Biotechnology department **LAB: Genetic engineering**

Collage of science **Al-Mustansiriyah University**

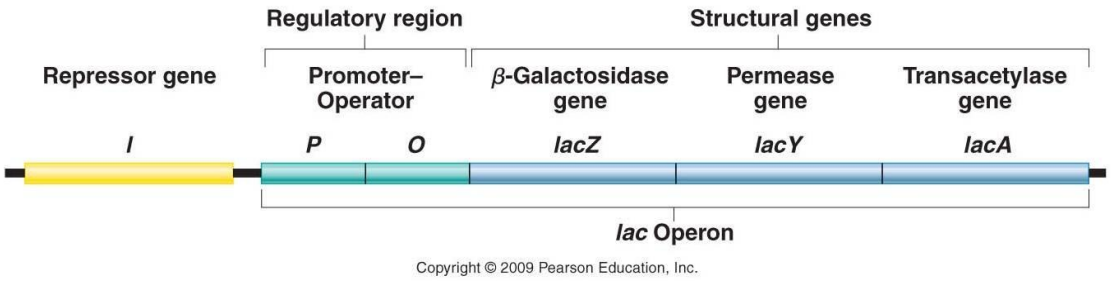
Lab 9

Recombinant selection

Blue-White Screening

Blue-white screening is a rapid and efficient technique for the identification of recombinant bacteria. It relies on the activity of β-galactosidase, an enzyme occurring in *E. coli*, which cleaves lactose into glucose and galactose.

The presence of lactose in the surrounding environment triggers the lacZ operon in *E. coli*. The operon activity results in the production of β-galactosidase enzyme that metabolizes the lactose.



Most plasmid vectors carry a short segment of lacZ gene that contains coding information for the first 146 amino acids of β-galactosidase. The host *E. coli* strains used are competent cells containing lacZΔM15 deletion mutation. When the plasmid vector is taken up by such cells, due to α-complementation process, a functional β-galactosidase enzyme is produced.

The plasmid vectors used in cloning are manipulated in such a way that this α-complementation process serves as a marker for recombination. A multiple cloning

site (MCS) is present within the lacZ sequence in the plasmid vector. This sequence can be nicked by restriction enzymes to insert the foreign DNA. When a plasmid vector containing foreign DNA is taken up by the host *E. coli*, the α-complementation does not occur, therefore, a functional β-galactosidase enzyme is not produced. If the foreign DNA is not inserted into the vector or if it is inserted at a location other than MCS, the lacZ gene in the plasmid vector complements the lacZ deletion mutation in the host *E. coli* producing a functional enzyme.

For screening the clones containing recombinant DNA, a chromogenic substrate known as X-gal is added to the agar plate. If β-galactosidase is produced, X-gal is hydrolyzed to form 5-bromo-4-chloro-indoxyl, which spontaneously dimerizes to produce an insoluble blue pigment called 5,5'-dibromo-4,4'-dichloro-indigo. The colonies formed by non-recombinant cells, therefore appear blue in color while the recombinant ones appear white. The desired recombinant colonies can be easily picked and cultured.

Isopropyl β-D-1-thiogalactopyranoside (IPTG) is used along with X-gal for blue-white screening.

- IPTG is a non-metabolizable analog of allolactose that induces the expression of lacZ gene. It should be noted that IPTG is not a substrate for β-galactosidase but only an inducer.
- X-gal (5-bromo-4-chloro-3-indolyl-b-D -galactopyranoside) a lactose analog called which is broken down by β-galactosidase to a product that is colored deep blue. For visual screening purposes, chromogenic substrate like X-gal is required.

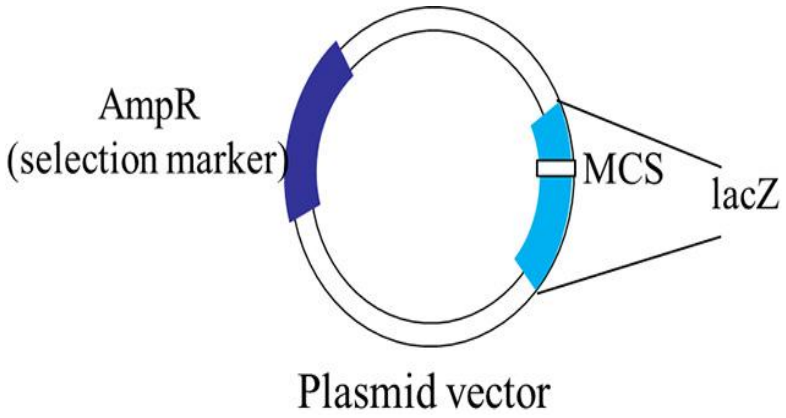


Figure 1: A schematic representation of a typical plasmid vector that can be used for blue-white screening.

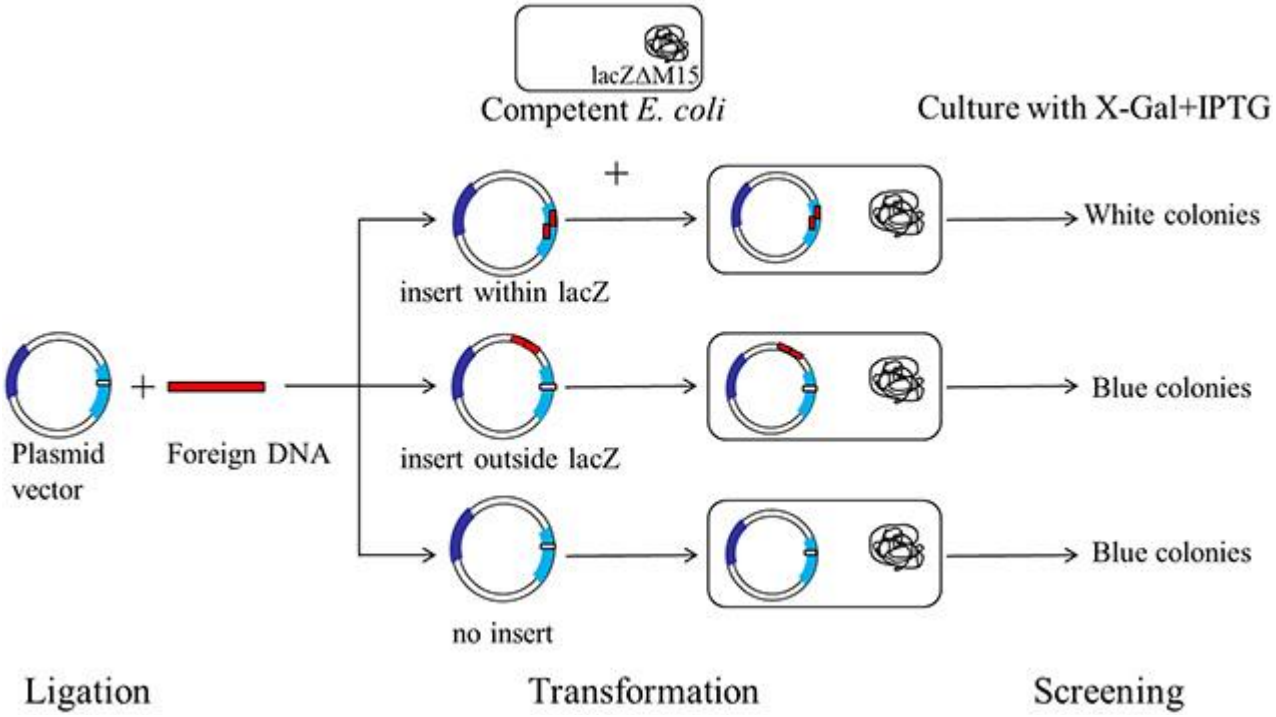


Figure 2 : A schematic representation of a typical blue-white screening procedure.

Protocol

Preparation of the plate

1. Pour sterile warm LB agar (about 25 mL) into a Petri dish.
2. Dry opened LB plates at room temperature under UV light for about 30 minutes.
3. Add 40 µL of the X-Gal Solution (20 mg/mL)
4. Add 40 µL of 100 mM of the IPTG Solution
5. Spread evenly on the plate with a sterile spatula.
6- Spread the transformation mixture on the plate
7- Following morning, check for blue and white colonies