TRANSFORMATION OF DNA INTO HOST CELL

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INTRODUCTION

- Transformation is a process by which bacteria is made to take up exogenous **DNA**.
- The word transformation is derived from Griffth's discovery of a "**Transforming Principle**".
- Transformation can occur in two ways: natural transformation and artificial transformation.
- Transformation is one of the most popular techniques of molecular genetics because it is often the best way to reintroduce experimentally altered DNA into cells.

There are two forms of transformation:

Each process depends on the ability of the organism to transform the DNA into the host cells.

- **Natural**
  - In natural transformation, bacteria are capable of DNA naturally which means they can take up DNA from their environment directly. That kind of bacteria is called as naturally transformable.
- **Artificial**
  - In artificial transformation, bacteria are not naturally transformable which they do not take up DNA from the environment.
  - Bacterial cells have been exposed to particular chemical or electrical treatments to make them more permeable and then only the cells can take up DNA efficiently.

INTRODUCTION CONTD...

- In general transformation is the addition of foreign deoxyribonucleic acid (DNA) into a bacterium.

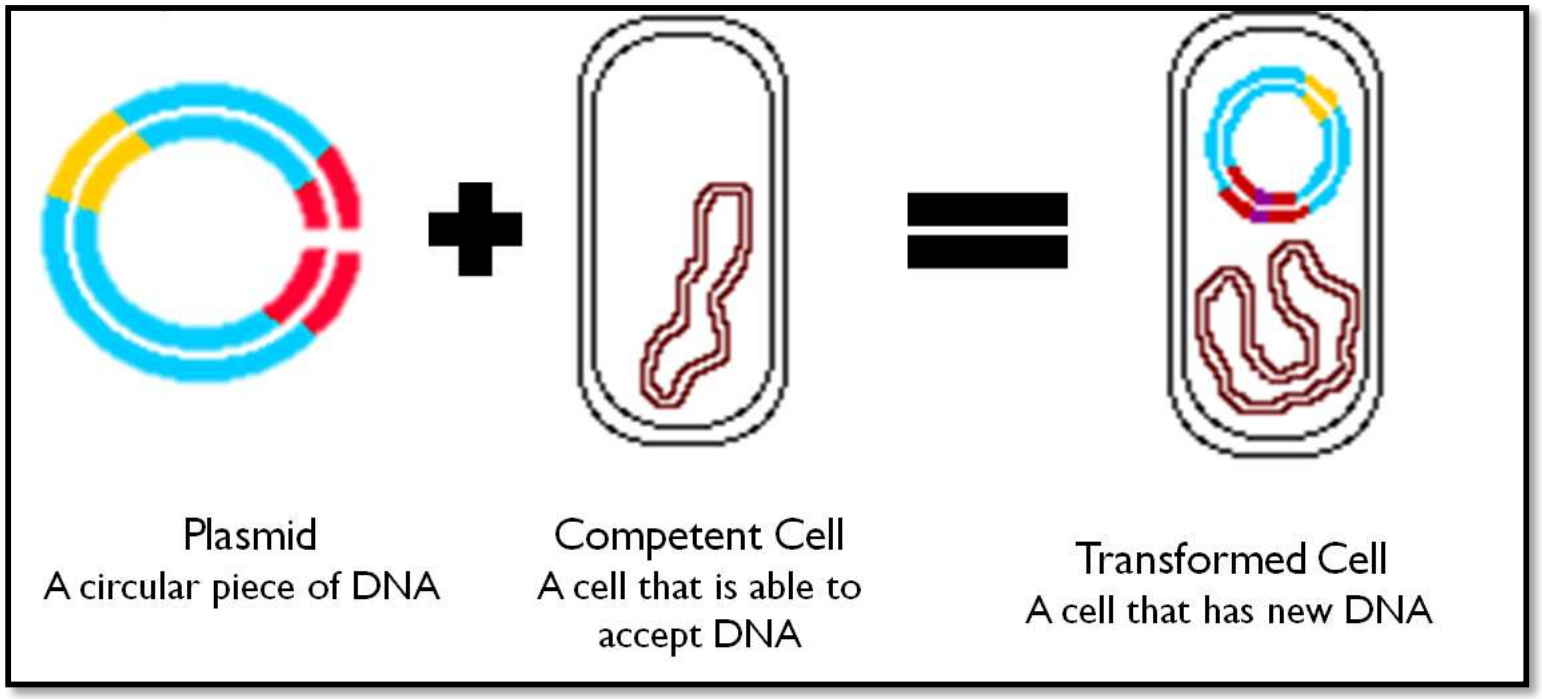

FIGURE.1. DIAGRAMMATIC REPRESENTATION.

HISTORY

- 1928 - **Frederick Griffith** transformed non-pathogenic pneumococcus bacteria into a virulent variety by mixing them with heat-killed pathogenic bacteria.
- Transformation principle was demonstrated in 1944 by **Oswald Avery, Colin MacLeod, and Maclyn McCarty**, who showed gene transfer in *Streptococcus pneumoniae* was pure DNA.
- Avery, Macleod and McCarty called the uptake and incorporation of DNA by **Bacterial transformation.**

METHODS

- BIOLOGICAL METHODS
- DIRECT GENE TRANSFER BY CHEMICAL AND BIOCHEMICAL MEANS
- DIRECT GENE TRANSFER BY PHYSICAL MEANS

TABLE.NO. 1: BIOLOGICAL METHODS

BIOLOGICAL METHODS	
METHOD	ORGANISM TRANSFORM
In vitro packaging and phage infection	**Bacteria**
Agrobacterium tumefaciens-mediated transformation	**Plants**
Plant-virus mediated transformation	**Plants**
Transposon-mediated transformation	**Insects**

TABLE NO. 2 :DIRECT GENE TRANSFER BY PHYSICAL MEANS

DIRECT GENE TRANSFER BY PHYSICAL MEANS	
METHOD	**ORGANISM TRANSFORMED**
Microprojectile bombardment	Plants
Electroporation	Plants, Animals Bacteria
Microinjection	Plants, Animals
Silicon carbide fibers- mediated transformation	Plants
Ultrasonication	Plants
UV laser microbeam irradiation	Plants

GENE GUN MEDIATED TRANSFORMATION (MICRO-PROJECTILE BOMBARDMENT TRANSFORMATION)

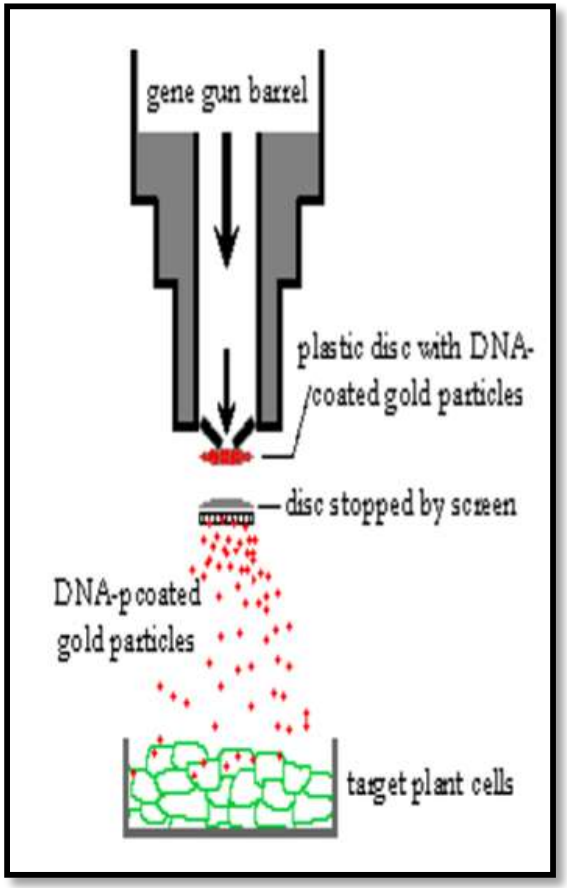

FIG.2. MECHANISM

FIG. 3. INSTRUMENT

ELECTROPORATION

FIG. 4. ELECTROPORATION INSTRUMENT AND MECHANISM

MICROINJECTION

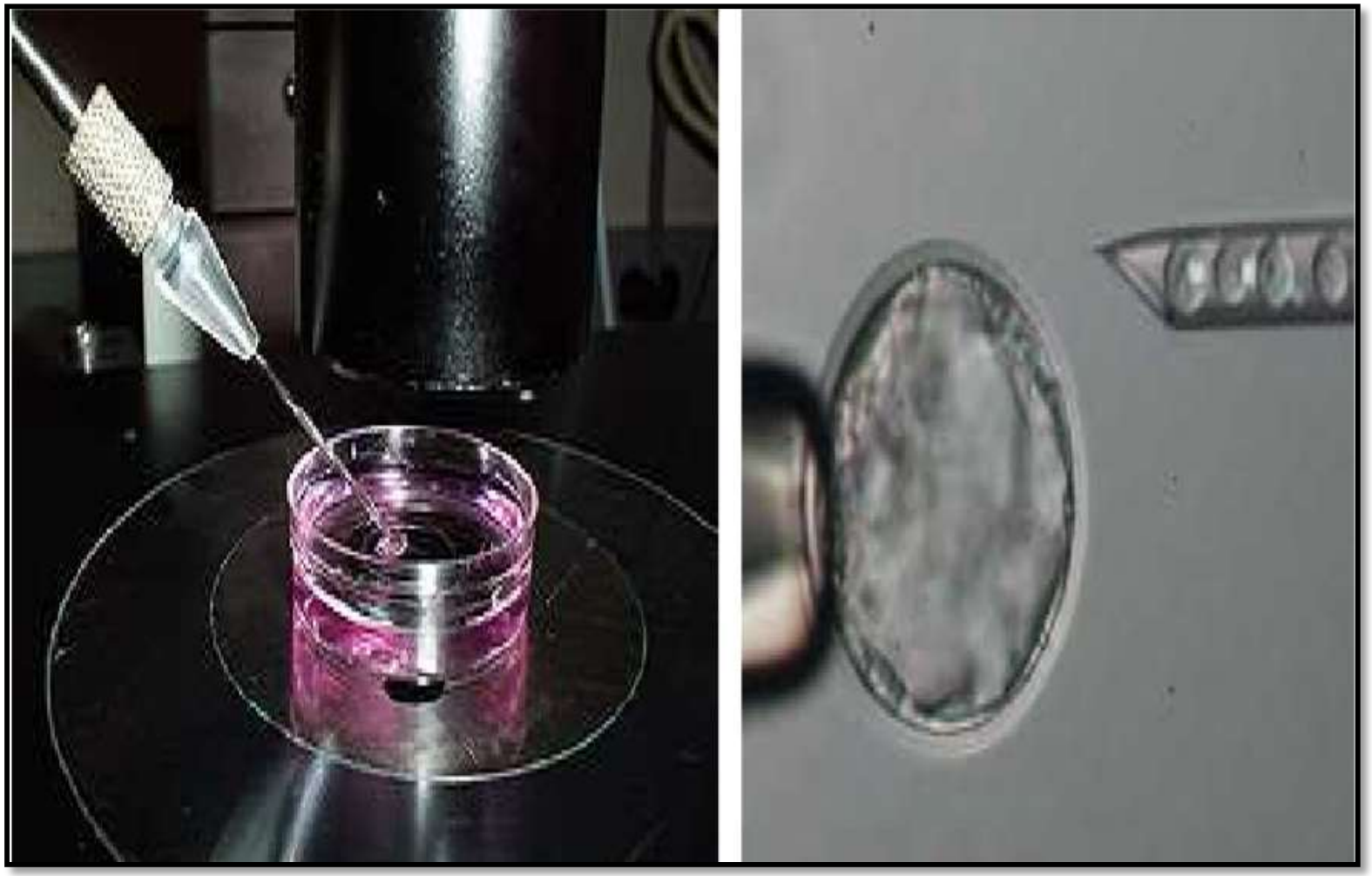

FIG.5 . MICROINJECTION

TABLE NO.3. : DIRECT GENE TRANSFER BY CHEMICAL MEANS

DIRECT GENE TRANSFER BY CHEMICAL MEANS	
METHOD	**ORGANISM TRANSFORMED**
$CaCl_2$ / Heat shock	Bacteria, Yeast
Ca-DNA co-precipitation	Plants, Animals
Liposome packaging and fusion or Endocytosis	Plants, Animals
DEAE-dextran transfection	Plants, Animals
Poly cation-mediated DNA uptake	Plants, Animals

IMPORTANT ELEMENTS FOR TRANFORMATION

- Two elements are required in a transformation system:

  - First element:

    First element is a suitable host bacterium. For this, commonly we use *E.coli* as host organism. The strain of *E.coli* has been cultured in the laboratory and it has been selected for characteristics that make it especially useful in the molecular biology laboratory.

CONTD....

- Second element:

- Plasmid is the other important element in the transformation system. Plasmid encodes some enzymes and antibiotic resistant markers which are expressed in the bacterium after transformation.
- When transformation occurs, the DNA transferred is often a plasmid: small, circular DNA found naturally in many bacteria.
- Plasmid is found as extra chromosomal DNA and it contains some genes that the bacterium would not normally possess. These extra genes can provide a growth advantage to bacteria by providing the gene for an enzyme such as amylase, beta-lactamase.

WORKING

- There are two major parameters involved in efficiently transforming a bacterial organism.
- The first is the method used to induce competence for transformation.
- The second major parameter is the genetic constitution of the host strain of the organism being transformed.

- Chilling cells in the presence of divalent cations such as Ca2+ (in CaCl2) prepares the cell walls to become permeable to plasmid DNA.
- Cells are incubated with the DNA in ice and then briefly heat shocked (42°C for 30-120 seconds), and further plunging them in an ice., which causes the DNA to enter the cell.
- Then it is plated in an agar plate containing appropriate antibiotic. The presence of an antibiotic marker on the plasmid allows for rapid screening of successful transformants.

- This method works well for circular plasmid DNAs but not for linear molecules such as fragments of chromosomal DNA.
- An excellent preparation of competent cells will give highers number of colonies per μg of plasmid. A poor preparation will be about moderate or less.

$CaCl_2$ METHOD

- This method also alters the permeability of the cell membrane:
- Ca2+ interacts with the negatively charged phospholipid heads of the cell membrane, creating an electrostatically neutral situation.
- Lowering the temperature stabilizes the membrane, making the negatively charged phosphates easier to shield.
- Then a heat shock creates a temperature imbalance and thus a current, which helps get the DNA into the cell.

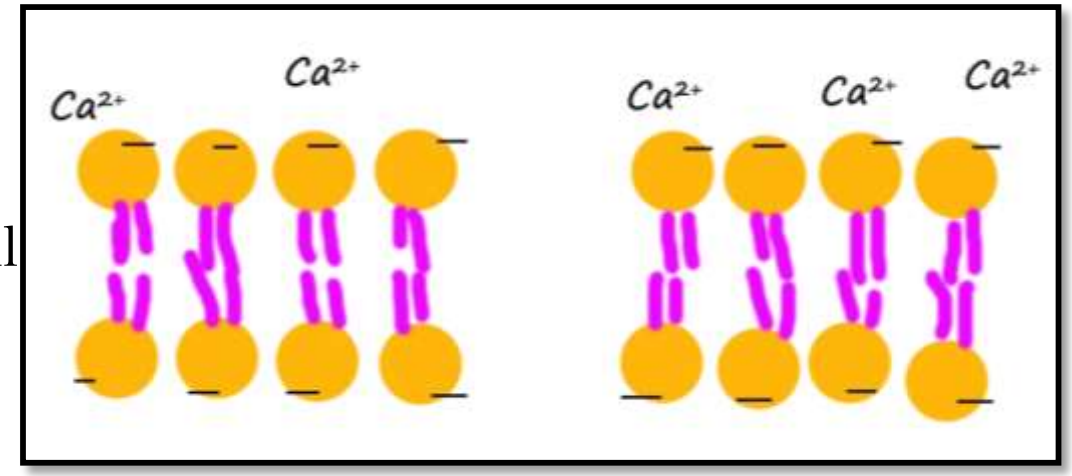

FIG. 6. INTERACTION BETWEEN Ca^{2+} IONS AND PHOSPHOLIPID LAYER

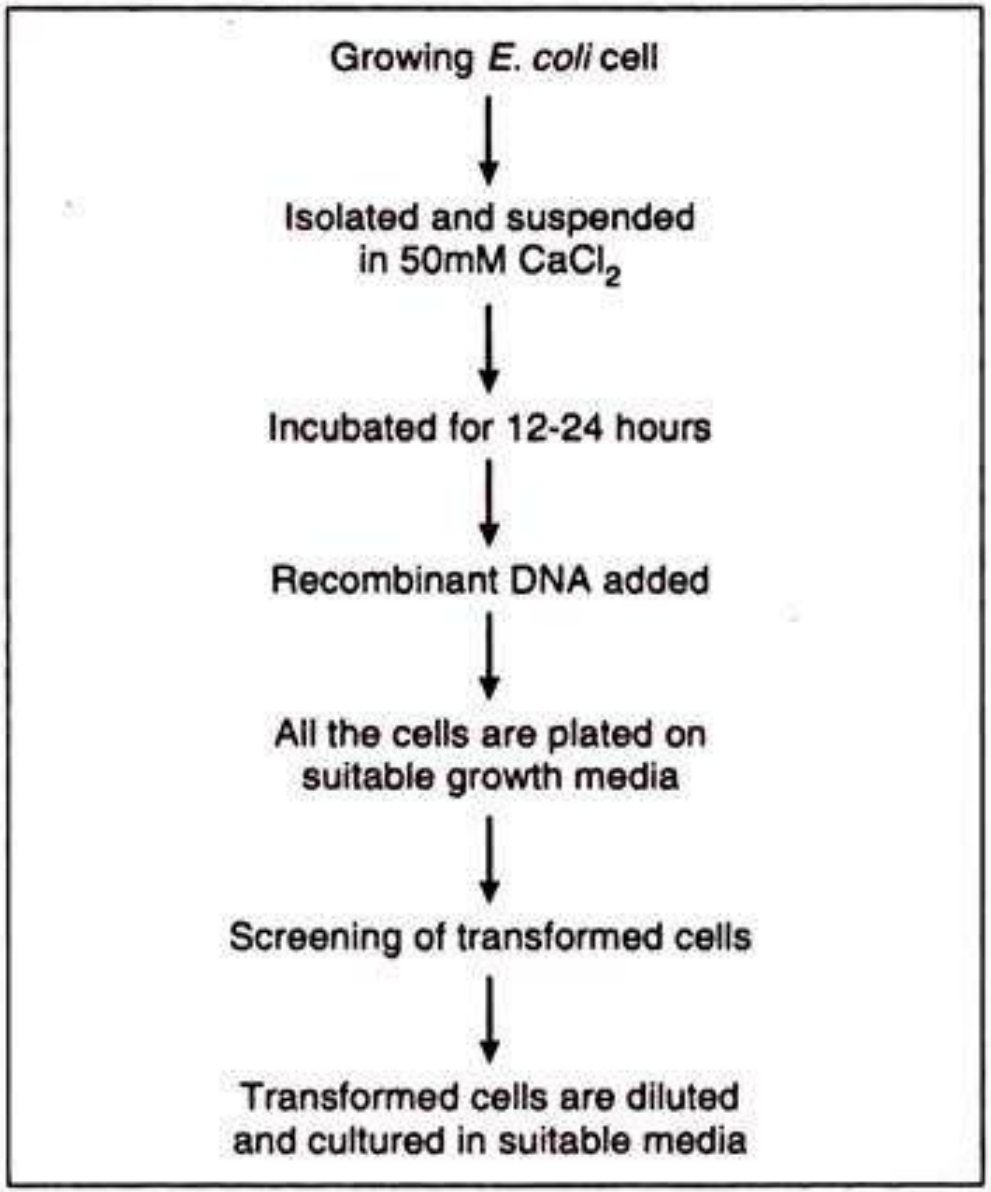

FIG. 7. : CALCIUM CHLORIDE ($CaCl_2$) MEDIATED DNA TRANSFER

BLUE AND WHITE SCREENING

- Blue –white selection (**Alpha complementation**) can be used to determine which plasmids carry an inserted fragment of DNA and which do not. These plasmids contain an additional gene (lac Z) that encodes for a portion of the enzyme β – galactosidase.
- When it transformed into an appropriate host, one containing the gene for the remaining portion of β –galactosidase, the intact enzyme can be produced and these bacteria form blue colonies in the presence of **X -gal** (5-bromo-4-chloro-3-indoyl-b-D-galactoside) and a gratuitous inducer called **IPTG** (Isopropyl β-D- Thiogalactopyronoside).
- These plasmids contains a number of cloning sites within the **lac Z gene**, and any insertion of foreign DNA into this region results in the loss of the ability to form active β –galactosidase. Therefore colonies that carry the plasmid with the insert,

  i.e. Transformants will remain white and the colonies without the foreign DNA (Non-Transformants) will remain **Blue.**

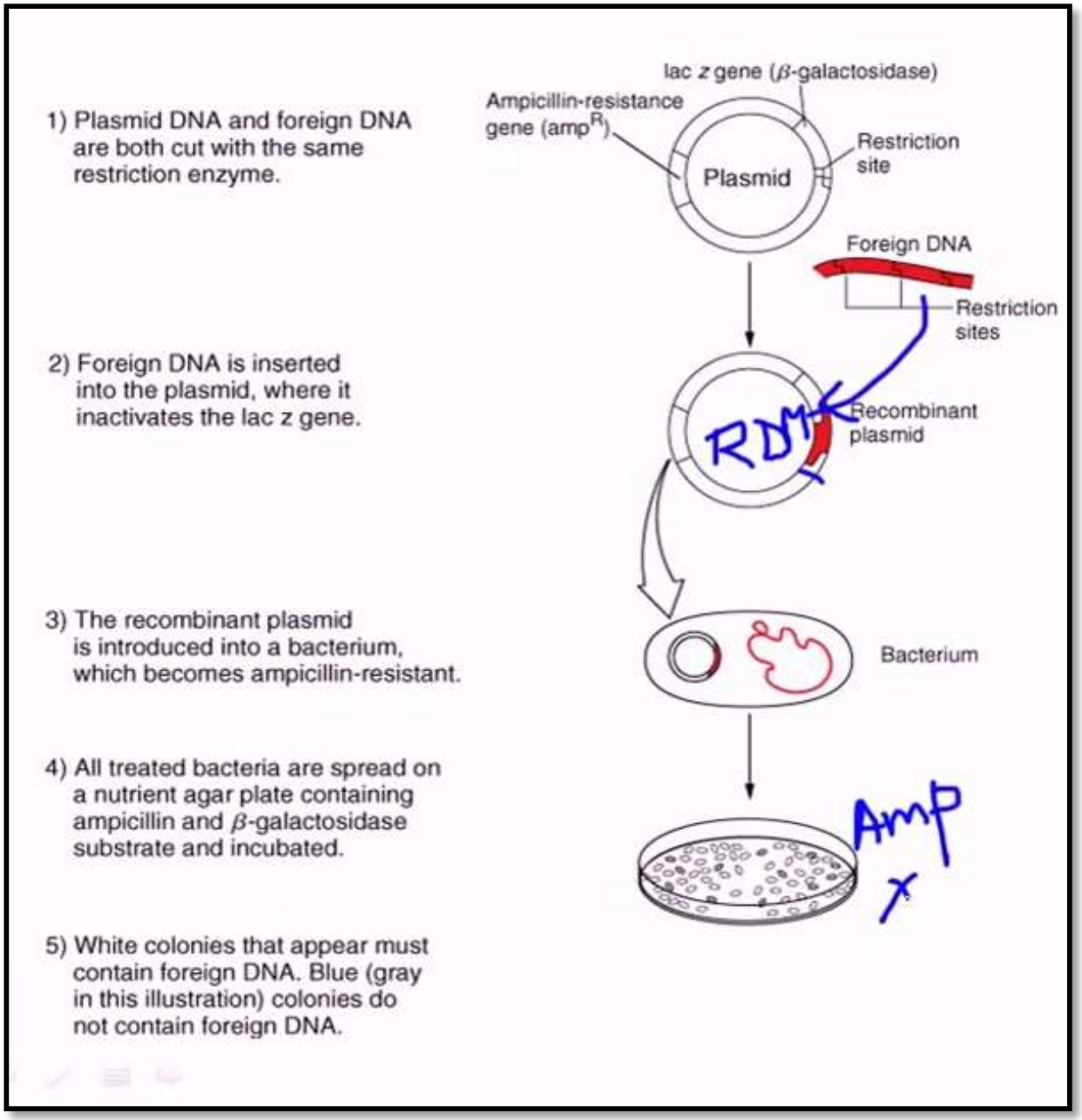

FIG. 9. DRAGRAMATIC REPRESENTATION

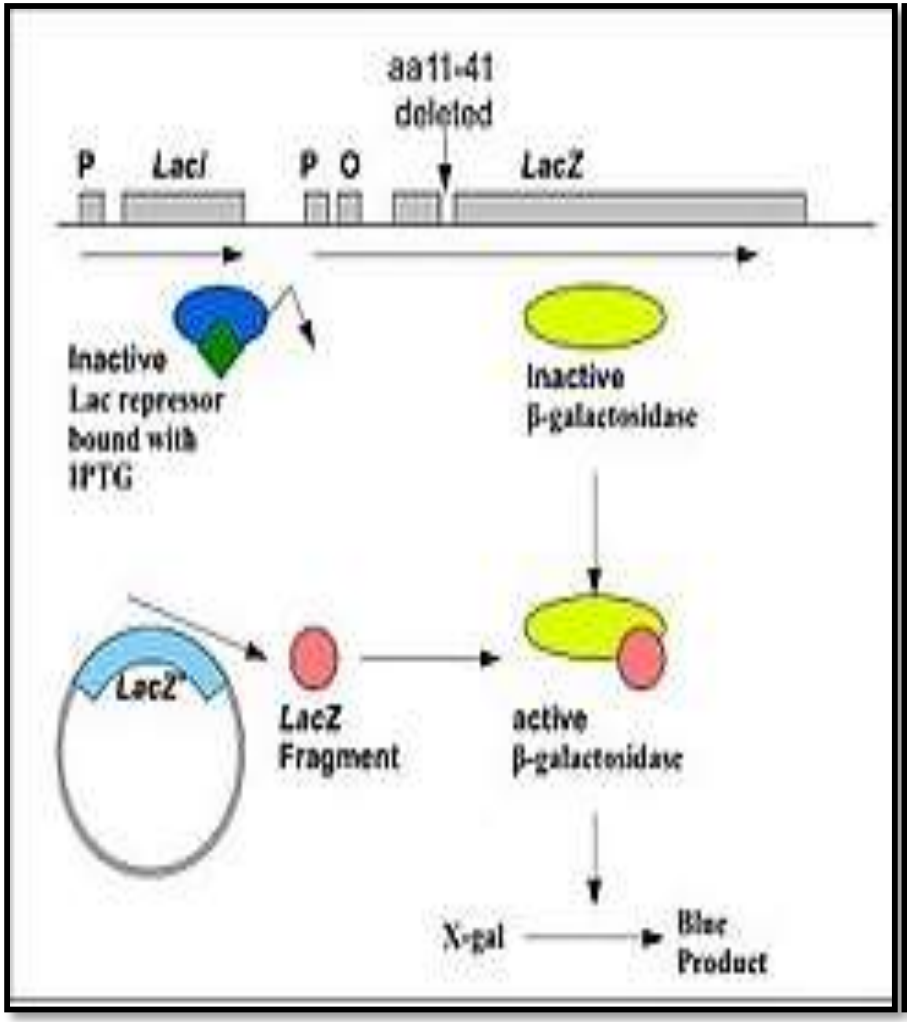

FIG. 9. : DIAGRAMMATIC REPRESENTATION

FIG.10.: BLUE AND WHITE COLONIES ON AGAR PLATE. (ALPHA COMPLEMENTATION)

TRANSFORMATION EFFICIENCY

- **Transformation efficiency** is the **efficiency** by which cells can take up extracellular DNA and express genes encoded by it.
- This is based on the competence of the cells. It can be calculated by dividing the number of successful transformants by the amount of DNA used during a **transformation** procedure.

TRANSFORMATION EFFICIENCY

$$\text{Transformation Efficiency (TE)} = \frac{\text{Number of Transformed Colonies}}{\text{Amount of Plasmid Added to Bacteria}}$$

- Transformation efficiency decreases with increase in size of DNA molecule.
- Transformation efficiency also increases with the increase in the number of transformed colonies.

FATORS AFFECTING TRANSFORMATION EFFICIENCY

- Temperature of competent cells during preparation, storage and transformation
- Concentration of $CaCl_2$.
- Culture medium
- Strain of bacteria
- Bacterial colony's phase of growth

USES OF BACTERIAL TRANSFORMATION

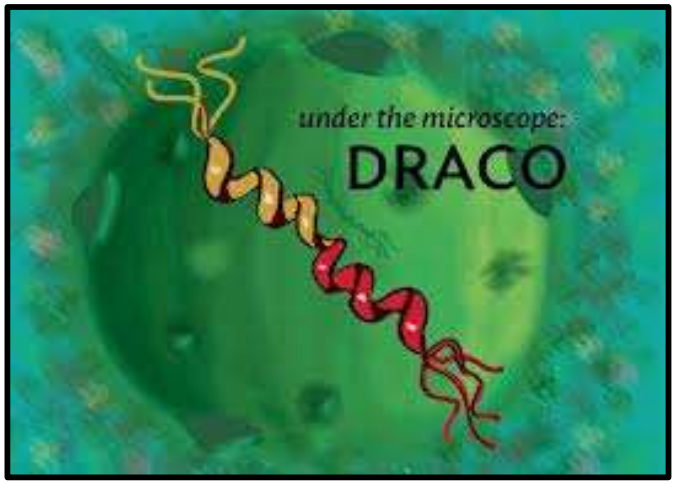

FIG.11. DRACO

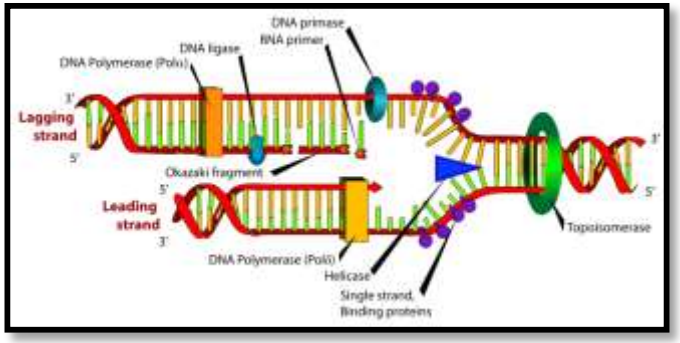

FIG. 12. MASS PRODUCTION OF DNA

To mass produce proteins
e.g. DRACO protein gives immunity to all viruses (e.g., cold, flu, Ebola)

To transfer traits among species e.g. Transfer of luminescence genes can be used to create secret codes

To mass produce DNA
DNA is multiplied each time a cell reproduces

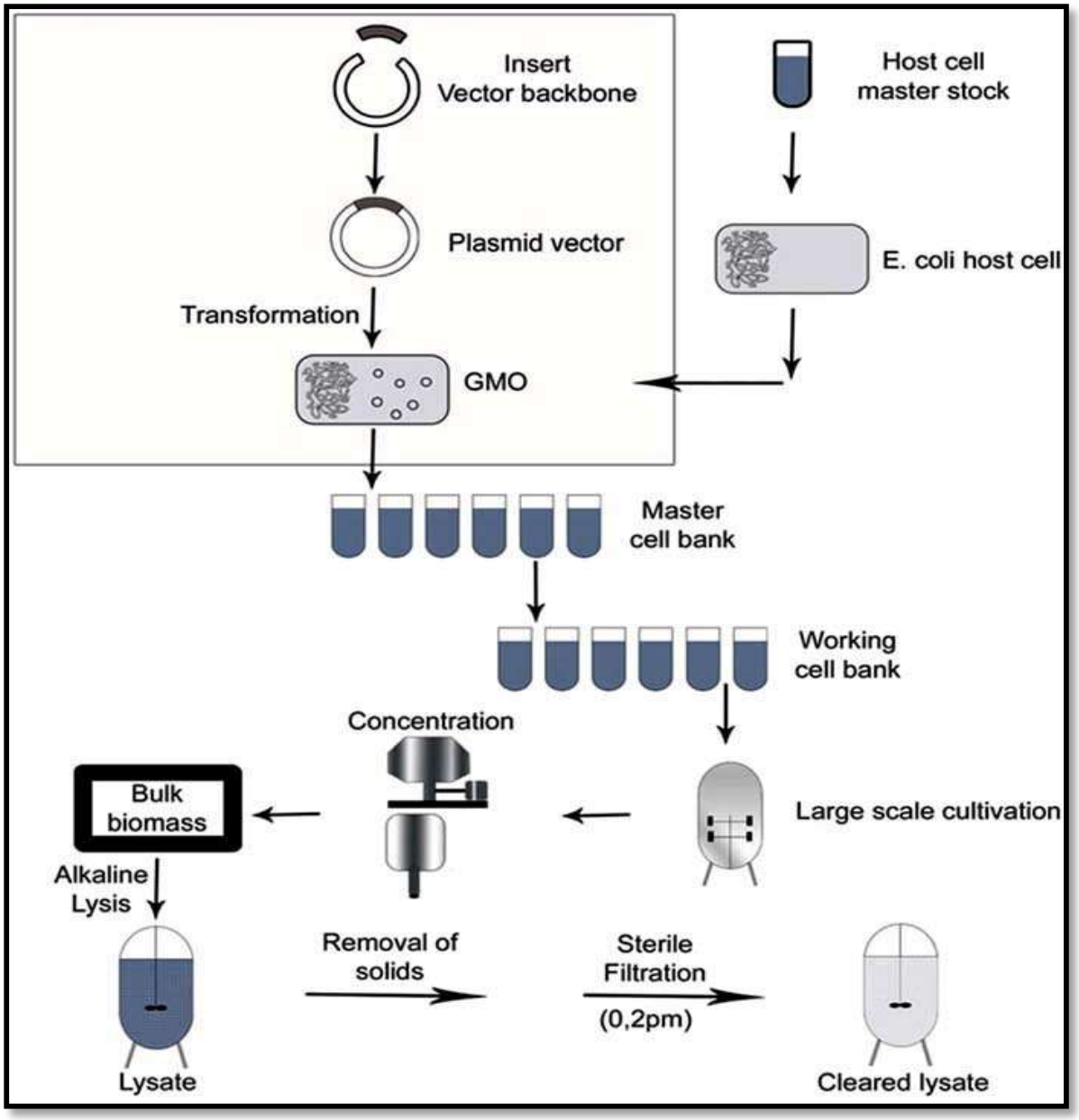

FIG.13 : LARGE SCALE PRODUCTION OF DNA

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“Thank You”

ANY QUESTIONS???