ZYMOLYASE™ PROTOCOLS

# Smash and Grab – Zymolyase™

**PROVIDED BY: DAVID AMBERG**

1. Grow cells in 3mls selective media o/n
2. Pellet cells by 2 quick spins in a microfuge
3. Re-suspend cells in 200 u1 of the following solution:
   ∠5 ml SCE*
   ∠60 ul l0 mg/ml l00T Zymolyase
   ∠10 ul 2-ME
4. Vortex and inc at 37oC for 30-60 min.
5. Add 400 ul 0.2N NaOH/1%SDS, invert to mix and inc, on ice for 5 minutes.
6. Add 300 ul 3M K/5M OAc pH4.5, invert to mix and inc on ice x 5 minutes.
7. Spin 2 minutes in microfuge, pour super into a fresh tube and repeat spin. Remove 500 ul to a fresh tube.
8. Add 300 ul iPrOH, vortex and let stand for 5 minutes at room temperature.
9. Pellet DNA 5 minutes. in a microfuge, then wash pellet with 70% EtOH and repeat spin.
10. Dry pellet in speed vac and reconstitute in 25ul TE.
11. Porate 2ul into electrocompetent coli, recover and plate all.

*SCE:

- 1M Sorbitol
- 0.1M Sodium Citrate pH 7.6
- 0.06M EDTA

# MOLECULAR BIOLOGY COMPARATIVE STUDIES

Methods and Reagents for the Transformation of Saccharomyces cerevisiae

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A Comparison of Methods and Reagents for the Transformation of Saccharomyces cerevisiae

## Introduction:

The transformation of yeast can be accomplished by several methods, which include treatment of the yeast with lithiumcations and by protoplast fusion. The efficiency of these methods vary greatly as do the effect of individual reagents on these methods. For instance, the quality of polyethylene glycol (PEG) on the efficiency of DNA uptake in the protoplast transformation method is known to be critical with researchers testing the PEG lots much the same way cell culturists test serum. This study compares lithium cation and protoplast fusion transformations of yeast, in addition to a comparison of Zymolyase and Lyticase as the cell wall degrading enzyme for the protoplast transformation of yeast.

## Materials and Methods:

### YEAST STRAIN

Saccharomyces cerevisiae INVSc1, from Invitrogen, was used for all transformation. The yeast phenotype is MAT his 3(delta)l leu2 trp 1-289 ura3-52. Transformation experiments will make use of the leuphenotype and selection of transformants using LEU2 complementation.

### PLASMID DNA

The plasmid YEp351/A/M was used as the control DNA for the transformation experiments. This plasmid contains the LEU2 and thus allows for leucine complementation for the selection of transformants. This plasmid also encodes an easily detectable marker for verification. The plasmid contains the ADC1 promoter fused to the MEL1 coding region. This results in the expression of MEL 1 and synthesis of β- galactosidase on glucose. β-Galactosidase is easily measured using a variety of colorimetric substrates, as with the enzymatic cleavage of X-β-Gal.

A Comparison of Transformation Methods and Reagents Transformation of Saccharomyces cerevisiae by the Lithium Cation Method

## Materials:

- YPD Broth
- TE Buffer
- 100 mM Lithium Acetate in TE Buffer, sterile
- Plasmid DNA
- PEG 4000, 50% in water, sterile
- 1 M Sorbitol in TE buffer
- Leu selective agar plates: 1% glucose, 0.67% yeast nitrogen base, 1 M sorbitol

## Protocol:

12. Culture yeast in YPD broth (2% glucose, 2% peptone, 1% yeast extract) with aeration overnight at 30°C. The culture should have approximately 1 x 107 cells/ml.

13. For each transformation, pellet yeast from 4.5 ml of broth by centrifuging for 5 minutes at 3000 rpm.

14. Re-suspend the yeast in 4 ml TE buffer and centrifuge at 3000 rpm for 5 minutes.

15. Carefully re-suspend the yeast in 3 ml or 100 mM lithium acetate, TE and shake gently at room temperature for 30 minutes.

16. Centrifuge the yeast for 5 min. at 2000 rpm. Carefully remove the supernatant and re-suspend the yeast in 100 ml of lithium acetate/TE and transfer to a 1.5 ml microfuge tube. The final density of the yeast is approximately 5 x 108 cells/ml.

17. Add up to 10 mg of plasmid to the yeast in a volume not greater than 10 ml. Incubate at 30°C without shaking for 30 minutes.

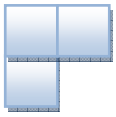
18. Add 300 ml of 50% PFG, mix and incubate at 30°C for 1 hr. without shaking.

19. Heat shock the cells for 5 minutes at 42°C.

20. Immediately centrifuge the cells for 20 sec. Remove the PEG solution and re-suspend the cells in 1 ml of sorbitol/TE solution.

21. Plate the cells on selective media. Allow the plates to dry before inverting to incubate.





A Comparison of Transformation Methods and Reagents Transformation of Saccharomyces cerevisiae by Protoplast Method

**Materials:**

- 1 M Sorbitol, 25 mM EDTA, pH 8, 50 mM DTT - The sorbitol/EDTA is prepared separately and sterilized. Filtered sterilized 1 M DTT stock solution (molecular biology grade) is prepared and frozen in 500 m1 aliquots. The DTT is added to 9.5 ml of sorbitol/EDTA just prior to use.
- 1 M Sorbitol - Sterilize by autoclaving.
- 1 M sorbitol, 1 mM EDTA, 10 mM sodium citrate buffer, pH 5.8 (for Zymolyase)
- 1 M sorbitol, 1 mM EDTA, 10 mM Tris buffer, pH 7.5 (for Lyticase)
- Zymolyase in water- 60 U/ml
- Lyticase in water - 1200 U/ml
- 1 M Sorbitol, 10 mM Tris, pH 7.5, 10 mM CaCl2
- 20% PEG/10 mM Tris, pH 7.5, 10 mM CaCl2 (freshly prepared)
- Leu selective agar plates: 1% glucose, 0.67% yeast nitrogen base, 1 M sorbitol
- Leu selective top agar tubes: 10 ml of 1% glucose, 0.67% yeast nitrogen base, 1 M sorbitol in a large foam stoppered test tube. Melt and store at 47oC when using.
- Water - Sterile, molecular biology grade.
- 5% SDS in water

**Protocol:**

1. Streak cryopreserved yeast on a YPD agar plate (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) and culture 24-48 hours at 28-30$^{o}$C.

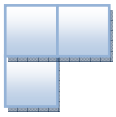
2. Inoculate 100 ml of YPD (1% yeast extract, 2% peptone, 2% dextrose) from an isolated colony into a 500 ml flask with yeast from the YPD plate. Culture the yeast overnight at 28$^{o}$C with shaking (250-300 rpm).

3. Measure the OD520 of the yeast culture the following morning using sterile YPD to zero the spectrophotometer. If necessary, dilute the yeast in YPD in order to attain a readable OD (i.e., between 0.1 and 1.0). If the yeast OD is between 0.2 and 0.3, then harvest the cells as described below. Alternatively, dilute the yeast to an OD of 0.2 and continue culturing. When the culture OD reaches 0.3, harvest the cells by centrifuging at room temperature for 10 minutes at 1500 x g. Pour off the supernatant and save the yeast pellet.

4. Thoroughly re-suspend the yeast pellet in 10 ml of sterile water. Transfer the cells to a sterile 15 ml screw capped tube.

5. Centrifugation the yeast at 1500 x g for 5 minutes at room temperature. Decant and save the yeast pellet.





6. Re-suspend the yeast in 10 ml of freshly prepared 1 M Sorhitol, 25 mM EDTA, pH8, 50 mM DTT. Immediately pellet the yeast by centrifuging at 1500 x g for 5 minutes at room temperature.

7. Re-suspend the yeast cells 10 ml of 1 M sorhitol, Pellet the yeast by centrifuging at 1500 x g for 5 mm.

8. Re-suspend the cells in 10 ml of sorbitol/citrate buffer (1 M sorbitol, 1 mM EDTA, 10 mM sodium citrate buffer, pH 5.8). To assess the time required and efficiency of protoplast formation, transfer 1.6 ml of cells to a small tube and add 2.5 ml of Zymolyase. Proceed to step 9 Save the remaining cells.

9. Re-suspend the cells in 10 ml of sorbitol/citrate buffer (1 M sorbitol, 1 mM EDTA, 10 mM Tris buffer, pH 7.5). To assess the time required and efficiency of protoplast formation, transfer 1.6 ml of cells to a small tube and add 2.5 ml of Lyticase. Proceed to step 9. Save the remaining cells.

10. Following the addition of cell wall lytic enzyme (i.e., either Zymolyase or Lyticase) incubate the cells at 300C. Remove 200 p aliquots of treated cells at 0, 5, 10, 20, 30, 40, 50, and 60 mm and add the aliquot to 2 ml of 1 IM Sorbitol. Add 800 m1 5% SDS, mix, and measure cell lysis by culturing clearing at OD800. The spectrophotometer should be blanked against 1 M sorbitol.

11. The efficiency and time required for protoplast formation can be determined by plotting OD800 of the treated yeast cells vs. time. To calculate efficiency, divide the OD800 at each time point by the starting OD800, then multiply by 100. A protoplast efficiency of 70% is needed for transformation.

12. To protoplast the remaining yeast, at 13 ml either Lyticase or Zymolyase to their respective tubes and incubate for the determined time to reach 70% protoplast efficiency. Handle the protoplasts carefully since they are fragile and will easily rupture.

13. Centrifuge the protoplasts at 750 x g for 10 mm. at room temperature. Decant and save the pellet.

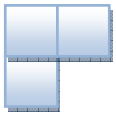
14. Gently re-suspend the protoplasts in 1 ml of 1 M sorbitol. Centrifuge at 750 x g at room temperature.

15. Re-suspend the protoplasts in 5 ml of 1 M Sorbital, 10 mM Tris, pH 7.5,10 mM CaCl2. Centrifuge as above and re-suspend the cells in 300 m1 of 1 M Sorbitol, 10 mM Tris pH 7.5, 10 mM CaCl2.

16. For each transformation, aliquot 100 m1 of the protoplasts into sterile 15 ml tubes. Add 10 mg of YEp351 /A/M control plasmid and incubate at room temperature for 10 minutes.

17. Add 1.0 ml of fresh 20% PEG/10 mM Tris, pH 7.5, 10 mM CaCl2 to the protoplasts, mix gently, and incubate at room temperature for 10 minutes.





18. Centrifuge the protoplasts at 750 x g for 10 minutes at room temperature and carefully decant the 20% PEG/10 mM Tris, pH 7.5, 10 mM CaCl2. Invert the tube and drain excess solution.

19. Gently re-suspend the protoplasts in 1 ml of 1 M sorbitol.

20. Add 100 m1 of protoplasts to 10 ml of molten top agar and pour onto the selective agar plates. Allow the top agar to solidify.

21. Invert plates and incubate at 28-3oEC. Transformed cells should appear in 4-6 days.

## Results and Discussions:

The transformation of Saccharomyces cerevisias with YEp351/A/M was successful. The efficiency of the transformation was affected by both the method employed, and in the case of protoplast transformation, the lytic enzyme used (i.e., Zymolyase and Lyticase). The protoplast transformation method was significantly more efficient than lithium cation transformation, with the enzyme employed for protoplast formation also having a significant effect. When Zymolyase was used for protoplast formation, efficiency was 652 transformants/10 µg plasmid DNA This efficiency dropped considerably when Lyticase was used, which yields of 53 transformants/10 µg. The transformation of yeast with lithium cations was relatively inefficient with 10 µg of plasmid yielding 6 transformants.

The efficiencies of each of the transformation methods employed can be correlated to the difficulty and cost of the technique. Protoplast transformation though relatively efficient, does require removal and regeneration of the yeast cell wall which can often be difficult. This is especially true when a variety of yeast strains are being transformed. Lithium acetate transformation is relatively easy to perform, however ease in the procedure results in loss in the relative efficiency of the transformation.

The effect of lytic enzyme source on protoplast efficiency is easily demonstrated with the differences observed between tranformations using Lyticase and Zymolyase. Previous experiments comparing Lyticase and Zymolyase show that the specificity of these enzymes differ significantly. Lyticase often is ineffective in completely lysing cells, thus demonstrating limited lytic specificity. Zymolyase is a mixture of several carbohydrates (i.e., β1, 3 glucan laminaripentaohydrolase, β-1 , 3 glucanase, protease, and mannase) all which work in conjunction for efficient cell wall degradation.

The success of protoplast transformation is two fold: 1) sufficient cell wall must be removed so to allow fusion between protoplasts, and 2) adequate cell wall must remain in order to successfully regenerate the complete cell wall. It is feasible that Zymolyase contains the correct enzymatic activities to allow for both of these conditions when yeast are partially protoplasted (i.e., to 70%). Lyticase could conceivably be under or over degrading a particular cell wall component which either prevents protoplast fusion or subsequent cell wall regeneration.