

TOPIC : RECOMBINANT DNA TECHNOLOGY – II

**(CLONING STRATEGIES AND METHODS OF
SELECTION OF TRANSFORMANTS)**

COURSE : M.SC. BOTANY PART II

PAPER : XVI (BIOTECHNOLOGY AND BIOINFORMATICS)

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CLONING STRATEGIES

- ✓ The process of uptaking foreign DNA by the host cell is known as transformation.
- ✓ The transforming ability can be induced artificially by various methods depending upon the host cell.
- ✓ **The various techniques to transfer cloning vector to host cell w.r.t. type of cells are discussed below :**

Calcium chloride (CaCl₂) method : In this, bacterial cells are treated with cold CaCl₂ which make the cell competent for transformation. After addition, cells are given heat shock treatment at 42°C for 90s which transiently create pores in the cell membrane allowing entry of foreign DNA.

BACTERIAL CELLS

Transfection : It is the transfer of foreign DNA into cultured host cell mediated through charged chemicals (cationic liposomes, calcium phosphate, DEAE dextran) which are taken & mixed with DNA molecules. The recipient host cell is overlaid by this mixture & foreign DNA is taken up by the host cell.

ANIMAL CELLS

Microinjection : A technique of delivering foreign DNA into a living cell (cell, egg, oocyte, embryos of animals) through a glass micropipette. (also used in plant cell)

Direct transformation : A foreign DNA fragment is precipitated with calcium phosphate & mix with the cells to be transformed. DNA molecule passes through the cell membrane & integrates with the mammalian chromosome.

Vector-mediated or indirect gene transfer method: This is mediated by Ti plasmid *Agrobacterium tumefaciens* which is known as natural genetic engineer of plants.

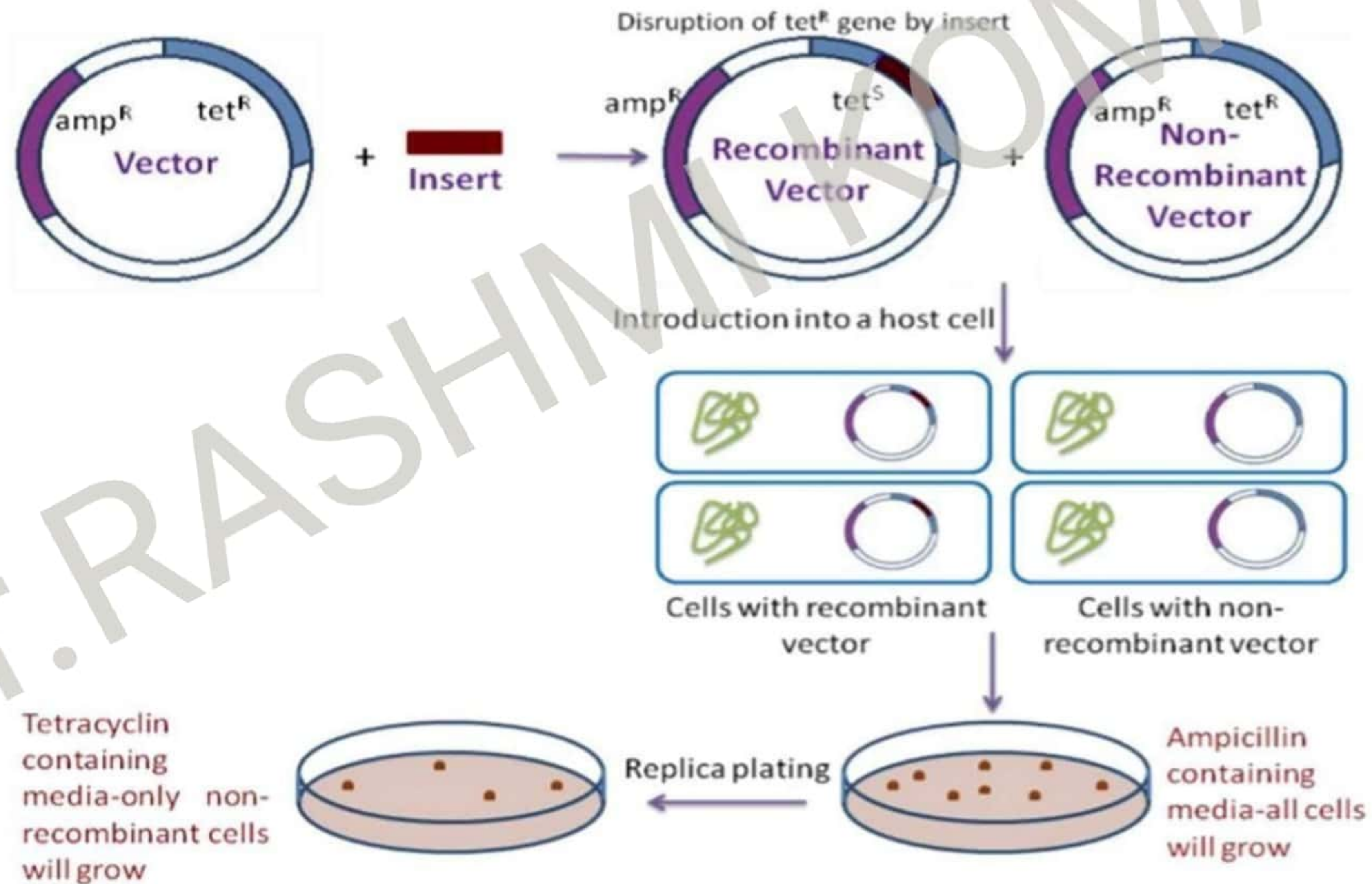
PLANT CELLS

Electroporation : This involves application of a pulse of high voltage (~350 V) to protoplasts/ cells/ tissues which induce the formation of temporary pores in the plasma membrane facilitating the uptake of foreign DNA.

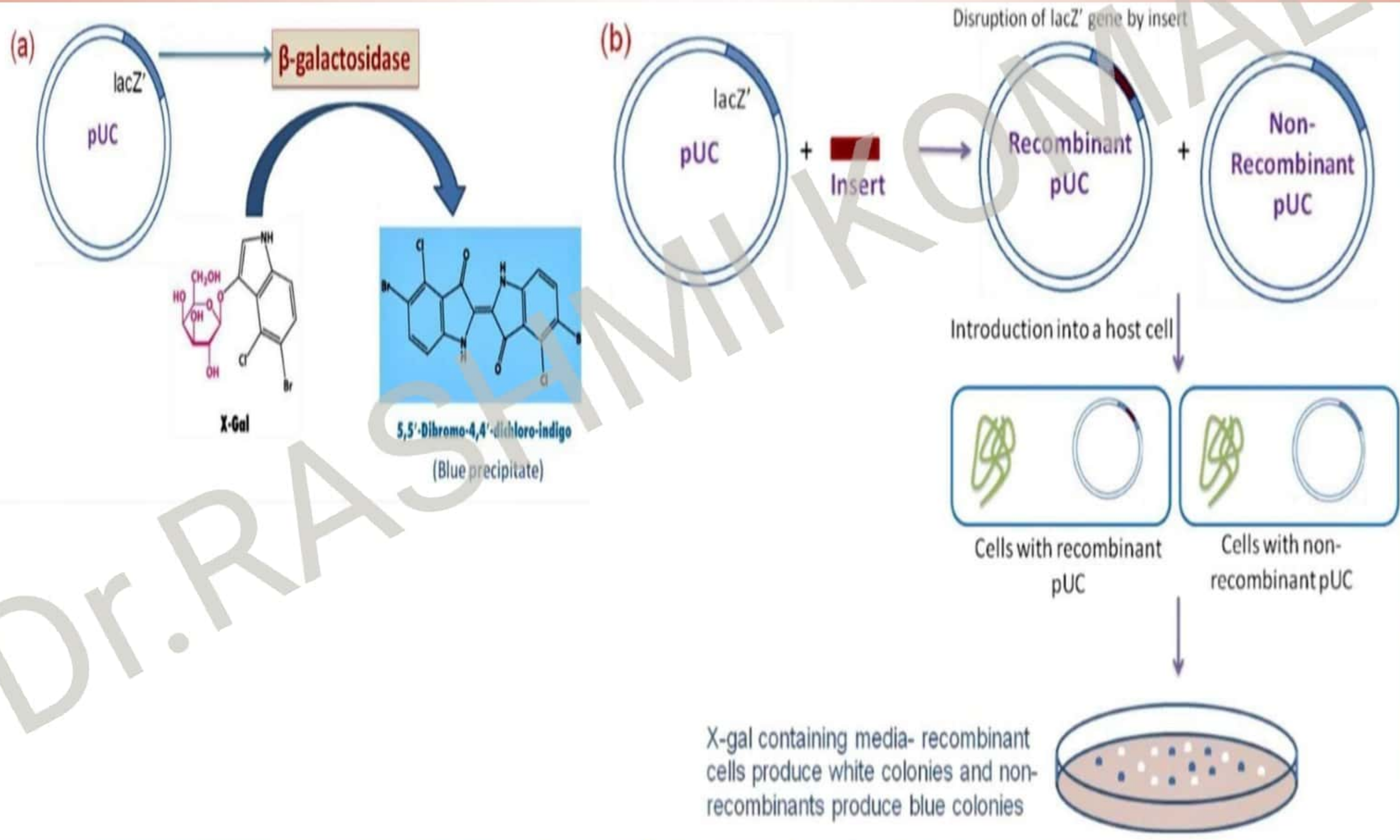
Particle bombardment method (biolistics) : The foreign DNA is coated onto the surface of minute gold or tungsten particles & bombarded (shot) onto the target tissue/ cells using a particle gun (gene gun/ shot gun/ microprojectile gun).

TECHNIQUES FOR SELECTION OF TRANSFORMED CELLS :

I. Using Selectable Marker by Insertional Inactivation Method :



II. Blue and White Method of selection :



THANK YOU