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-galactoisdase 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-galactosisdase. 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-galatosidase 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.

How Does Blue White Screening Work?

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 galactose that induces the expression of lacZ gene. It should be noted that IPTG is not a substrate for β-galactosidase but only an inducer. For visual screening purposes, chromogenic substrate like X-gal is required.

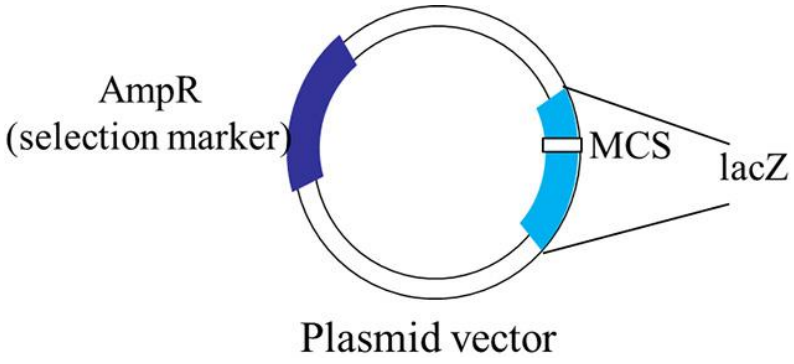

Figure 1: *A schematic representation of a typical plasmid vector that can be used for blue-white screening.*

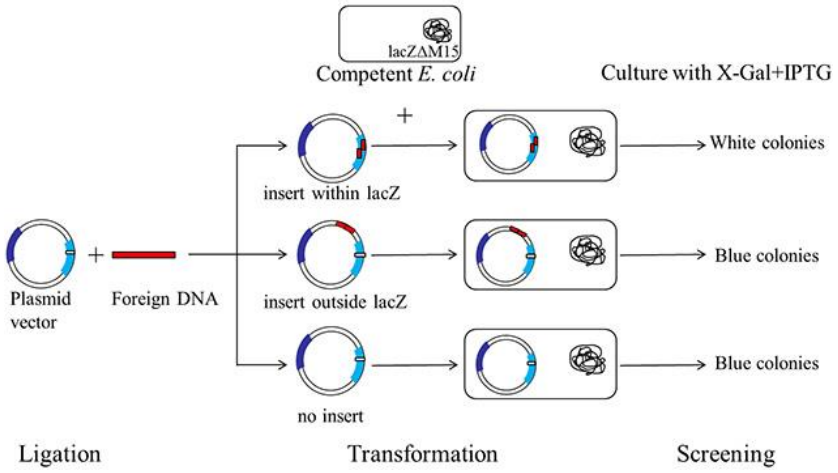

Figure 2: *A schematic representation of a typical blue-white screening procedure.*