Protocol

Blue-White Selection Protocol
Utilizing X-alpha-Gal

Introduction

In yeast, the enzyme α-galactosidase allows the use of melibiose, a disaccharide, as a carbon source during the growth phase. In *Saccharomyces cerevisiae*, α-galactosidase is encoded by the gene *MEL1*, which is regulated by the transcription factor GAL4. Upon GAL4 activation, α-galactosidase is produced. This mechanism has proved very useful in the study of protein-protein interactions and has allowed the generation of powerful assays, such as the GAL4-based yeast two-hybrid assay. In this assay, an effective protein-protein interaction results in functional GAL4. GAL4 then activates transcription and production of α-galactosidase, which hydrolyzes the chromogenic substrate X-α-Gal, yielding a blue precipitate. This blue precipitate enables the observation and detection of protein-protein interactions directly on agar selection plates. Here, we describe the use of X-α-Gal to detect protein-protein interactions.

Materials

- X-α-Gal (GoldBio Catalog # XA250)
- Dimethylformamide (DMF)
- dH_2O
- Agar media
- Screening antibiotic of choice
- 10 or 15 cm plates

Storage and Handling

- X-α-Gal should be stored at -20°C. Protect from light.
- This product may be shipped on blue ice and should be protected from light and stored at -20°C, immediately upon arrival.

Method

Preparation of X-α-Gal stock solutions for agar and pre-made plates.

1. X-α-Gal can be incorporated into agar media before pouring into plates or added onto pre-made plates.
    a. To incorporate into agar, dissolve 60 mg of X-α-Gal in 3 ml of DMF to obtain a final concentration of 20 mg/ml.

b. To add to pre-made plates, dissolve 24 mg of X-α-Gal in 6 ml of DMF to obtain a final concentration of 4 mg/ml.

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

Screening on agar media containing X-α-Gal (recommended)

1. Autoclave the growth media agar, then cool to 50°C.
2. Add 1 ml of 20 mg/ml X-α-Gal per 1 L of media to cooled agar.
3. Add the screening antibiotic.
4. Pour plates and allow them to cool to room temperature before use. This usually takes at least 30 minutes.

Screening on pre-made agar plates lacking X-α-Gal

1. Pour autoclaved growth media containing screening antibiotic on media plates and dry in a laminar flow hood.
2. Add 200 µl (15 cm plate) or 100 µl (10 cm plate) of X-α-Gal (4 mg/ml) to the surface of each plate, spreading 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-α-Gal-coated media plates in a laminar flow hood for approximately 30 minutes before use.
4. Spread transformed competent cells or yeast and incubate inverted at either 37°C or 30°C, respectively, until blue colonies form.

Associated Products

- X-α-Gal (GoldBio Catalog # XA250)
- Antibiotic

References

Fernández-Leiro, R., Pereira-Rodríguez, Á, Cerdán, M. E., Becerra, M., and Sanz-Aparicio, J. (2010). Structural Analysis of Saccharomyces cerevisiae α-Galactosidase and Its Complexes with Natural Substrates Reveals New Insights into Substrate Specificity of GH27 Glycosidases. *Journal of Biological Chemistry*, 285(36), 28020-28033. Doi:10.1074/jbc.m110.144584.

Fields, S., and Song, O. (1989). A novel genetic system to detect protein–protein interactions. *Nature*, 340(6230), 245-246. Doi:10.1038/340245a0.