Yeastmaker™ Yeast Transformation System 2 User Manual

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I. Introduction

The **Yeastmaker™ Yeast Transformation System 2** provides a high-efficiency polyethylene glycol (PEG)/LiAc-based method for preparing and transforming competent yeast cells. Though originally developed for use with our Matchmaker™ Library Construction & Screening Kits for yeast two-hybrid and one-hybrid screening (Cat. Nos. 630445 & 630304), the System 2 protocol is suitable for any yeast transformation experiments.

The Yeast Transformation System 2 protocol provides a higher and more reliable frequency of transformation than many other commonly used methods. Achieving a high transformation efficiency is especially important if you are preparing a one-hybrid or two-hybrid library for screening. The more clones your library contains, the more likely you are to detect rare and potentially novel interactions. One reason why the Yeast Transformation System 2 yields more transformants (per µg of DNA) than many other commonly used methods is because it includes an uncommon but crucial incubation step: After the addition of DNA and treatment with DMSO, yeast cells are incubated in **YPD Plus Liquid Medium**—a formulation that enhances the uptake of plasmid DNA. Using this protocol with yeast strain AH109 or Y187, we typically obtain $\geq 3 \times 10^5$ transformants per µg of plasmid DNA.

II. List of Components

Store Carrier DNA and the pGBT9 Control Plasmid at –20°C.

Store all other components at room temperature.

The following reagents are suitable for a maximum of 50 small-scale or 15 library-scale transformations.

- **50 ml 1 M LiAc (10X)**
- **50 ml 10X TE Buffer**
- **50 ml YPD Plus Liquid Medium**
- **2 x 1 ml 10 mg/ml Herring Testes Carrier DNA, denatured***
- **2 x 50 ml 50% PEG 3350 (Sigma, Cat. No. P4338)**
- **20 µl pGBT9 (positive control plasmid), 0.1 µg/µl**

* Although the Herring Testes Carrier DNA has been denatured, we recommend denaturing the Carrier DNA again upon receipt and periodically thereafter (boil for 20 min, then chill on ice).

III. Additional Materials Required

Growth and maintenance of yeast

Stocks of yeast strains in **15–30% glycerol** must be stored at –70°C. A small amount of frozen cells can be scraped from this stock with a sterile loop or wooden stick and streaked onto YPD plates to recover the strain. Avoid thawing stock.

- **YPD medium**

  YPD Medium (Cat. No. 630409) and YPD Agar Medium (Cat. No. 630410) are available in convenient powder form from Clontech. Our YPD Medium is a blend of peptone, yeast extract, and dextrose in optimal proportions for growth of most strains of *Saccharomyces cerevisiae*. If you purchase our YPD media, prepare the medium according to the instructions provided. If you prefer, you can prepare your own YPD mixture as follows:

20	g/L	Difco peptone
10	g/L	Yeast extract
20	g/L	Agar (for plates only)

  Add H_2O to 950 ml. Adjust pH to 6.5 if necessary, and autoclave. Allow medium to cool to ~ 55°C and then add dextrose (glucose) to 2% (50 ml of a sterile 40% stock solution).

  Note: If you add the sugar solution before autoclaving, autoclave at 121°C for 15 min; autoclaving at a higher temperature, for a longer period of time, or repeatedly may cause the sugar solution to darken and will decrease the performance of the medium. Note that YPD from Clontech already contains glucose.

- **YPDA Medium**

  Prepare YPD medium as above. After autoclaved medium has cooled to 55°C, add 15 ml of a 0.2% adenine hemisulfate solution per liter of medium. (Final concentration is 0.003%, in addition to the trace amount of Ade that is naturally present in YPD).

  – For kanamycin-containing medium, prepare YPD or YPDA as above. After autoclaved medium has cooled to 55°C, add 0.2–0.3 ml of 50 mg/ml kanamycin (final concentration 10–15 µg/ml).

- **SD medium**

  Synthetic dropout (SD) is a minimal medium used in yeast transformations to select and test for specific phenotypes. SD medium is generally prepared by combining a minimal SD base (providing a nitrogen base, a carbon source, and in some cases, ammonium sulfate) with a stock of "dropout" solution that contains a specific mixture of amino acids and nucleosides.

  Minimal SD Base and Minimal SD Agar Base are available from Clontech in convenient powder form. If you purchase our Minimal SD Base, prepare the medium according to the instructions provided.

- **Dimethyl Sulfoxide** (DMSO; Sigma Cat. No. D8418)
- **Adenine Hemisulfate Salt** (Sigma Cat. No. A9126)

IV. Solutions Required for Yeast Transformation

- **1X TE/LiAc Solution**

  Prepare fresh just prior to transformation using the 10X stock solutions provided. Dilute in sterile H_2O.

- **1.1X TE/LiAc Solution**

  Prepare fresh just prior to transformation using the stock solutions provided. Combine 1.1 ml of 10X TE Buffer with 1.1 ml of 1 M LiAc (10X). Bring the total volume to 10 ml using sterile, deionized H_2O.

- **PEG/LiAc Solution** (polyethylene glycol 3350/lithium acetate)

  Prepare fresh just prior to transformation using the stock solutions provided.

	Final Conc.	To prepare 10 ml of solution
PEG 3350	40%	8 ml of 50% PEG 3350
TE buffer	1X	1 ml of 10X TE Buffer
LiAC	1X	1 ml of 1 M LiAc (10X)

- **0.9% (w/v) NaCl Solution**

  Dissolve 0.9 g of NaCl in 100 ml of deionized H_2O and filter-sterilize the solution.

V. Yeast Cell Stock Maintenance

For those who are not familiar with yeast manipulations or would like more information, we recommend *Guide to Yeast Genetics and Molecular Biology*, by Guthrie & Fink (1991) and *Molecular Biology and Genetic Engineering of Yeasts*, edited by Heslot & Gailardin (1992).

- Yeast strains can be stored for up to 2 months at 4°C on YPD medium in petri dishes sealed with Parafilm. However, fresh colonies (1–3 weeks) will give better results when inoculating a liquid culture.
- Storage of new yeast transformants
  1. To prepare stock cultures of new yeast transformants for storage, use a sterile inoculation loop to scrape an isolated colony.
  2. Thoroughly suspend the colony in 0.5 ml of YPD medium (or the appropriate SD medium) containing 15–30% sterile glycerol. We recommend using 2-ml vials for storing these cultures.
  3. Ensure that the cap is closed tightly. Shake the vial. Freeze immediately at –70°C.
  4. To recover the strains, streak a small portion of the frozen stock onto a YPD (or appropriate SD medium) agar plate. (If the tube has thawed prior to streaking a small portion, vortex to insure even distribution of the yeast cells.)

VI. Yeast Transformation Protocol

A. Preparation of Competent Yeast Cells

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING.

Before strarting:

- Prepare the appropriate selection media, and pour the required number of agar plates.
- Prepare YPDA liquid medium (Section III).
- Prepare 1.1X TE/LiAc Solution (Section IV).
- Prepare PEG/LiAc Solution (Section IV).
- Prepare 0.9% (w/v) NaCl Solution (Section IV).

1. Streak a YPDA agar plate with a small portion of frozen yeast stock—e.g., AH109 or Y187.

   Notes:

   - If the tube has thawed prior to streaking, vortex to ensure even distribution of the yeast cells.
   - Yeast strains can be stored for up to 1 month at 4°C on YPDA medium in culture plates sealed with parafilm.

2. Incubate the plate upside down at 30°C until colonies appear (~ 3 days).
3. Inoculate one colony (≤ 4 weeks old, 2–3 mm in diameter) into 3 ml of YPDA medium in a sterile, 15-ml centrifuge tube.
4. Incubate at 30°C with shaking for 8–12 hr.
5. Transfer 5 µl of the culture to a 250-ml flask containing 50 ml of YPDA.
6. Incubate at 30°C with shaking at 230–250 rpm for 16–20 hr. The OD_{600} should reach 0.15–0.3. If not, continue incubating until this density is reached and then proceed, taking care not to overgrow the culture.
7. Centrifuge the cells at 700 x g for 5 min at room temperature.
8. Discard the supernatant and resuspend the cell pellet in 100 ml of YPDA.
9. Incubate at 30°C for 3–5 hr (final OD_{600} = 0.4–0.5).
10. Centrifuge the cells at 700 x g for 5 min at room temperature.
11. Discard the supernatant and resuspend the cell pellet in 60 ml of sterile, deionized H_2O.
12. Centrifuge the cells at 700 x g for 5 min at room temperature.
13. Discard the supernatant and resuspend the cells in 3 ml of 1.1X TE/LiAc Solution.
14. Split the resuspension between two 1.5-ml microcentrifuge tubes (1.5 ml per tube).
15. Centrifuge each tube at high speed for 15 sec.

VI. Yeast Transformation Protocol *continued*

16. Discard the supernatant and resuspend each pellet in 600 µl of 1.1X TE/LiAc Solution.

    Note: Competent cells should be used for transformation immediately following preparation; however, if necessary they can be stored at room temperature for a few hours without significantly affecting the competency.

B. Transformation of Competent Yeast Cells

Directions for both small- and library-scale yeast transformation protocols are provided below. Volumes and quantities for the library-scale protocol are given in brackets.

1. Set up the required number of sterile 1.5-ml [15-ml] tubes for the planned transformations.
2. In a sterile, prechilled, 1.5-ml [15-ml] tube combine the following:
   - 0.1–1 µg [1–10 µg] plasmid DNA
   - 5 µl [20 µl] Herring Testes Carrier DNA, denatured*

   * Transfer ~50 µl of Herring DNA to a microcentrifuge tube and heat at 100°C for 5 min. Then, immediately chill the DNA by placing the tube in an ice bath. Repeat once more before adding the DNA to the 15-ml reaction tube.
3. Add 50 µl [600 µl] of competent cells.
4. Gently mix by vortexing.
5. Add 0.5 ml [2.5 ml] PEG/LiAc Solution.
6. Mix thoroughly by gently vortexing.
7. Incubate at 30°C for 30 min [45 min]. Mix cells every 10 min [15 min].
8. Add 20 µl [160 µl] DMSO, mix, and then place the tube in a 42°C water bath for 15 min [20 min]. Vortex gently every 5 min [10 min].
9. Centrifuge to pellet yeast cells:
   - For small-scale reactions, centrifuge at high speed in a microcentrifuge for 15 sec.
   - For library-scale reactions, centrifuge at 700 x g for 5 min.
10. Remove the supernatant and resuspend in 1 ml [3 ml] of **YPD Plus Liquid Medium.**

    Note: YPD Plus is specially formulated to promote transformation, increasing efficiency by 50–100%. **Do not use** standard YPD medium for this step.
11. Incubate at 30°C with shaking for 90 min. (For small scale transformations, omit this step.)
12. Centrifuge to pellet yeast cells (For speeds and times, see Step 9).
13. Discard the supernatant and resuspend in 1 ml [15 ml] of 0.9% (w/v) NaCl Solution.

VI. Yeast Transformation Protocol *continued*

C. Plating the Transformation Mixture

1. Spread 100 µl [150 µl] of the transformation mixture onto a 100-mm [150-mm] plate containing the appropriate SD selection medium. For pGBT9, the positive control, use SD/–Trp medium. To obtain well-isolated colonies, you may need to dilute the mixture (e.g., 1:10, 1:100, or 1:1000) before spreading.
2. Incubate plates (upside down) at 30°C until colonies appear (~3–6 days).
3. Pick the largest colonies and restreak them on the same selection medium. Seal these master plates with Parafilm and store at 4°C (not longer than 1 month).

VII. References

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Guthrie, C. & Fink, G. R. (1991) Guide to yeast genetics and molecular biology. *Methods in Enzymology* (Academic Press, San Diego, CA)**194**:1–932.

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VIII. Related Products

For a complete listing of all Clontech products, please visit **www.clontech.com**

Matchmaker™ Products	**Cat. No.**
• (Two-Hybrid) Library Construction & Screening Kit	630445
• One-Hybrid Library Construction & Screening Kit	630304
• Two-Hybrid System 3	630303
• Mammalian Two-Hybrid Assay Kit	630301
• Mammalian Assay Kit 2	630305
• Pretransformed cDNA Libraries	many
• GAL4 cDNA Libraries	many
• Random Peptide Library	638853
• Co-IP Kit	630449

Related Products	
• Yeastmaker Plasmid Isolation Kit	630441
• Yeastmaker Carrier DNA	630440
• YPD Medium	630409
• YPD Agar Medium	630410
• Minimal SD Base (contains glucose)	630411
• Minimal SD Base/Gal/Raf (contains galactose and raffinose)	630420
• Minimal SD Agar Base (contains glucose)	630412
• Minimal SD Agar Base/Gal/Raf (contains galactose and raffinose)	630421
• Dropout (DO) Supplements for use with any SD Base	many
• pCMV-Myc & pCMV-HA (Epitope-Tagged Mammalian Expression) Vector Set	631604
• pBridge Three-Hybrid Vector	630404