METHODS FOR SELECTION AND SCREENING OF RECOMBINANT TRANSFORMANTS

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SCREENING OF RECOMBINANTS

- A **genetic screen** or **mutagenesis screen** is an experimental technique used to identify and select for individuals who possess a phenotype of interest in a mutagenised population.

I) BLUE-WHITE SCREENING

- The use of chromogenic substrate to detect a particular enzymatic activity is the basis to screen the desired clone.

- The colourless compound X-gal or 5-bromo-4-chloro-3-indolyl-β-D-galactoside used in this screening method is a substrate for β-galactosidase.

- The enzyme β-galactosidase is the product of lacZ gene of the lac operon.

- β-galactosidase is a tetrameric protein and an initial N-terminal region of the protein is important for activity of the protein.

- In this system, host contains lacZ gene without the initial region where as vector contains α-peptide to complement the defect to form active enzyme. As a result, if a vector containing α-peptide will be transformed into the host containing remaining lacZ, the two fragment will reconstitute to form active enzyme.

- In addition, the α-peptide region in vector contains multiple cloning site and as a result of insertion of gene fragment, consequently α-peptide will not be synthesized to give fully active β-galactosidase.

- The enzyme β-galactosidase oxidizes x-gal to form 5-bromo-4-chloro-indoxyl and galactose. The indoxyl derivative is oxidized in air to give a blue colored dibromo-dichloro derivative.

- Hence, blue colored colonies indicate the absence of insert where as colorless colonies indica̶t̶e̶ ̶p̶r̶e̶s̶e̶n̶c̶e̶ ̶o̶f̶ ̶i̶n̶s̶e̶r̶t̶
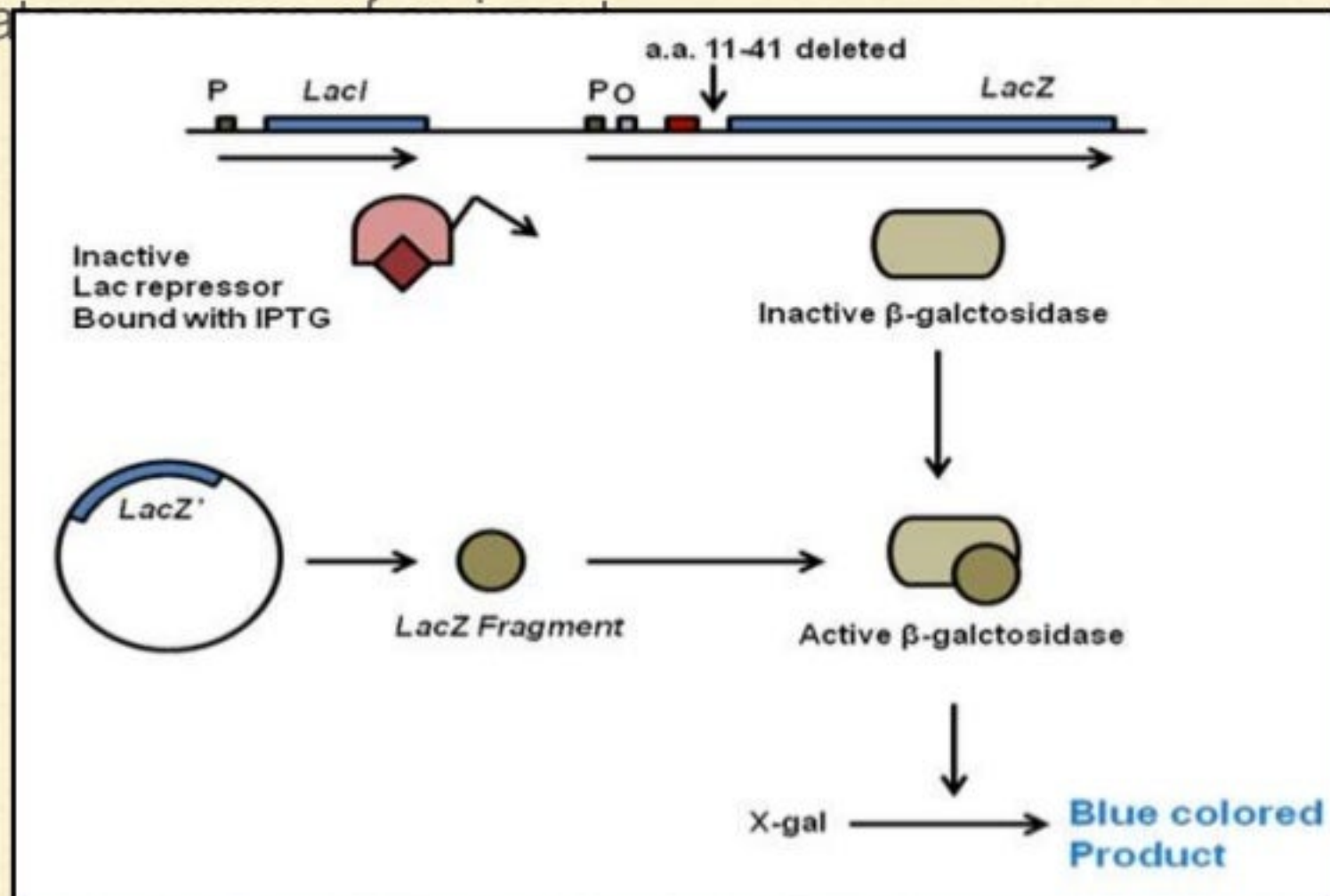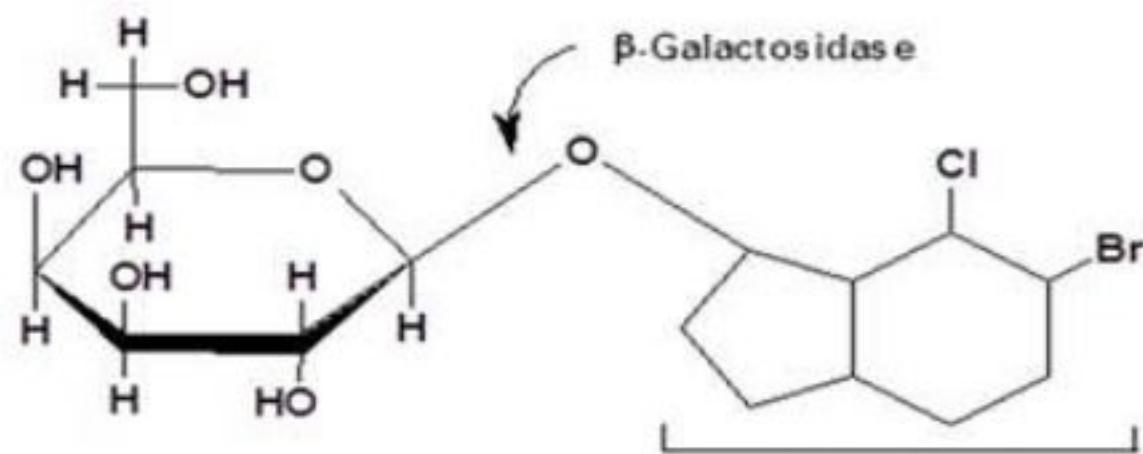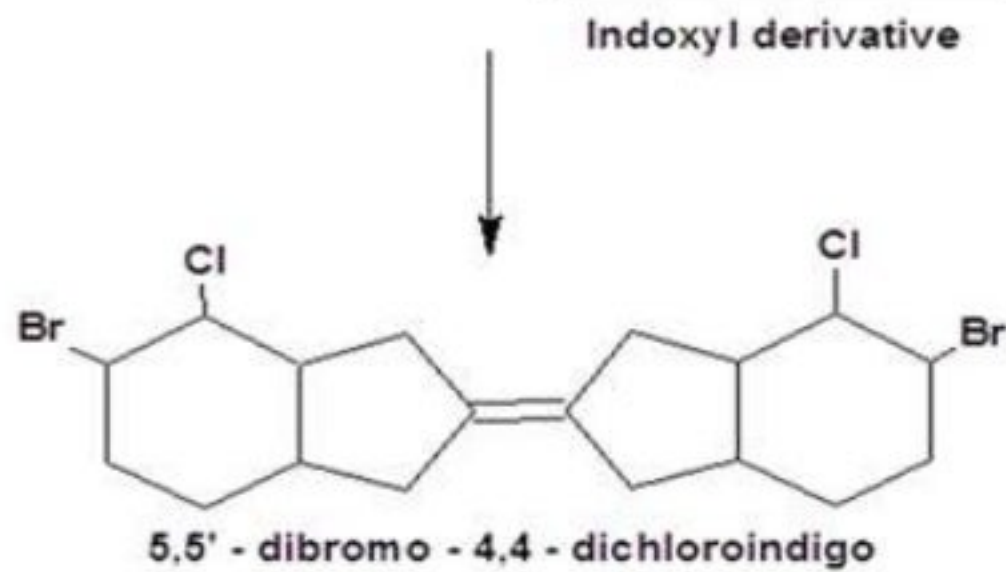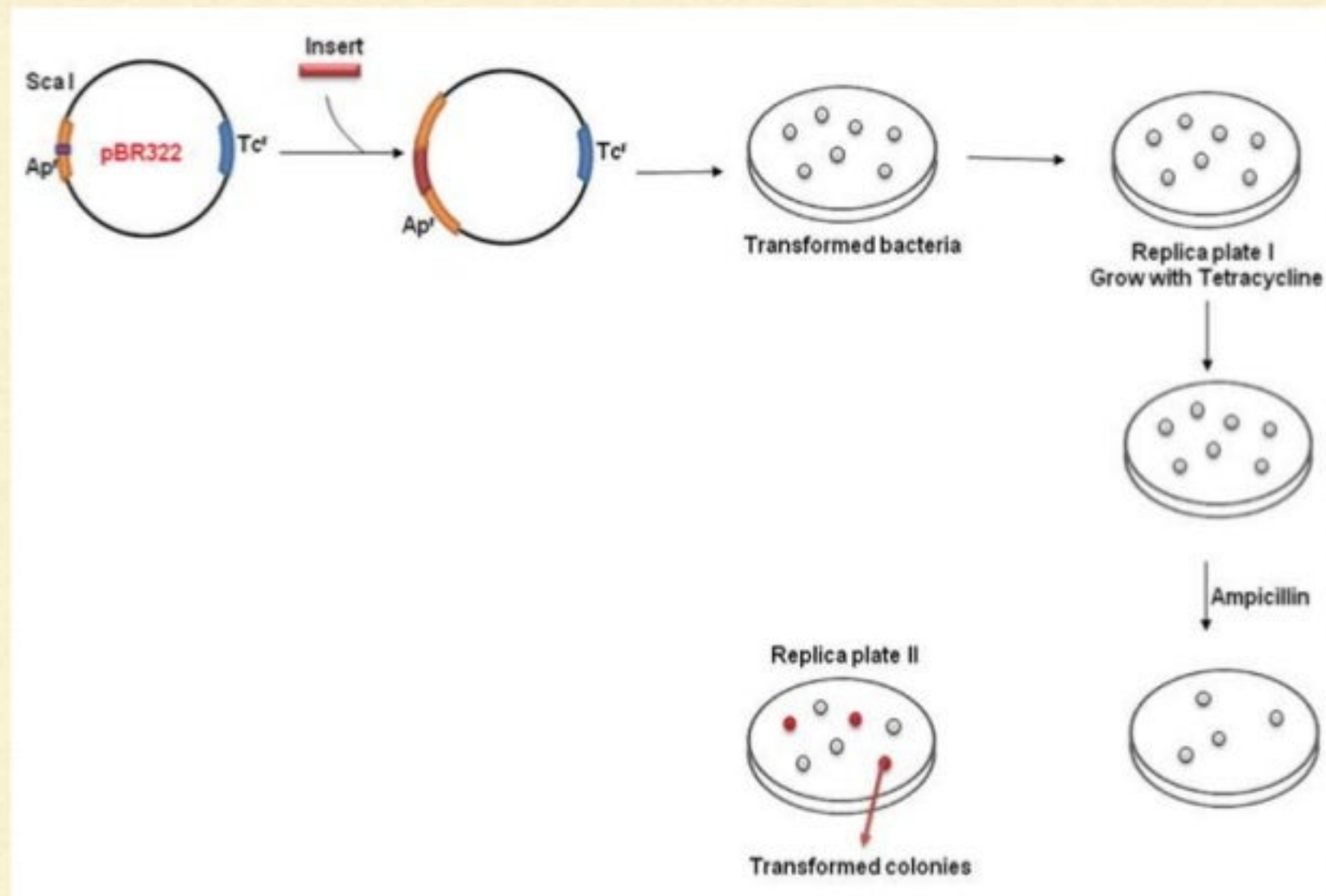
Chemical conversion of X-gal

II) INSERTIONAL INACTIVATION

1) Insertional Inactivation of antibiotic resistance gene-

Bacterial plasmid PBR322 has two antibiotic resistance gene, Ap and Tc. If a gene fragment will be cloned in ScaI, it will disrupt the Ap gene. As a result, the clone will be ampicillin sensitive and Tc. where as the original plasmid will be Ap and Tc.

2) Insertional inactivation of cl gene-

During an infection cycle, virus undergoes a lytic and lysogenic stages. The cl gene encodes for cl repressor which is responsible for the formation of lysogens. In the presence of functional cl, the plaques contains unlysed host cells and has a turbid appearance where as in the absence it will clear.

This feature can be use to screen the clone to detect functional cl (absence of clone) or absence of cl (presence of insert).
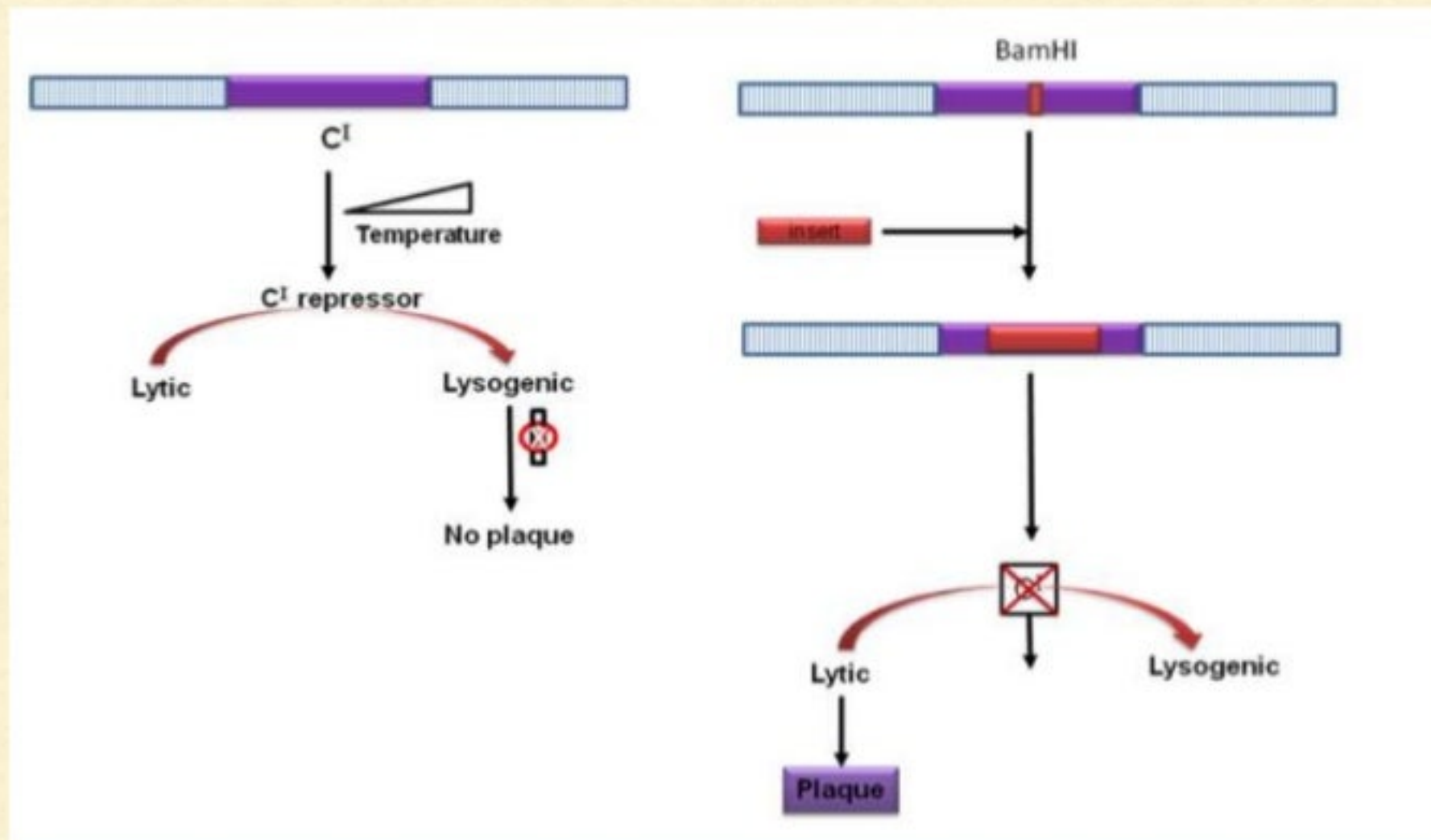
III) ANTIBIOTIC SENSITIVITY

- In this approach, a circular plasmid containing antibiotic resistance gene can be able to replicate into the host cell plated on a antibiotic containing media. In the cloning of a fragment into this plasmid, the plasmid is cut with restriction enzymes and a fragment in ligated to give circular plasmid with insert.

- Cut plasmid and circularized clone into the host and plated onto the antibiotic containing solid media.

- Only circularised clone will give colonies where as cut plasmid will not grow as it has lost antibiotic resistance gene.
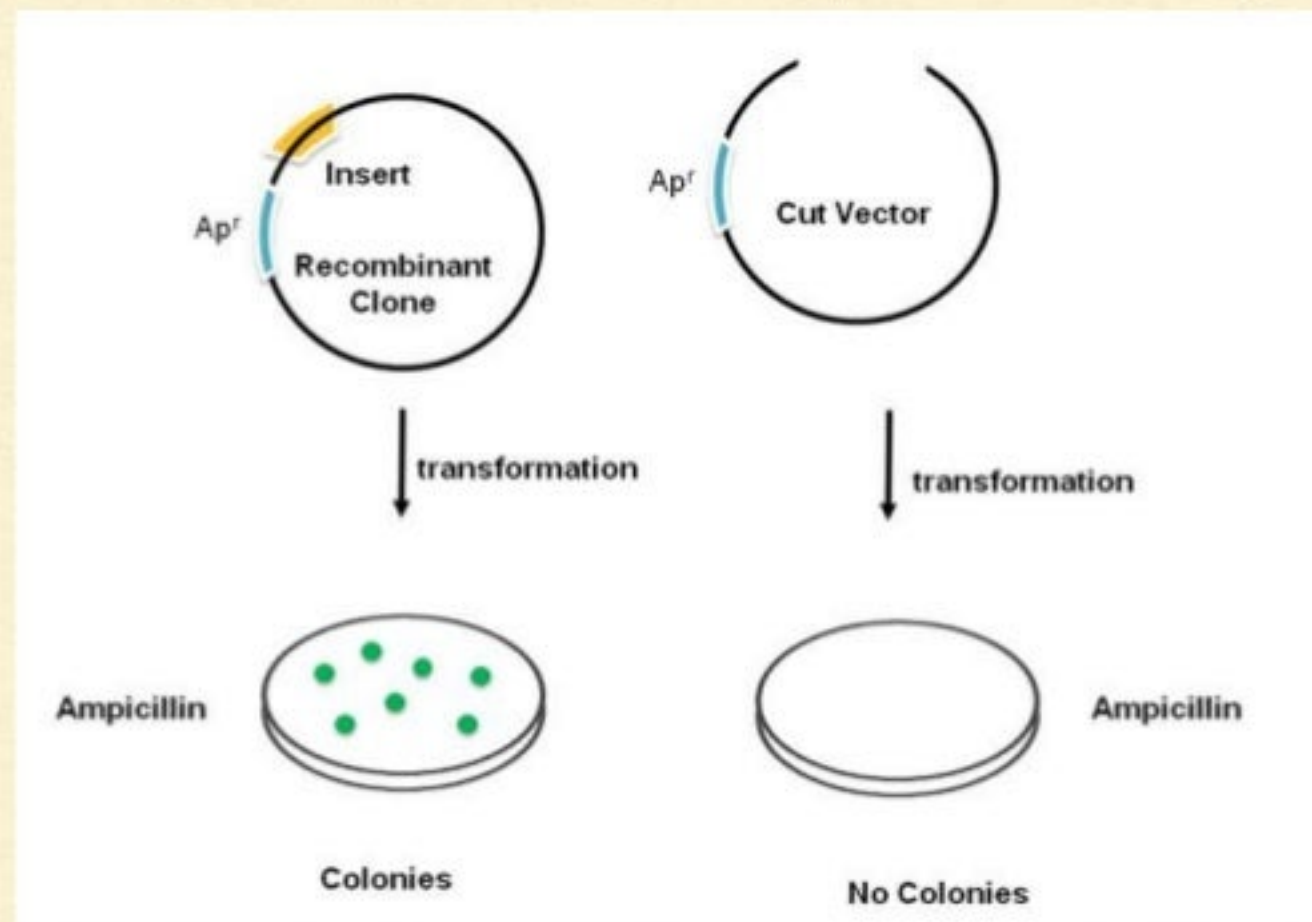
IV) AUXOTROPHIC YEAST STRAIN

- Yeast vector has 4 different gene His3, Leu2, Trp1 and Ura3 as selectable marker.

- Yeast host with a mutation in these gene are available and can be use with the yeast vector to screen the recombinant clone.

- Ura3 and Lys2 marker offer both positive and negative selection.

  - **POSITIVE SELECTION-** In the positive selection, host strain doesn't grow on the media lacking the functional gene but the host transformed with the recombinant clone can be able to supply the gene product required to grow in the media.

SELECTION BASED ON GROWTH ON MINIMAL MEDIUM

- In yeast, an auxotrophic mutant that has non-functional *leu2* gene is used as a host. Such a mutant is able to survive only if leucine is supplied in the growth medium. However, transformants are able to grow on a minimal medium (contains no added leucine) due to presence of *leu2* gene in the vector

- **NEGATIVE SELECTION-** In the negative selection, a chemical compound is added to the media which will be converted to the cyto-toxic agent in the presence of gene product,.

- Ura3 codes for orotine-5'-monophosphate (OMP) decarboxylase and an active enzyme process the 5-fluoro-orotic acid to the toxic fluorodeoxyuridine. Generation of this cyto-toxic agent kills the cells carrying functional Ura3 gene product.
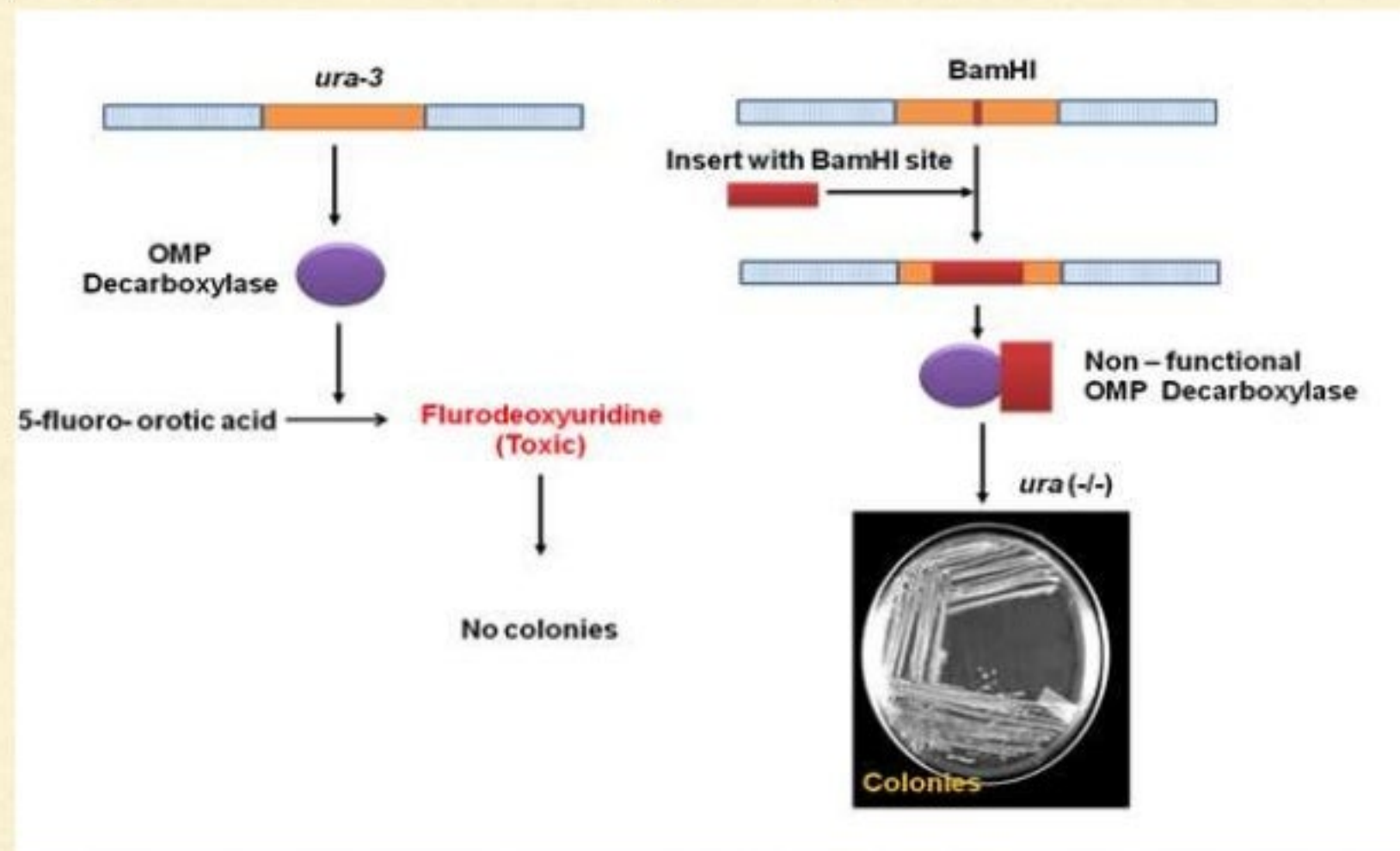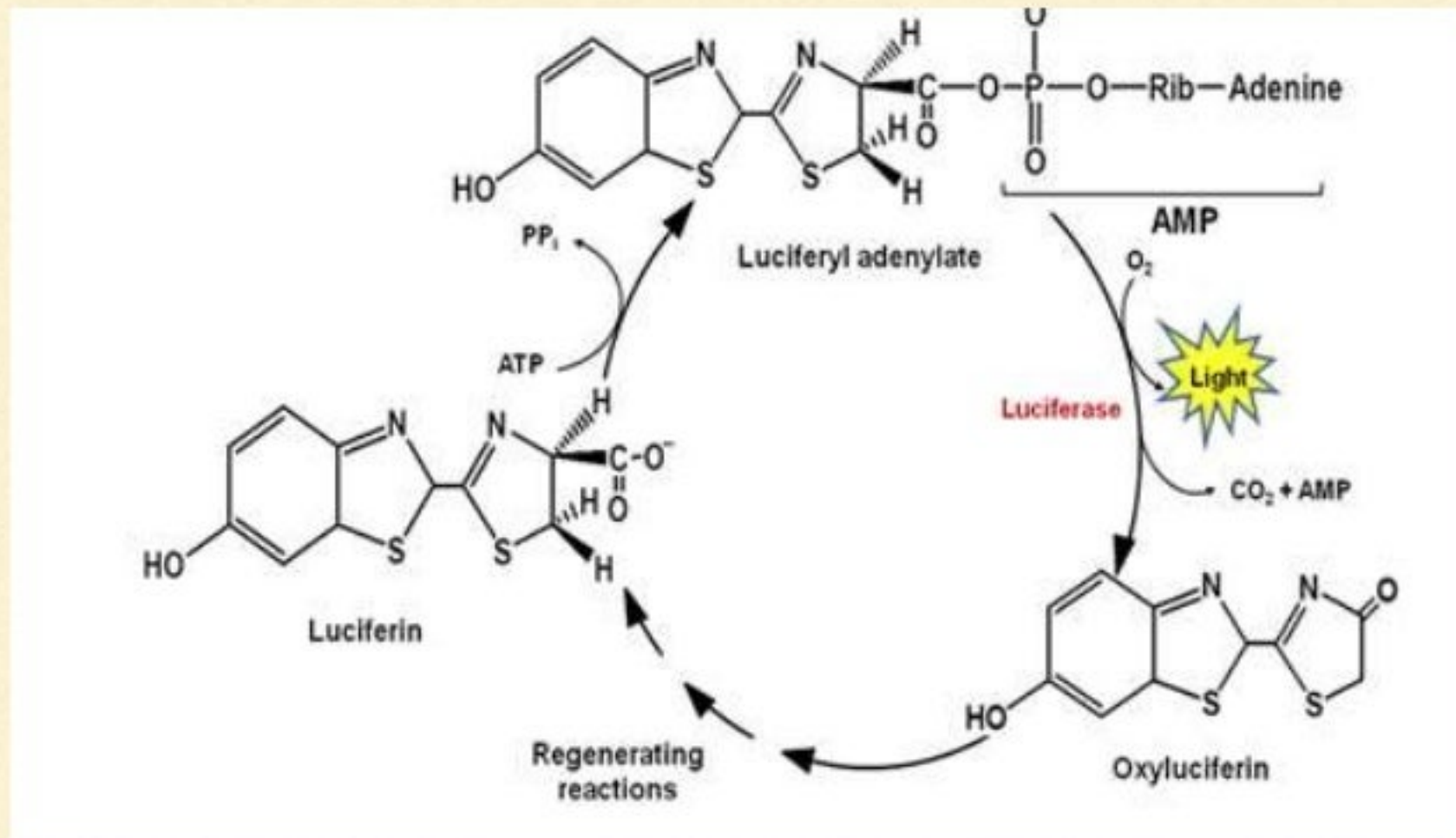
V) REPORTER GENE ASSAY

- In the reporter gene assay system, a chimeric construct is produced with an enzyme gene is cloned in front of the promoter of gene of interest.

- The general reporter gene construct contains a eukaryotic promoter and a enzyme for easy read out
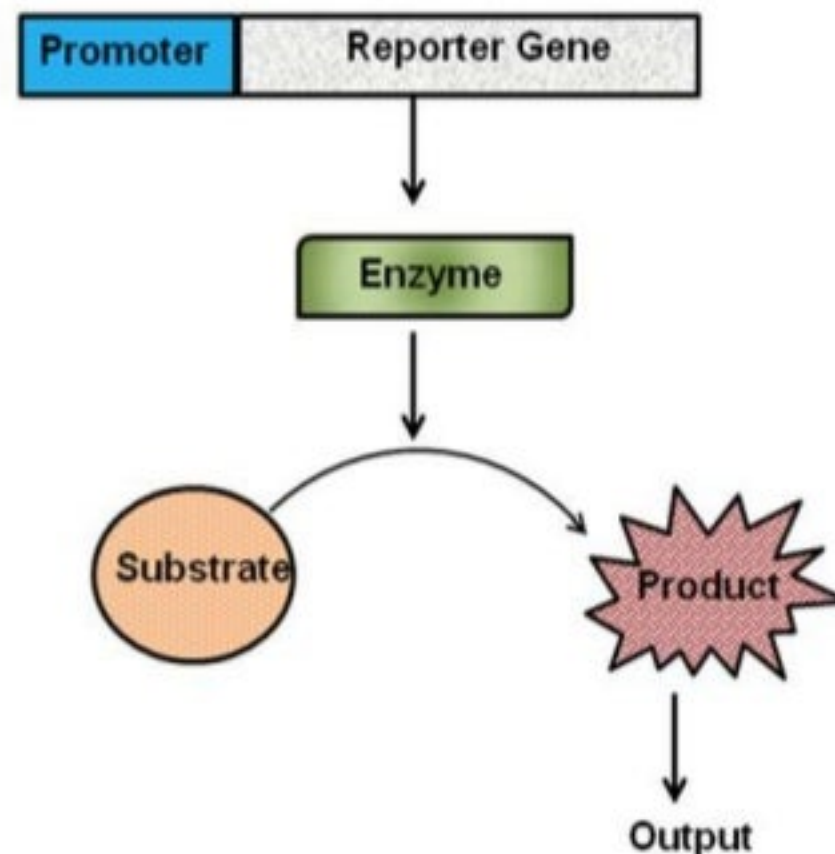
Luciferase reporter gene system- Luciferase is an enzyme present in the abdomen of firefly *photinus pyralis*. The enzyme utilizes D-luciferin as a substrate to form oxyluciferin. In the presence of ATP, Mg^{2+}, luciferin is converted into the luciferinadenylate involving pyrophosphate cleavage and transfer of AMP to luciferin. Luciferin adenylate undergoes oxidative decarboxylation to oxyluciferin with simultaneous emission of light.

- **Chimeric Construct with green fluorescent protein (GFP) -** In the live cell, green fluorescent protein is a good choice as reporter gene to screen cells containing recombinant protein fluorescently tagged with the GFP at their c-terminus. The cell receiving recombinant DNA will give green fluorescence and it can be visualized with an inverted fluorescence microscope and it can be analyzed in flow cytometer to separate the GFP containing cells from the untransfected cells.

- Flow cytometer analysis the cell based on its shape, size and fluorescence level.

- A non-fluorescent cell is giving separate peak as compare to the fluorescently labeled cells and with the help of flow cytometer, both of these peaks can be collected in separate tubes.

- Besides, GFP, red fluorescent protein, yellow fluorescent protein, cyan fluorescent protein are also popular to use to label the protein.

REFERENCE

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