Blue White Screening

Blue White Screening for Colony Selection

Introduction

Blue-white screening of bacterial colonies is a popular and effective molecular biology tool often used to detect recombinant bacteria in cloning experiments. Central to this technique is the enzymatic activity of β-galactosidase, a tetrameric enzyme encoded by the lacZ α gene in E.coli that metabolizes lactose to form glucose and galactose. Alternatively, β-galactosidase can hydrolyze a different substrate, X-Gal, resulting in 5-bromo-4-chloro-indoxyl, which dimerizes to form a blue pigment.

The Basic idea and α-complementation

The phenomenon of α-complementation has made β-galactosidase a powerful molecular cloning tool. In α-complementation, the deletion of a specific fragment of the lacZ ω gene in bacteria resulting in an inactive β-galactosidase is resolved by the presence of a plasmid containing the deleted fragment. In cloning, the plasmids routinely used contain a segment of the lacZ α gene, while the E. coli host strain contain a lacZ ω deletion mutation. Thus, during transformation, when bacteria containing the deletion take up the plasmid containing the deleted lacZ α segment, functional β-galactosidase is produced. However, if the plasmid taken up by the bacteria is carrying a piece of DNA (DNA of interest ligated into the plasmid using restriction sites during the cloning process) that disrupts the lacZ α gene segment, recombinant bacteria result. Then, alpha complementation cannot occur, and a functional β-galactosidase does not form.

To perform blue-white screening after transformation, X-Gal is added along with Isopropyl β-D1 thiogalactopyranoside (IPTG), an inducer of lacZ ω gene expression. The blue colonies contain bacteria with functional β-galactosidase, indicating the plasmid taken up during transformation did not contain the DNA of interest. Conversely, the white colonies cannot metabolize X-Gal to produce the blue color, because they do not produce functional β-galactosidase after taking up plasmid carrying the inserted DNA and disrupting the lacZ α gene. These white colonies contain the recombinant bacteria and should be selected (Figure 1). Here, we describe a protocol to perform effective blue-white colony screening to select the recombinant bacteria carrying your DNA of interest.

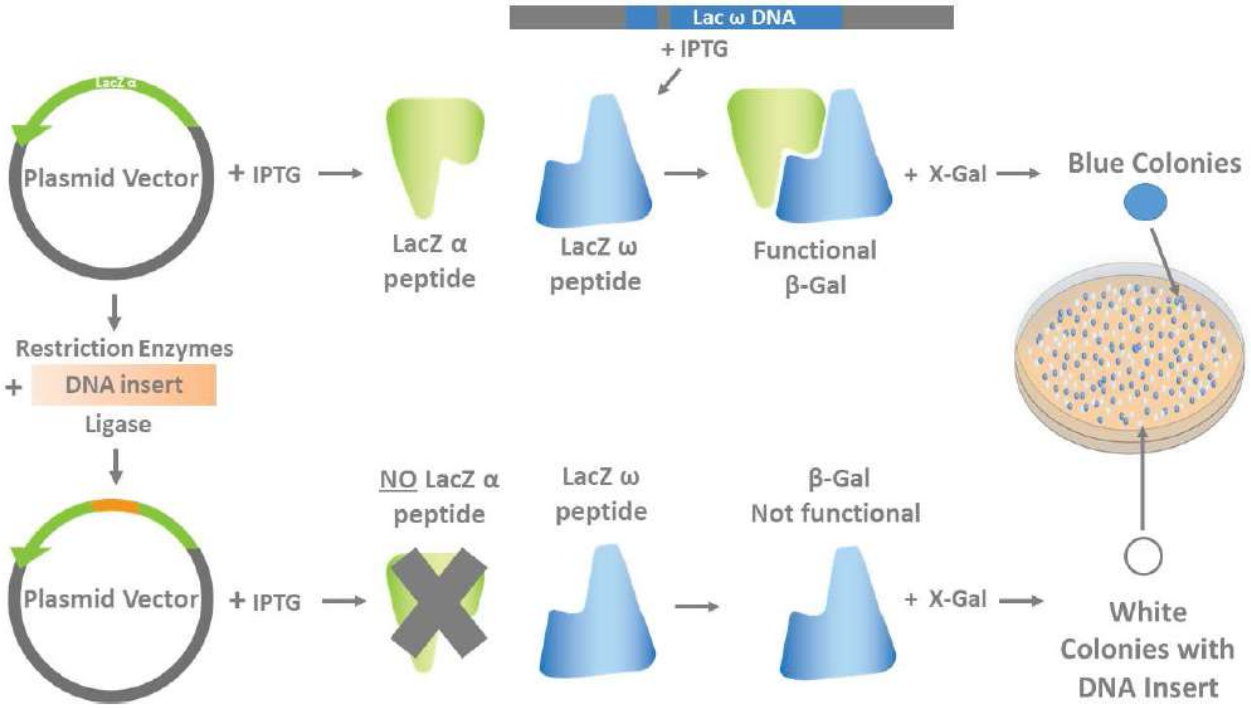
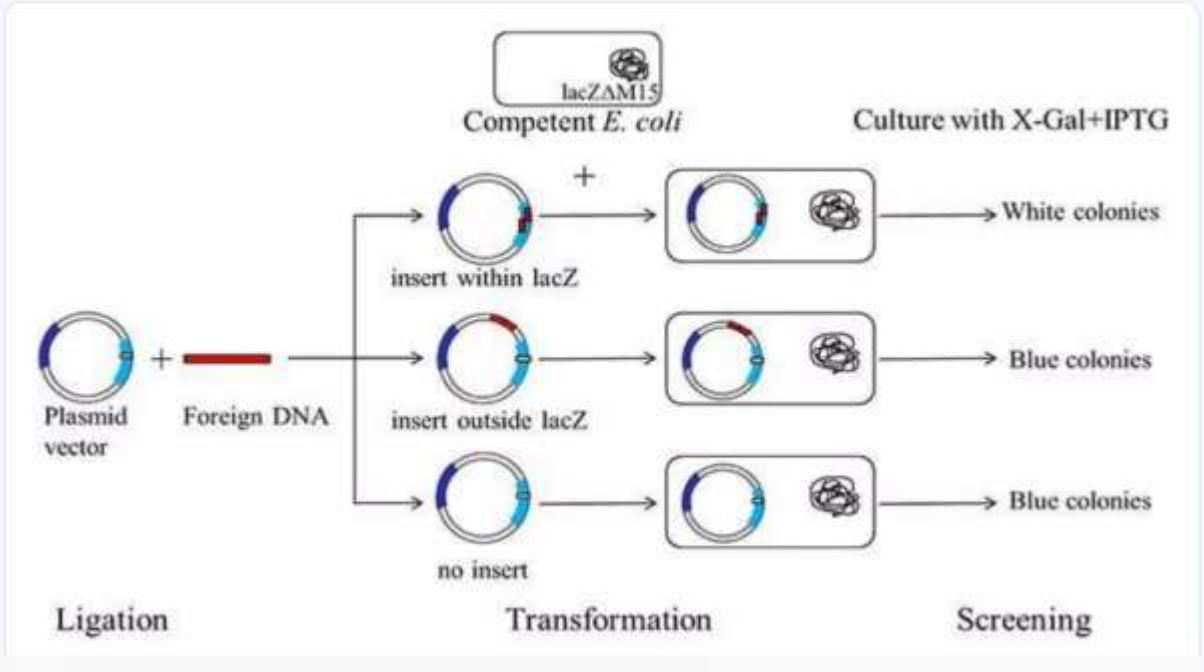


Figure 1. Blue-white screening of bacterial colonies using IPTG and X-Gal.

Materials

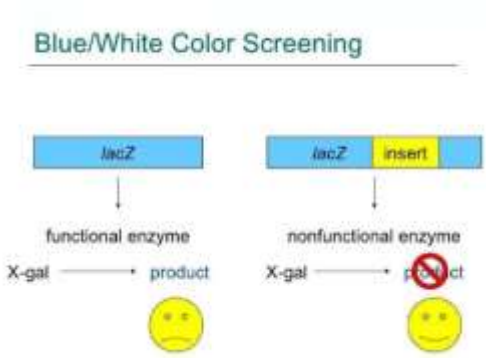
- X-Gal
- Dimethylformamide (DMF)
- dH2O
- Isopropyl β-D-1-thiogalactopyranoside, IPTG
- Screening antibiotic of choice
- Agar media (optional)
- Plates



Quick Points

- 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.

Fig: Blue-white color selection of recombinant bacteria using X-gal.



References:

- https://www.sigmaaldrich.com
- https://www.learnsci.com/resources/blue-white-screening-theory#skills-list
- Web: www.goldbio.com