Protocol

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Blue-White Screening of Bacterial Colonies

Utilizing X-Gal and IPTG Plates

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 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-D-1-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.

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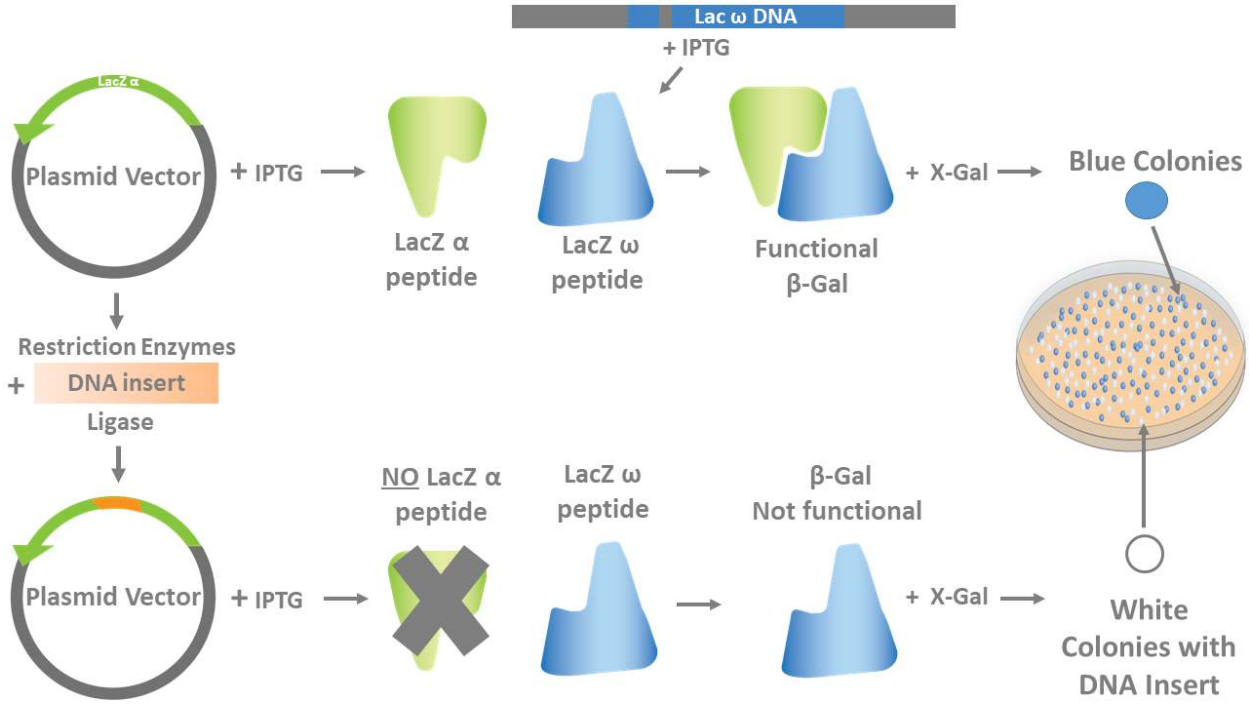

Figure 1. Blue-white screening of bacterial colonies using IPTG and X-Gal.

Materials

- X-Gal (GoldBio Catalog # X4281C)
- Dimethylformamide (DMF)
- dH_2O
- Isopropyl β-D-1-thiogalactopyranoside, IPTG (GoldBio Catalog # I2481C[E])
- Screening antibiotic of choice
- Agar media (optional)
- Plates

Method

Preparation of X-Gal and IPTG. X-Gal and IPTG can be incorporated into agar media before pouring into plates or added onto pre-made plates.

1. Prepare 20 mg/ml X-Gal in DMF (see X-Gal Stock Solution Procedure).

Note: This stock solution should be stored in a polypropylene or glass tube, protected from light, at -20°C. This solution is stable for 6-12 months. Aliquots (1 ml) should be made to prevent degradation due to handling.

E: EZ-Pak available

Note: For reduced DMF toxicity in media, increase the concentration to 100 mg/ml X-Gal in DMF.

Note: The higher concentration solution is only stable for 1 week at -20°C.

2. Prepare 100mM IPTG solution in dH_2O (see IPTG Stock Solution Procedure) or dilute from a 1M IPTG solution.

This solution is stable for 1 year. Aliquots (1 ml) should be made to prevent degradation due to handling.

Screening on agar media containing IPTG and X-Gal (recommended)

1. Autoclave the growth media agar, then cool to 50°C.

2. Add 10 µl of 20 mg/ml X-Gal solution per 1 ml of media or 2 µl of 100 mg/ml X-Gal solution per 1 ml of media.

Note: GoldBio recommends using a higher concentration of X-Gal than most protocols as the higher concentration increases blue color intensity and in turn decreases the number of ambiguous colonies needing rescreening. Higher X-Gal concentration also reduces blue color development time and refrigeration time.

3. Add 10 µl IPTG (100mM) per 1 ml of media for a final concentration of 1mM.

4. Add the screening antibiotic.

5. Pour plates and allow them to cool to room temperature before use. This usually takes at least 30 minutes.

6. Spread transformed competent cells as desired.

Note: Blue-white selection plates are generally stable for only 1 week if stored at 4°C in clear sleeves, but may be stored in the dark, or in dark sleeves, at 4°C for up to 1 month.

Screening on pre-made agar plates lacking IPTG and X-Gal

1. Pour autoclaved growth media containing screening antibiotic on media plates and dry in a laminar flow hood.

2. Add 40 µl 100mM IPTG and 120 µl X-Gal (20 mg/ml) to the surface of each plate and spread over the entire surface.

   Note: The plate edges are difficult to spread evenly and may give false positives. We advise picking colonies in the middle of the plate, if possible, for best results.

3. Dry X-Gal/IPTG-coated media in a laminar flow hood for approximately 30 minutes before use.

4. Spread transformed competent cells and incubate inverted at either 37°C until blue colonies form (usually ~24 hours).

Associated Products

- X-Gal (GoldBio Catalog # X4281C)
- IPTG (GoldBio Catalog # I2481C [E])
- Applicable antibiotic

References

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