Protocol

TD-P Revision 3.0

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Yeast Plate Preparation and Transformation with Antibiotic Screening

Modified from Hahn Lab Protocols

Introduction

Yeast transformation is a common technique used in molecular biology to introduce a plasmid into a specific yeast strain. Currently, there are various methods of transformation of yeast. The high efficiency method, or single-stranded carrier DNA LiAc/SS-DNA/PEG method, yields a small number of transformants and is suited for very specific purposes. This method can be used to screen for plasmids that complement a specific mutation. It can also be used to transform a yeast strain with two plasmids, or to transform a specific yeast strain with integrating plasmid or oligonucleotide. Finally, it can be used to transform a plasmid library into a two-hybrid yeast strain. Here, we outline the antibiotics, yeast recipes, and plate preparations needed for successful screening of transformed yeast. This procedure can also be used with yeasts modified with the KanMX cassette.

Materials

- Antibiotics:
  Hygromycin B
  (GoldBio Catalog # H-270) or
  G-418 Sulfate
  (GoldBio Catalog # G-418) or
  Nourseothricin Sulfate
  (GoldBio Catalog # N-500)
- Amino Acid Mix (see recipe below)
- Tris-HCl (GoldBio Catalog # T-095)
- Lithium Acetate (LiOAc)
- Polyethylene Glycol (PEG) 3350
  (GoldBio Catalog # P-290)
- Yeast Nitrogen Base
- Ammonium Sulfate
- Molecular biology Grade water
- Sodium Hydroxide (NaOH)
- Bacto-Agar/Plant-Agar
  (GoldBio Catalog # P1001)
- Adenine Sulfate in HCl
- Uracil
- Glucose
- Bacto Peptone
- 5-FOA (GoldBio Catalog # F-230)

Antibiotic Stock Solutions:

- For Hygromycin B (use 50 mg/ml stock solution protocol) (GoldBio Catalog # H-270)
- For G-418 (200 mg/ml)
- For Nourseothricin Sulfate (200 mg/ml) (GoldBio Catalog # N-500)

For Amino Acid Mix (40x), use 0.6 g/L:

- 2 g Tyrosine (GoldBio Catalog # T-130)
- 4 g Arginine (GoldBio Catalog # A-840)

- 2 g Serine
- 2 g Valine
- 4 g Threonine
- 2 g Isoleucine
- 2 g Phenylalanine
- 2 g Aspartic Acid
- 2 g Proline (GoldBio Catalog # P-230)

For Tris-EDTA (TE)/Lithium Acetate (LiOAc) Buffer:

- 10x TE
    1. 0.1M Tris-HCl (GoldBio Catalog # T-095)
    2. 0.01M EDTA at pH 7.5
- 10x LiOAc
    1. 1M LiOAc at pH 7.5, adjusted with diluted acetic acid.

For Sterile Polyethylene Glycol (PEG):

- 40% PEG (GoldBio Catalog # P-290)
- 1x TE
- 1x LiOAc

For Sabouraud Dextrose Chloramphenicol Agar (SDCA) Plates (1 liter):

- 1.7 g Yeast Nitrogen Base without Amino Acids and Ammonium Sulfate
- 5.0 g Ammonium Sulfate
- 1 liter Molecular Biology Grade H_2O
- Adjust pH to 7.0 using NaOH

Add prior to autoclaving:

- 0.6 g Amino Acid Mix (mix well before adding)
- 20 g bacto-agar

Autoclave 25 minutes, then add:

- 10 ml (10 mg/ml) of any other yeast specific amino acids
- 10 ml 0.4% Adenine Sulfate in 0.1M HCl (if required)
- 10 ml 0.2% Uracil (if required)
- 50 ml 40% Glucose

For Yeast Extract Peptone Dextrose (YPD) Plates (1 liter):

- 10 g Yeast Extract
- 20 g Bacto Peptone
- 20 g Bacto-Agar
- 1 liter Molecular Biology Grade H_2O

Then autoclave for 25 minutes, before adding:

- 50 ml 40% Glucose
- 5 ml 0.4% Adenine Sulfate in 0.1M HCl (if required)
- If plates will be used for tetrad dissection, add 10 ml of 0.2% uracil

Note: YPD is a complete medium for yeast growth and can be used as liquid or solid medium.

For 5-FOA Plates (for testing the presence of URA3 gene) (0.5 liter):

Agar:

- 15 g Bacto-Agar
- 300 ml Molecular Biology Grade H_2O in 500 ml flask

Autoclave for 25 minutes.

FOA Mix:

- 0.85 g Yeast Nitrogen Base without ammonium sulfate and amino acids
- 2.5 g Ammonium Sulfate
- 0.3 g Amino Acid Mix
- 25 ml 40% Glucose
- 17.5 mg Uracil
- 0.5 g 5-FOA
- Any other required amino acids for yeast
- 225 ml H_2O

Microwave the above mixture until heated to 65°C, approximately 3 minutes. Stir on stir plate until dissolved (approx. 10-15 minutes). Filter through 0.2 micron bottle top filter into sterile bottle. Add to the autoclaved agar, mix well and pour plates. Do not pH the plates as this will inactivate the 5-FOA.

Antibiotics Yeast Plates

For G418 Plates:

- Use G418 for yeast containing the KanMX allele. Use 200 µg/ml for checking cells already containing resistance to G418 or add 500 µg/ml for selection of gene disruptions using yeast transformation.

For Hygromycin B Plates:

- Use Hygromycin B at 200 µg/ml for yeast containing *hph*, the hygromycin resistance gene. Add 4 ml of 50 mg/ml stock solution per liter to achieve this concentration.

For Nourseothricin (NAT) Plates:

- Use NAT at 100 µg/ml for yeast containing *nat* , the NAT resistance gene.

Method

High Efficiency Yeast Transformation

1. Inoculate 5 ml of YPD with a single yeast colony. Grow overnight on a shaker at 30°C.

2. Add 0.5 ml culture to 4.5 ml fresh media, check absorbance at A_{660}. Add appropriate amount of cells to 60 ml fresh media to achieve an A_{660} reading of 0.2 (approx. 2×10^6 cells).

3. Grow the cells until A_{660} is equal to 1 (approximately 2×10^7 cells) on a shaker at 30°C. This takes approximately 5 hours.

   Note: This incubation will yield enough cells for ~10 transformations.

4. Collect 50 ml of the culture in a sterile 50 ml centrifuge tube.

5. Centrifuge the cell suspension at 5000 rpm for 5 minutes.

6. Pour off medium and wash cells with 10 ml sterile water, ensure that cells are resuspended.

7. Centrifuge again at 5000 rpm for 5 minutes, then resuspend in 1 ml of sterile water and transfer to a sterile 1.5 ml microcentrifuge tube.

8. Centrifuge at 5000 rpm for 5 minutes and resuspend in 1 ml TE/LiOAc.

9. Centrifuge at 5000 rpm for 5 minutes and resuspend in 0.25 ml TE/LiOAc. At this point there should be 4×10^9 cells/ml.

10. Mix 50 µl yeast cells with transforming DNA and 5 µl single-stranded carrier DNA (10 mg/ml, boiled and chilled on ice) in a 1.5 ml microcentrifuge tube.

11. Add 300 µl sterile PEG and mix thoroughly.

    Note: PEG protects the cells from high concentrations of LiAc.

12. Incubate at 30°C for 60 minutes with occasional gentle shaking.

13. Add 40 µl DMSO and mix thoroughly.

Note: This increases transformation between 5 to 10 fold.

14. Heat shock at 42°C for 15 minutes.

15. Microcentrifuge for 10 seconds, remove supernatant, and resuspend in 1 ml of 1x TE. Repeat this step once.

16. Plate 200 µl on selective media.

17. Incubate the plates at 30°C for 3-4 days and count number of transformants.

Associated Products

- Hygromycin B (GoldBio Catalog # H-270)
- G-418 Sulfate (GoldBio Catalog # G-418)
- Nourseothricin Sulfate (GoldBio Catalog # N-500)
- Tris-HCl (GoldBio Catalog # T-095)
- Polyethylene Glycol (PEG) 3350 (GoldBio Catalog # P-290)
- 5-FOA (GoldBio Catalog # F-230)
- Tyrosine (GoldBio Catalog # T-130)
- Arginine (GoldBio Catalog # A-840)
- DL-Proline (GoldBio Catalog # P-230)
- Bacto-Agar (GoldBio Catalog # P1001)
- Duchefa YPD Agar (Duchefa YPD)

References

Gietz, R. D., & Woods, R. A. (n.d.). Yeast Transformation by the LiAc/SS Carrier DNA/PEG Method. *Yeast Protocols,* 107-120. Doi:10.1385/1-59259-958-3:107.

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Yeast Plates. (2016, September 21). Hahn Laboratory. Retrieved May 9, 2018, from https://research.fhcrc.org/content/dam/stripe/hahn/methods/yeast_genetics/YeastPlates.pdf.