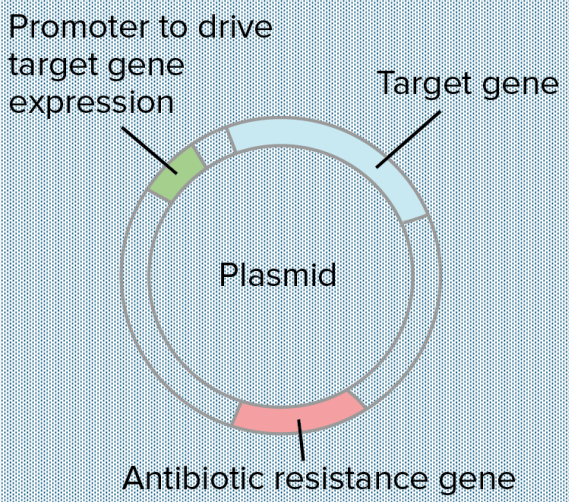
Lab#2

BCH 462

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



Outlines:

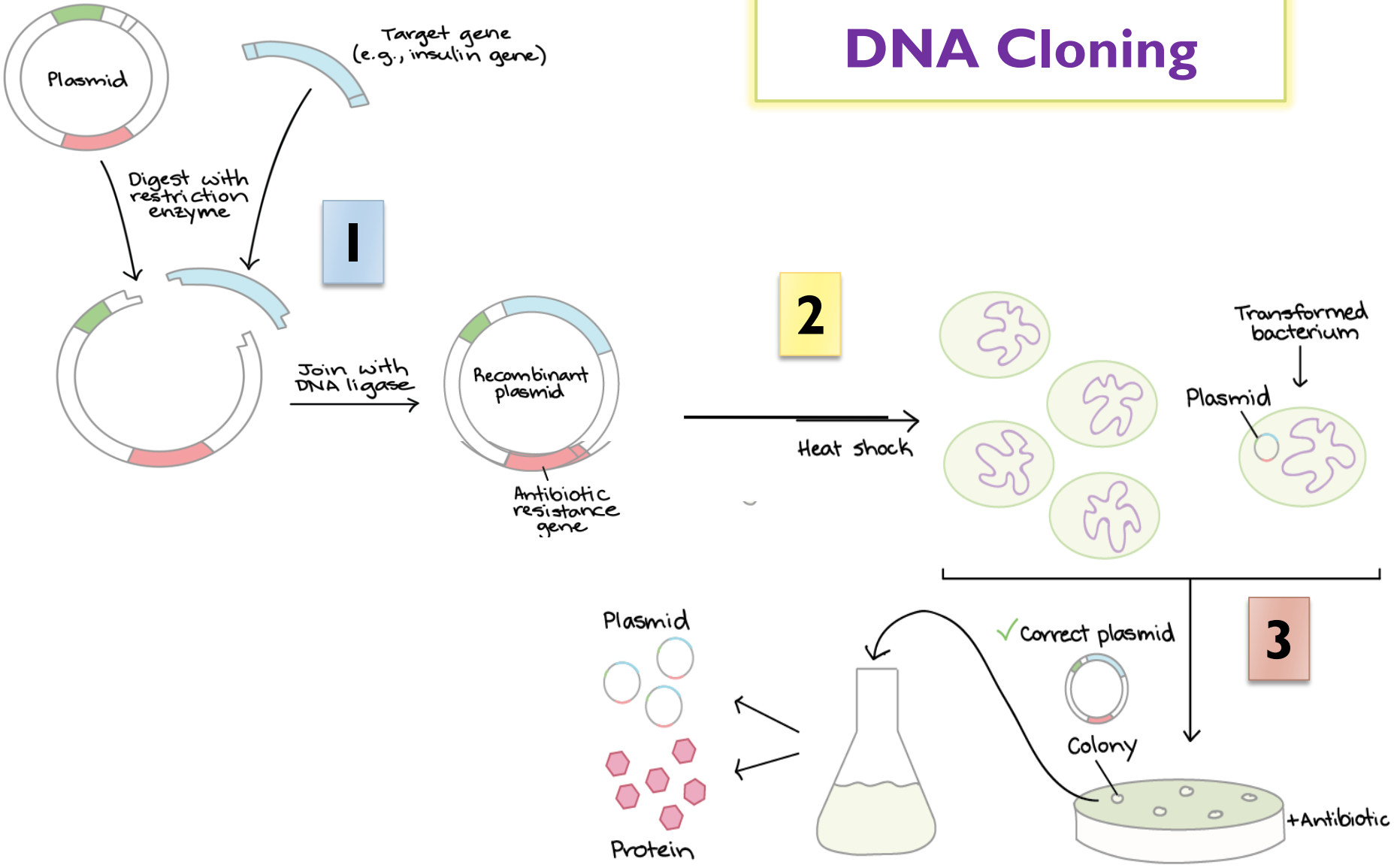
1-Insertion of foreign gene to the plasmid.

2-Competent cell.

3-Transformation of bacterial cell.

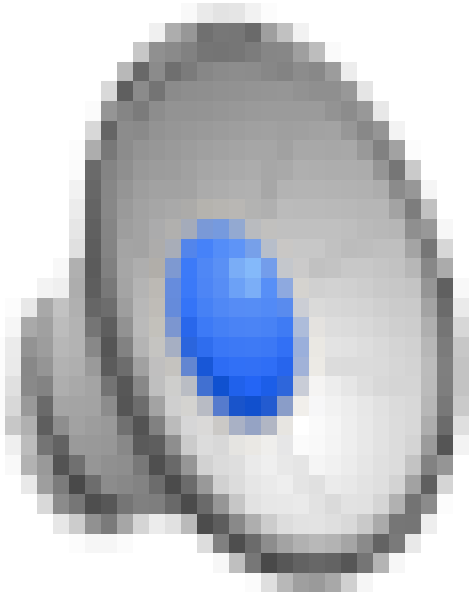
4-transformation efficiency.

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:

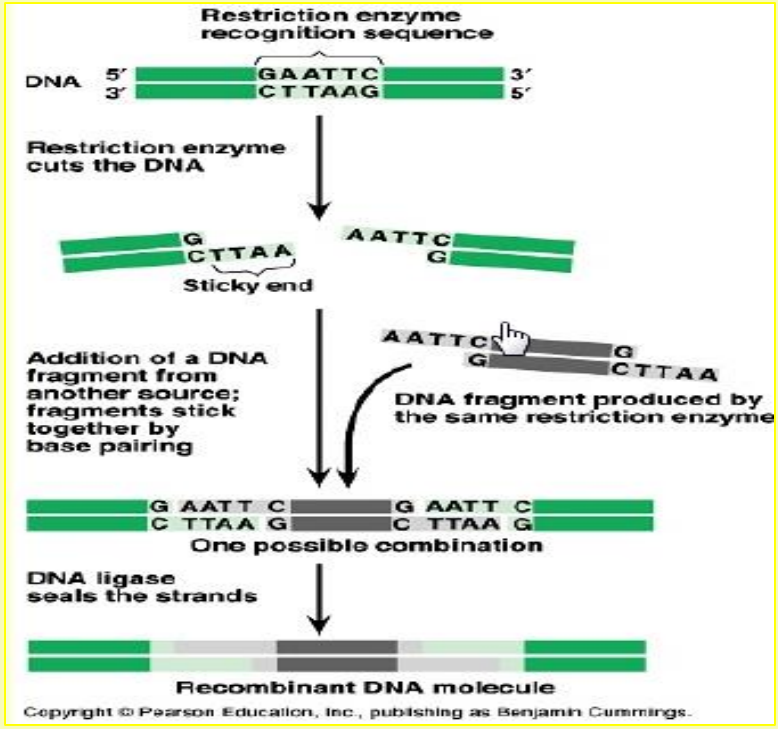
1) Restriction Enzymes[R.E]:

-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**.

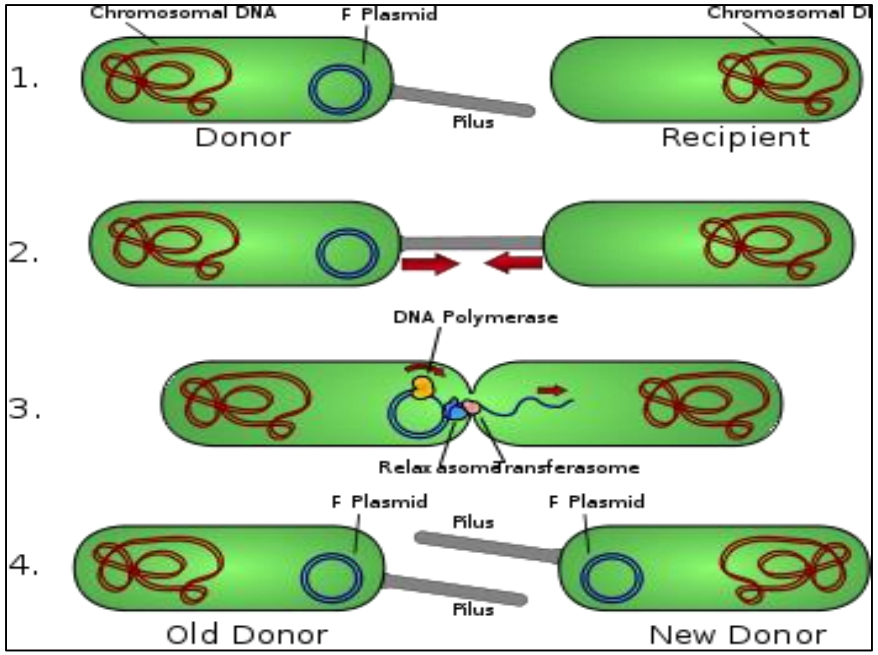
-They are harvested from bacteria for use in various genetic engineering techniques.



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.

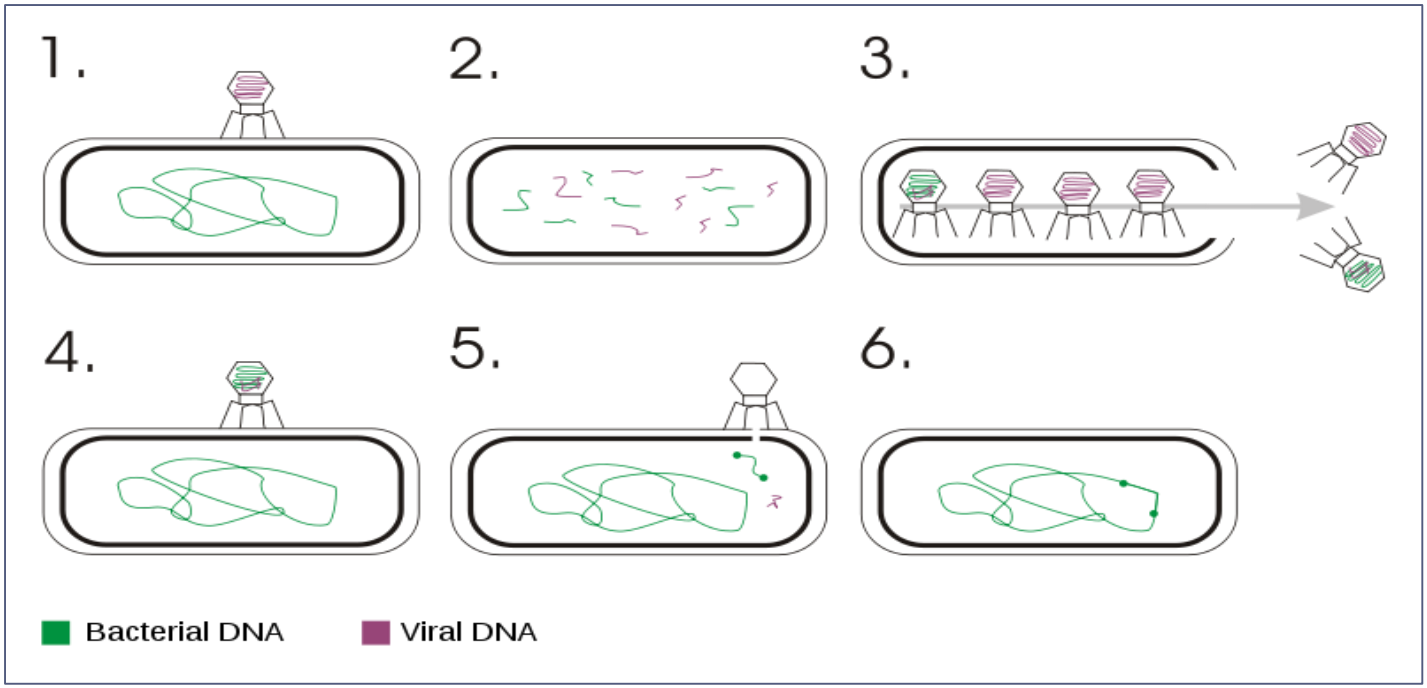
Bacteria in general can acquire new genetic information by:



During conjugation → direct contact.

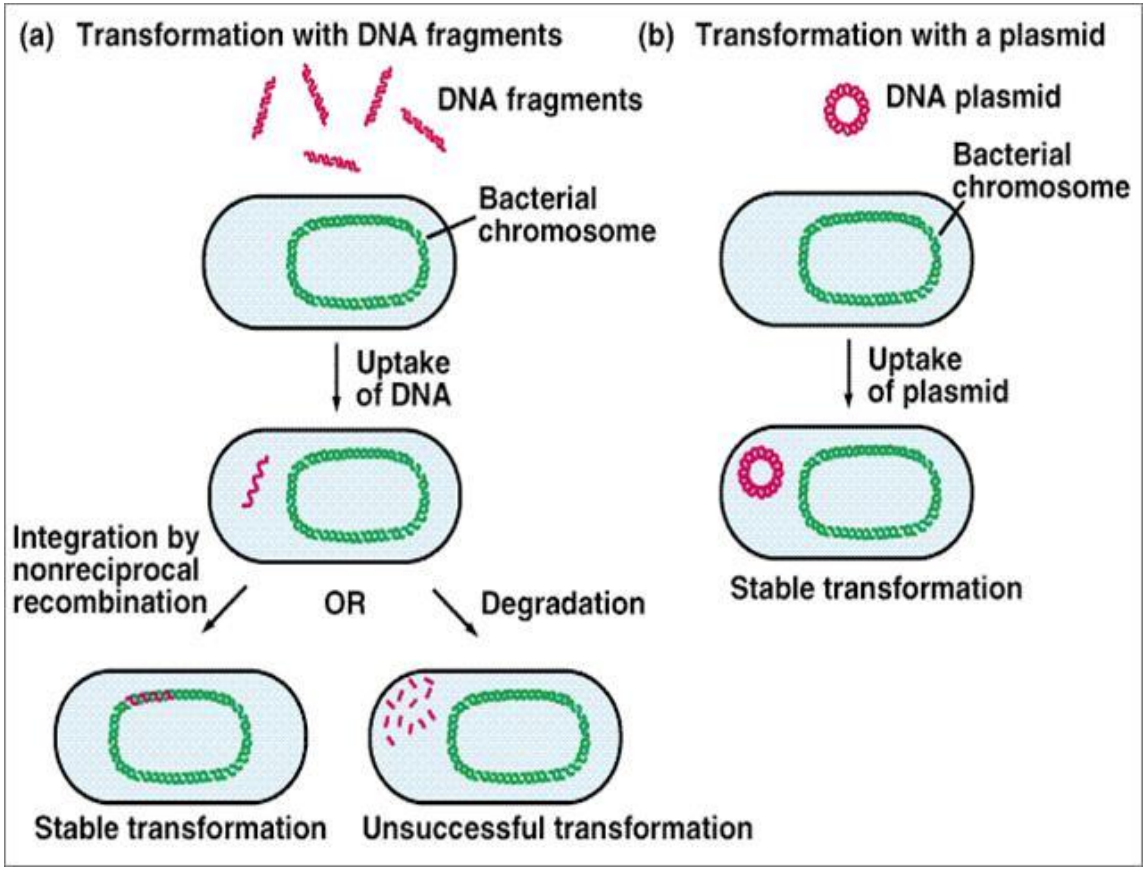
1

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



2

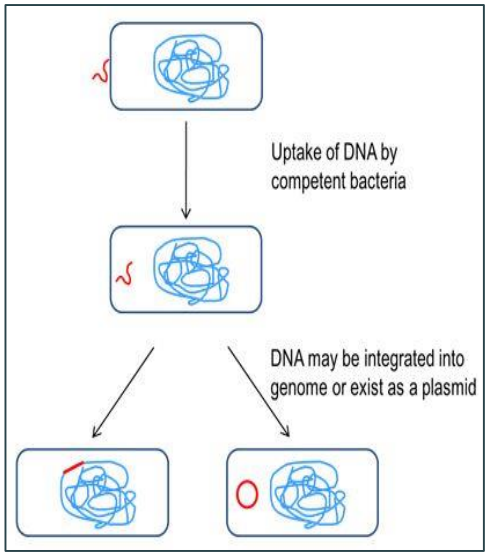
Transduction: is the process by which DNA is transferred from one bacterium to another by a virus [bacteriophges].



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 :

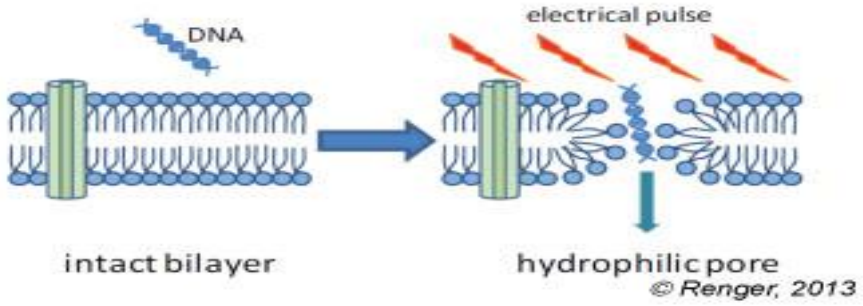
Natural competence: a genetically specified ability of bacteria that is occur under natural condition.

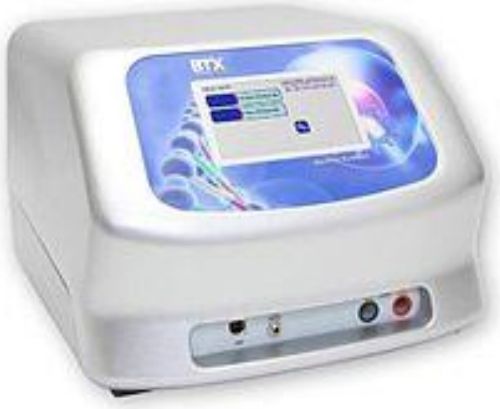
Artificial competence: when cells in laboratory cultures are treated to be permeable to DNA.

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.





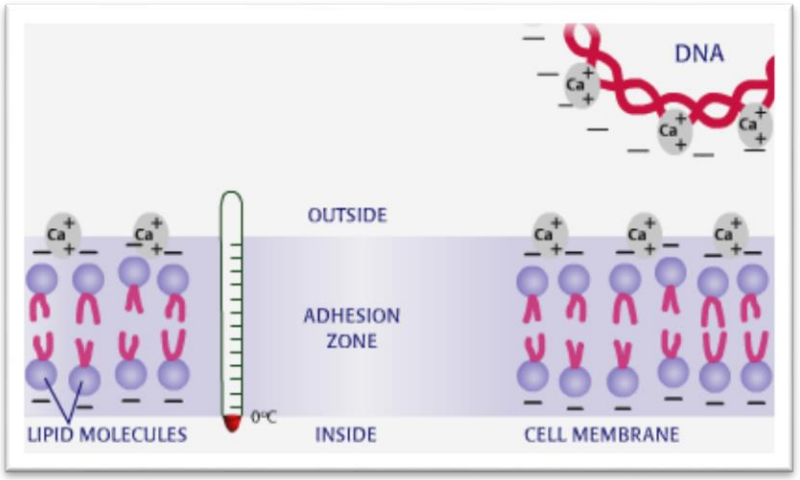
2.Chemical transformation.

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 (µg) 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.

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

CFU: colony –forming units.