User Manual

Yeastmaker™ Yeast Transformation System 2 User Manual

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Cat. Nos. 630439,
630489 & 630490
PT1172-1 (PR0Y3570)
Published November 2010

Table of Contents

Customer Service/Ordering

tel: 800.662.2566 (toll-free)

fax: 800.424.1350 (toll-free)

web: www.clontech.com

e-mail: orders@clontech.com

Technical Support

tel: 800.662.2566 (toll-free)

fax: 650.424.1064

e-mail: tech@clontech.com

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 experiment.

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 require library-scale transformations. The more clones your library contains, the more likely you are able 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, we typically obtain ≥3 x 10^5 transformants per µg of plasmid DNA.

II. List of Components

Store Box 1 at –20° C.

Store Box 2 at room temperature.

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

Box 1:

- 2 x 1 ml 10 mg/ml Yeastmaker Carrier DNA, denatured
- 20 µl pGBT9 (positive control plasmid), 0.1 µg/µl

Box 2:

- 2 x 50 ml 50% PEG 3350 (Sigma, Cat. No. P4338)
- 50 ml 1 M LiAc (10X)
- 50 ml 10X TE Buffer
- 50 ml YPD Plus Liquid Medium

III. Additional Materials Required

A. Ready-to-Go Media Pouches Available from Clontech

Clontech offers media sets with a complete assortment of mixes in convenient, "ready-mixed" foil pouches, suitable for use with any yeast transformation system.

Table I: Individual Yeast Media Pouches for Yeast Transformation Experiments

Yeast Media Pouches	Clontech Cat. No.	Volume of Media
Rich Media (for routine culturing of untransformed yeast)		
YPDA Broth	630306	10 x 0.5 L
YPDA With Agar	630307	10 x 0.5 L
Minimal Media Single Dropouts (SDO)		
SD–Trp Broth	630308	10 x 0.5 L
SD–Trp with Agar	630309	10 x 0.5 L
SD–Leu Broth	630310	10 x 0.5 L
SD–Leu with Agar	630311	10 x 0.5 L
SD/–His Broth	630312	10 x 0.5 L
SD/–His with Agar	630313	10 x 0.5 L
SD/–Ura Broth	630314	10 x 0.5 L
SD/–Ura with Agar	630315	10 x 0.5 L
Minimal Media Double Dropouts (DDO)		
SD–Leu/–Trp Broth	630316	10 x 0.5 L
SD–Leu/–Trp with Agar	630317	10 x 0.5 L
Minimal Media Triple Dropouts (TDO)		
SD–His/–Leu/–Trp Broth	630318	10 x 0.5 L
SD–His/–Leu/–Trp with Agar	630319	10 x 0.5 L
SD/–Leu/–Trp/–Ura Broth	630320	10 x 0.5 L
Minimal Media Quadruple Dropouts (QDO)		
SD–Ade/–His/–Leu/–Trp Broth	630322	10 x 0.5 L
SD–Ade/–His/–Leu/–Trp with Agar	630323	10 x 0.5 L
SD/–His/–Leu/–Trp/–Ura Broth	630324	10 x 0.5 L
SD/–His/–Leu/–Trp/–Ura with Agar	630325	10 x 0.5 L

B. General Media Preparation Instructions

- Prepare media by dissolving pouch contents in 500 ml dd$H_2$0, autoclave for 15 min at 121˚ C, and allow to cool before use (or filter-sterilize broth media). Do not over-autoclave.
- This media does not usually require pH adjustment, but if your source water is particularly acidic, you may need to adjust the pH of the media to 5.8.
- For additional information on preparing media from the pouches, please see the Clontech Yeast Media Protocol-at-a-Glance (PT4057-2) at **www.clontech.com**

IV. Solutions Required for Yeast Transformation

- **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).

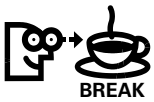
- Yeast strains can be stored for up to 2 months at 4° C on YPD or YPDA medium in petri dishes sealed with Parafilm. However, fresh colonies (1–3 weeks) will give better results when inoculating a liquid culture.
- Storage of new yeast transformants
  1. To prepare stock cultures of new yeast transformants for storage, use a sterile inoculation loop to scrape an isolated colony.
  2. Thoroughly suspend the colony in 0.5 ml of YPD or YPDA 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 YPDA (or appropriate SD medium) agar plate. (If the tube has thawed prior to streaking a small portion, vortex to ensure even distribution of the yeast cells.)

VI. Yeast Transformation Protocol

Protocol
5 days

A. Protocol: Preparation of Competent Yeast Cells

1. Materials:
   - Yeastmaker Yeast Transformation System 2 [provided with the Two-Hybrid Kit or available separately (Cat. No. 630439)]
   - 1.1x TE/LiAc (Section IV)
   - YPDA agar plates
   - YPDA liquid medium
   - Appropriate SD selective medium
   - Frozen stock of yeast cells (*S. cerevisiae*)
   - Sterile, deionized water
2. Streak a YPDA agar plate with your chosen yeast cells from a frozen yeast stock. Incubate the plate upside down at 30° C until colonies appear (~3 days).



NOTE: If you wish, you may stop the experiment at this step and resume work later. The plates can be stored at 4° C in subdued lighting for up to one month.

3. Inoculate one colony (diameter 2–3 mm, < 4 weeks old) into 3 ml YPDA medium in a sterile 15 ml culture tube.

TIP: Set up four separate 3 ml cultures from four separate colonies and choose only the fastest growing 3 ml culture to proceed. We find that faster growing cultures tend to result in higher transformation efficiencies.

4. Incubate at 30° C with shaking at 250 rpm for 8–12 hr.
5. Transfer 5 µl of the culture to 50 ml of YPDA in a 250 ml flask.
6. Incubate shaking until the OD_{600} reaches 0.15–0.3 (16–20 hr).

   NOTE: Continue incubating until OD is reached, but do not over grow the culture.
7. Centrifuge the cells at 700 g for 5 min at room temperature. Discard the supernatant and resuspend the pellet in 100 ml of fresh YPDA.
8. Incubate at 30° C until the OD_{600} reaches 0.4–0.5 (3–5 hr).

   NOTE: Continue incubating until OD is reached. Do not overgrow the culture.
9. Divide the culture into two 50 ml sterile Falcon conical tubes. Centrifuge the cells at 700 g for 5 min at room temperature. Discard the supernatant and resuspend each pellet in 30 ml sterile, deionized H_20.
10. Centrifuge the cells at 700 g for 5 min at room temperature. Discard the supernatant and resuspend each pellet in 1.5 ml of 1.1xTE/LiAc.
11. Transfer the cell suspensions to two respective 1.5 ml microcentrifuge tubes; centrifuge at high speed for 15 sec.
12. Discard the supernatant and resuspend each pellet in 600 µl of 1.1xTE/LiAc. The cells are now ready to be transformed with plasmid DNA.

NOTE: For best results, competent cells should be used for transformation immediately, although they can be stored on ice for a few hours without significant loss in efficiency.

VI. Yeast Transformation Protocol continued

Protocol
3 hr

B. Protocol: Transformation of Competent Yeast Cells

1. Materials:
   - Yeastmaker Yeast Transformation System 2
   - Competent Yeast Cells (Section VI.A)
   - PEG/LiAc (Section IV)
   - 0.9% (w/v) NaCl
   - DMSO

	Small-Scale	Library-Scale
2. Combine the following in a **pre-chilled, sterile tube**:	1.5 ml tube	15 ml tube
• **Plasmid DNA** (For best results, be sure to use a high-quality maxi prep plasmid DNA.)	100 ng	5–15 µg*
• **Yeastmaker Carrier DNA** (denatured**; 10 µg/µl)	5 µl	20 µl

NOTES:
* For example, use 5 µg of bait + 10 µg of prey for yeast two-hybrid library cotransformation.

**To denature carrier DNA, heat to 95–100°C for 5 min, then cool rapidly in an ice bath. Repeat once more just before use.

3. Add **competent cells** and gently mix.	50 µl	600 µl
4. Add **PEG/LiAc** and gently mix.	500 µl	2.5 ml
5. Incubate at **30° C**.	30 min	45 min

NOTE: Mix cells every 10 min (for small-scale) or 15 min (for library-scale) by tapping or gently vortexing.

6. Add **DMSO** and mix.	20 µl	160 µl
7. Place the tube in a **42° C** water bath.	15 min	20 min

NOTE: Mix cells every 5 min (for small-scale) or 10 min (for library-scale) by gently vortexing.

8. Centrifuge to pellet yeast cells.	high speed 15 sec	700 g 5 min
9. Remove the supernatant and resuspend in **YPD Plus Medium.**	1 ml	3 ml

NOTE: YPD Plus is specially formulated to promote transformation, increasing efficiency by 50–100 %. **Do not use** standard YPD medium for this step.

10. [Optional for small-scale transformations]: Incubate at **30° C** with shaking.	OPTIONAL	90 min
11. Centrifuge to pellet yeast cells. For speeds and times, see step 8.		
12. Discard the supernatant and resuspend in **0.9% (w/v) NaCl Solution**.	1 ml	15 ml

VI. Yeast Transformation Protocol continued

Protocol 3–5 days

C. Protocol: Plating and Determination of Transformation Efficiency

1. Spread 100 µl of 1/10 and 1/100 dilution onto a 100 mm plate containing the appropriate SD selection medium. For example:
   - For pGBKT7, use SD/-Trp
   - For pGADT7, use SD/-Leu
   - For cotransformations of both, use SD/-Leu/-Trp

NOTE: Do not plate undiluted transformed cells.

2. Incubate plates upside down at 30° C until colonies appear (3–5 days).
3. Calculate transformation efficiency.

Example Calculation

$$\text{Transformation Efficiency} = \frac{\text{cfu x Suspension Volume (ml)}}{\text{Vol. plated (ml) x amount of DNA (µg)}}$$

(If 1/10 or 1/100 dilutions were plated, multiply by 10 and 100 respectively.)

After transformation using 100 ng of pGBT9 (control plasmid from Yeastmaker Yeast Transformation System 2), 100 µl of a 1/10 dilution was plated (from 1 ml total) and yielded 300 colonies after 3 days on SD/Trp.

$$\text{Transformation Efficiency} = \frac{300 \times 1}{0.1 \times 0.1} \times 10 \text{ (dilution factor)} = 3\times10^5 \text{ cfu/µg}$$

VII. References

Bartel, P. L., Chien, C.-T., Sternglanz, R., & Fields, S. (1993) Using the two-hybrid system to detect protein-protein interactions. In *Cellular Interactions in Development: A Practical Approach*, D.A. Hartley, ed., Oxford University Press, Oxford; pp 153–179.

Chien, C. T., Bartel, P. L., Sternglanz, R. & Fields, S. (1991) The two-hybrid system: A method to identify and clone genes for proteins that interact with a protein of interest. *Proc. Nat. Acad. Sci. USA*, **88**:9578–9582.

Dower, W. J., Miller, J. F. & Ragsdale, W. W. (1988) High efficiency transformation of *E. coli* by high-voltage electroporation. *Nucleic Acids Res.* **16**:6127–6145.

Fields, S. & Song, O. (1989) A novel genetic system to detect protein-protein interactions. *Nature* **340:**245–247.

Fritz, C. C. & Green, M. R. (1992) Fishing for partners. *Current Biol.* **2:**403–405.

Guarente, L. (1993) Strategies for the identification of interacting proteins. *Proc. Natl. Acad. Sci. USA* **90**:1639–1641.

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

Heslot, H. & Gaillardin, C., eds. (1992) *Molecular Biology and Genetic Engineering of Yeasts*, CRC Press, Inc.