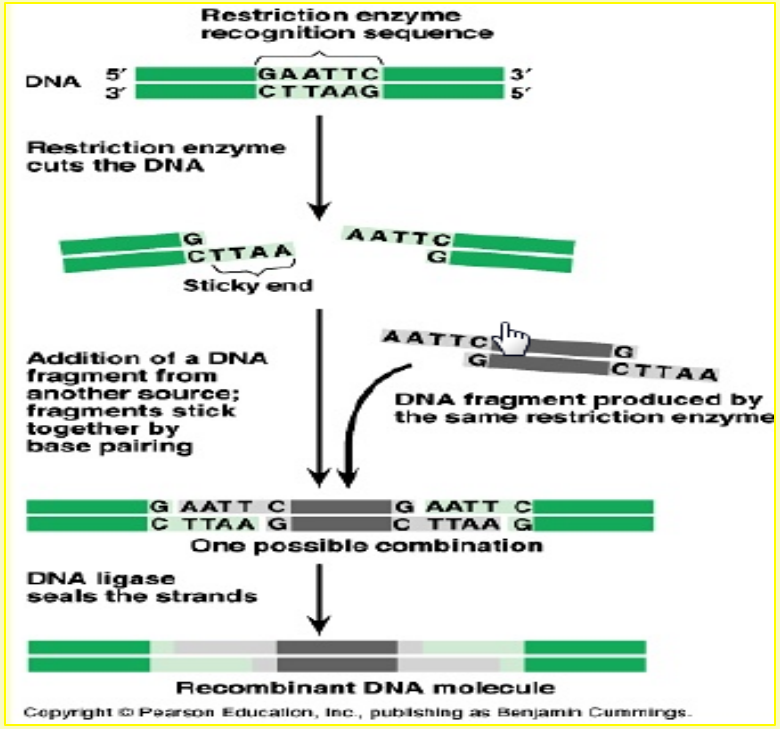
## 1) Restriction Enzymes[R.E]:

-Are **DNA-cutting enzymes** found in bacteria to protect them from intruding DNA.

-They cut only at very specific nucleotide sequences known as restriction sites.

-Because they cut within the molecule, they are often called **endonucleases**.

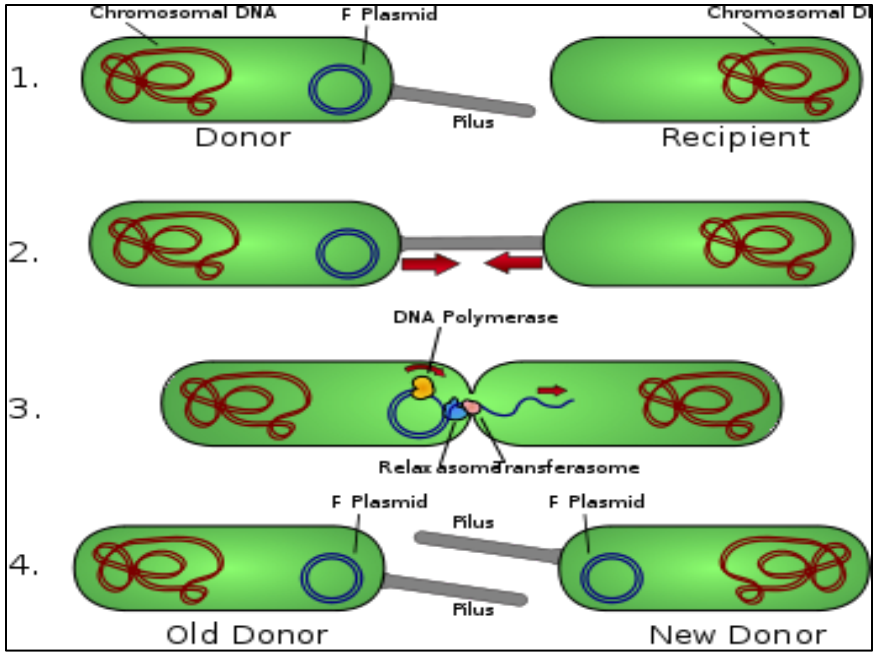
-They are harvested from bacteria for use in various genetic engineering techniques.




## 2) DNA ligase:

is a specific type of enzyme, that facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond.

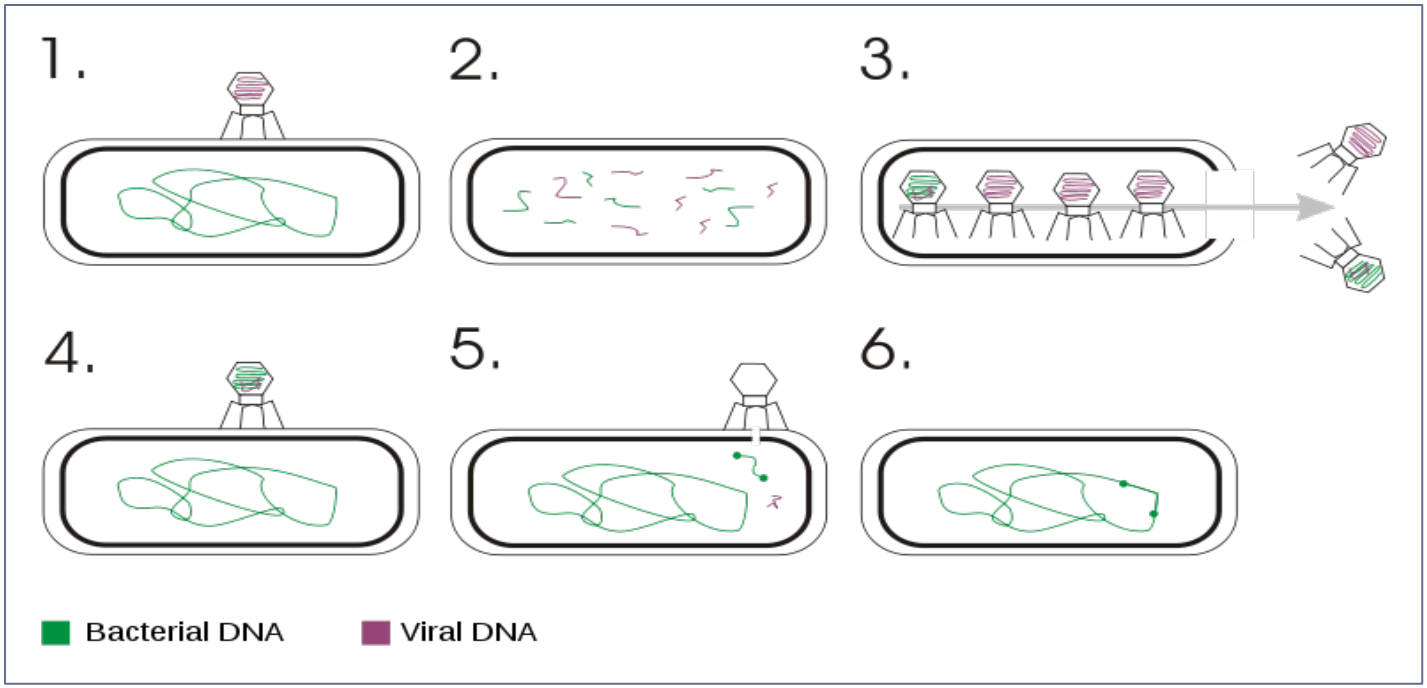
## Bacteria in general can acquire new genetic information by:




During conjugation → direct contact.

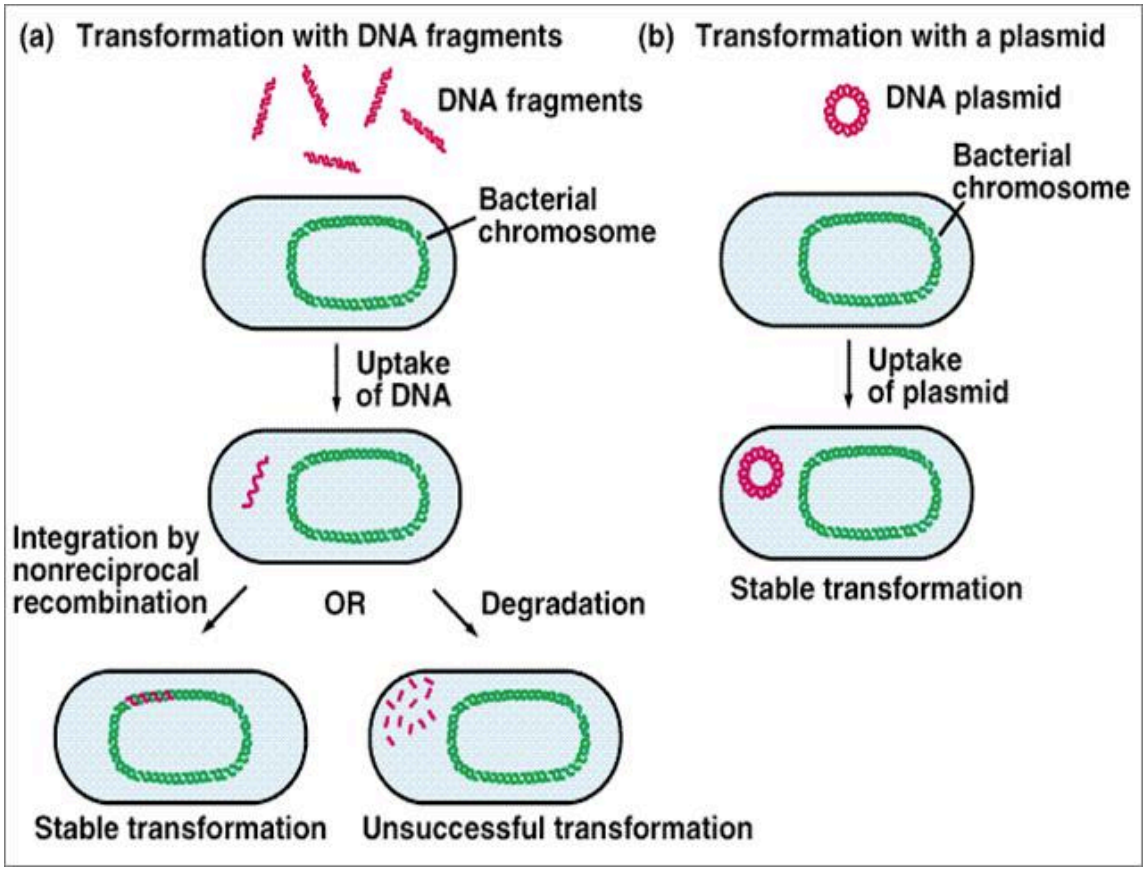
**1**

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




**2**

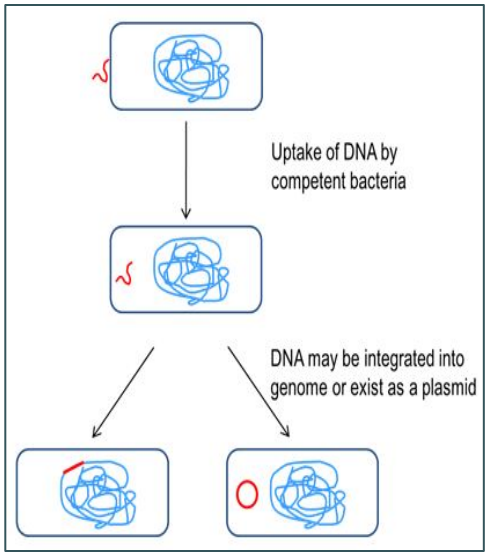
Transduction: is the process by which DNA is transferred from one bacterium to another by a virus [bacteriophges].




**3**

Transformation: acquisition of extracellular DNA from the environment.

# Competence




-**It** is the ability of a cell to undergo transformation, which means the ability to take up extracellular ("naked") DNA from its environment.

**-There are two classes of competent cells :**

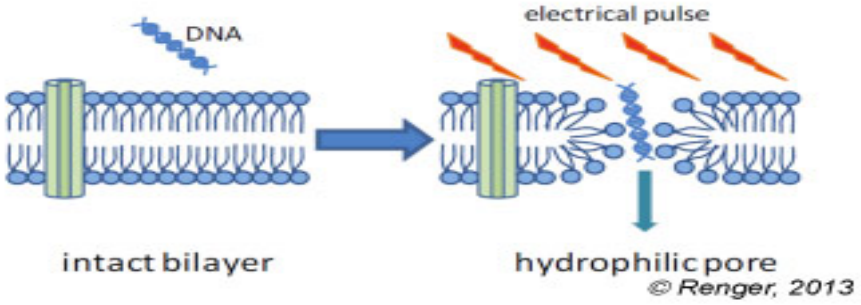
**Natural competence:** a genetically specified ability of bacteria that is occur under natural condition.

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

# Methods of Artificial transformation:

## 1. Electroporation, or Electropermeabilization

During electroporation the lipid molecules are not chemically altered but simply shift position, opening up a pore which acts as the conductive pathway through the bilayer as it is filled with water.



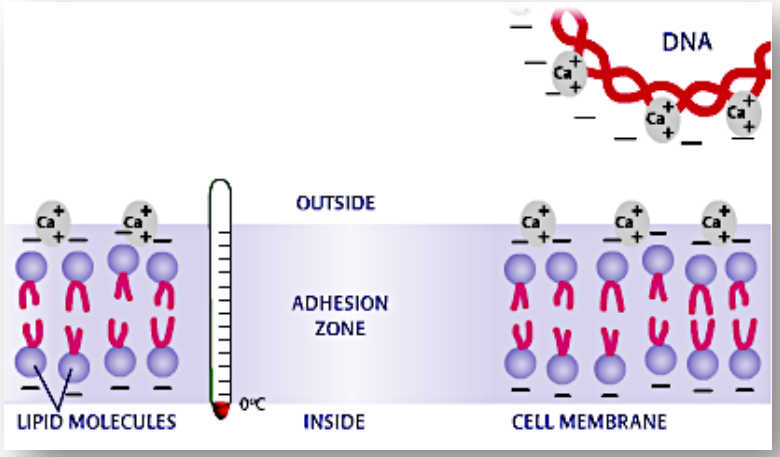

# 2.Chemical transformation.

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.




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

# Practical part

# Principle of the experiment:

**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.

**Transformation of competent cells with DNA**

- By applying brief heat shock will facilitate the DNA up take

**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.

# Calculations

-**Transformation efficiency,** is a quantitative value that describes how effective you were at getting plasmid DNA into your *E. coli* bacteria.

-The number represents how many cells were transformed per microgram ( $\mu$ g) of plasmid DNA used.

-This calculation requires two values: the number of colonies that were successfully transformed and the amount of plasmid DNA used for the transformation.

**Transformation efficiency=** $\frac{\text{total number of colonies on LB/Amp plate}}{\text{amount of DNA plated [µg/ml]}}$ CFU/µg

CFU: colony –forming units.