Blue/White Screening

Aim: To Clone a DNA fragment in the pUC18 vector and select colonies that have DNA inserts based on colour selection.

Introduction

The blue-white screen is a screening technique that allows for the detection of successful ligations in vector-based gene cloning. 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 induces the lacZ operon in *E. coli*. The operon activity results in the production of β-galactoisdase enzyme that metabolizes the lactose. A multiple cloning site (MCS) is present within the lacZ sequence in the plasmid vector (Figure1). 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, then 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 produce functional β-galactosidase.

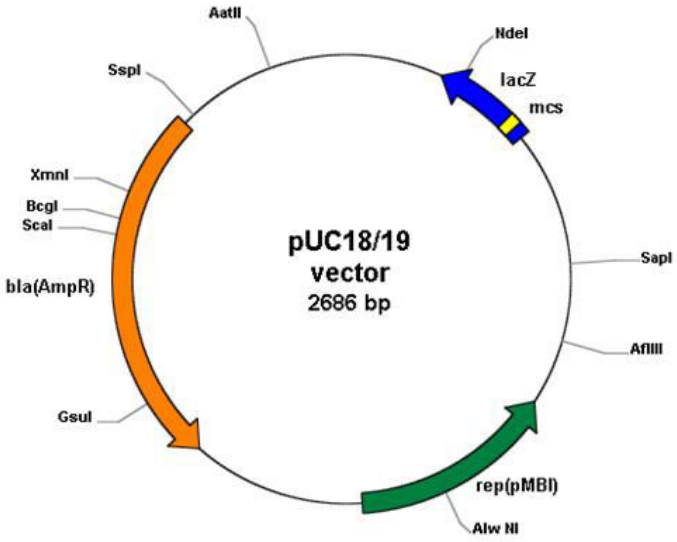

Figure 1: A schematic representation of a plasmid vector that can be used for blue-white screening.

For screening the clones containing recombinant DNA, a chromogenic substrate known as X-gal along with Isopropyl β-D-1-thiogalactopyranoside (IPTG) are used and added to the agar plate. 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. 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.

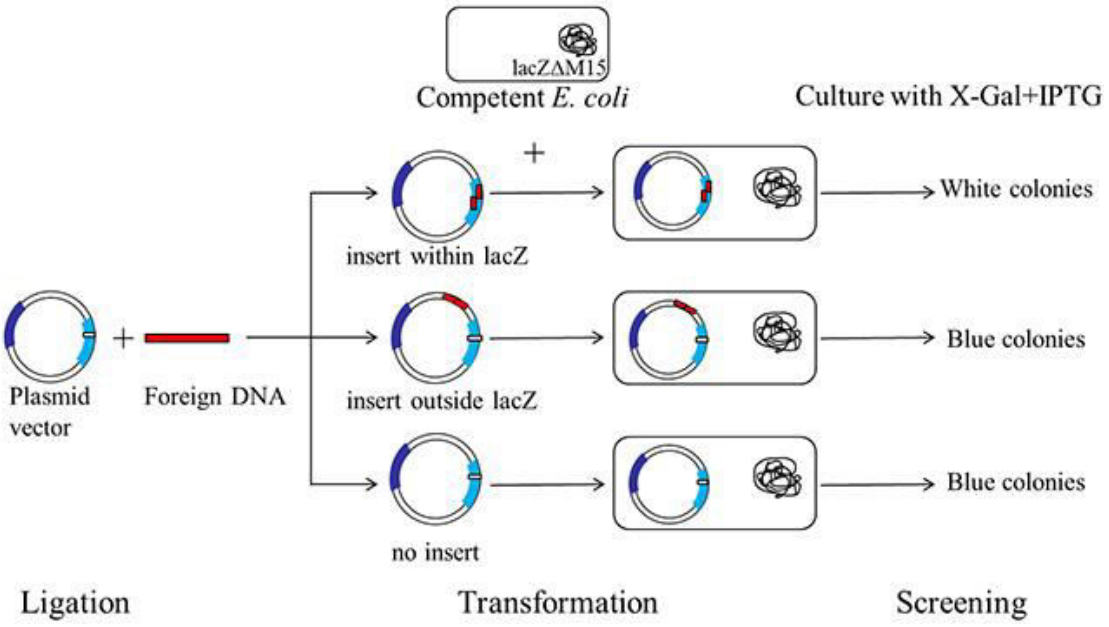

Figure 2: A schematic representation of a typical blue-white screening procedure.

Protocols

The complete protocol of blue-white screening includes 3 important steps:

Ligation: ligation of foreign DNA into MCS of the plasmid vector

Transformation: introduction of plasmid vector with foreign DNA insert into competent E. coli

Screening: blue-white screening to identify recombinant bacterial colonies

The following is the reaction setup for ligation

Component	Volume (µL)	Final concentration
T4 DNA ligase buffer	2	1X
Vector	x	1-10 ng/µL
Insert	x	1-10 ng/µL
T4 DNA ligase	1	6U/µL
Sterile water	x	NA
Total volume	20	

Add all the above components into a clean reaction tube.
Incubate for 1-2hr at 25°C (can be performed in a thermo cycler).

Transform 0.1-10 ng of the ligation product into competent cells that are compatible with the vector.

Screening protocol

1. Spread 40 µL from 20mg/ml stock solution of chromogenic substrate (X-Gal) and 10 µL of IPTG from 0.1 M stock solution on LB agar plates using a sterile spreader.
2. The plates should include those with appropriate antibiotic and without antibiotic as controls. Leave the plates to dry in laminar flow chamber with lids slightly open.
3. Spread 100-200 µL of transformed E. coli cells onto the LB agar plates using sterile spreader.
4. Incubate the plates at 37°C for overnight.

Observation

Blue and white colonies appear on the agar surface. Select the recombinant cells in the white colonies to culture.

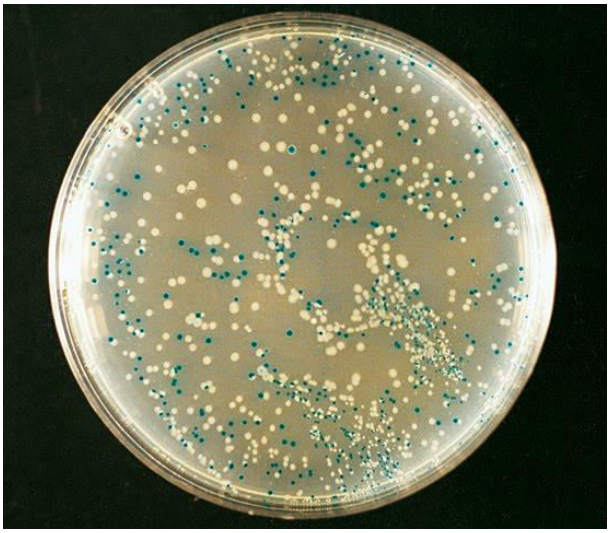

Figure 3: A typical Blue/white screening plate.