[4] Transformation of Yeast by Lithium Acetate/Single-Stranded Carrier DNA/Polyethylene Glycol Method

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Introduction

The introduction of exogenous DNA into yeast by transformation has become an essential technique in molecular biology. Transformation is used to investigate the genomics and proteomics of yeast itself and also when yeast is employed as a system to study the genes and gene products of other organisms. Intact yeast cells can be transformed by a number of procedures: the lithium acetate/single-stranded carrier DNA/polyethylene glycol (LiAc/SS-Carrier DNA/PEG) method,[1–3] electroporation,[4–6] agitation with glass beads,[7] and bombardment with DNA-coated microprojectiles.[8] Cells can also be transformed after conversion to spheroplasts by treatment with Zymolyase.[9] We have reviewed the application of these techniques to *Saccharomyces cerevisiae* and other yeasts.[10] We focus here on the transformation of intact cells by the LiAc/SS-Carrier DNA/PEG method since it is the most widely applicable.

LiAc/SS-Carrier DNA/PEG Transformation

In our experience this method is the most efficient, generating yields up to 5×10^6 transformants/μg plasmid DNA/10^8 cells with many commonly used laboratory strains of yeast. This makes it particularly suitable for the assay of plasmid libraries for protein : protein interactions by the yeast two-hybrid system.[11] We have developed four protocols that can be applied to various transformation needs:

[1] R. D. Gietz, R. H. Schiestl, A. R. Willems, and R. A. Woods, *Yeast* **11,** 355 (1995).
[2] R. D. Gietz and R. A. Woods, *in* "Methods in Microbiology," Vol. 26 (A. J. P. Brown and M. F. Tuite, eds.), p. 53. Academic Press, San Diego, 1998.
[3] R. A. Woods and R. D. Gietz, *in* "Gene Transfer Methods: Introducing DNA into Living Cells and Organisms" (P. A. Norton and L. F. Steel, eds.), p. 25. Eaton Publishing, Natick, MA, 2000.
[4] D. M. Becker and L. Guarente, *Methods Enzymol.* **194,** 182 (1991).
[5] R. H. Schiestl, P. Manivasakam, R. A. Woods, and R. D. Gietz, *Methods* **5,** 79 (1993).
[6] J. R. Thompson, E. Register, J. Curotto, M. Kurtz, and R. Kelly, *Yeast* **14,** 565 (1998).
[7] M. C. Costanzo and T. D. Fox, *Genetics* **120,** 667 (1988).
[8] S. A. Johnston and M. J. DeVit, *Methods Mol. Biol.* **53,** 147 (1996).
[9] F. Spencer, G. Ketner, C. Connelly, and P. Hieter, *Methods* **5,** 161 (1993).
[10] R. D. Gietz and R. A. Woods, *Biotechniques* **30,** 816 (2001).
[11] R. D. Gietz, B. Triggs-Raine, A. Robbins, K. C. Graham, and R. A. Woods, *Mol. Cell Biochem.* **172,** 67 (1997).

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(1) the rapid transformation protocol for the introduction of a plasmid into a yeast strain; (2) the high-efficiency transformation protocol for recovering large numbers of transformants; (3) the large-scale transformation protocol for screening complex plasmid libraries such as those required for a two-hybrid screen; and (4) the microtiter plate transformation protocols[3] for the simultaneous transformation of multiple strains or multiple samples of a single strain.

In all of these protocols the number of reagents and steps has been reduced from previous versions. Growth and transformation efficiency are improved if broth cultures are grown in double-strength YPAD.[3] Cells for transformation are harvested, washed in sterile water, resuspended in Transformation Mix (PEG, LiAc, SS-carrier DNA, and plasmid DNA) without pretreatment and immediately incubated at 42°. After centrifugation and removal of the Transformation Mix the cells are resuspended in sterile water and sampled onto Synthetic Complete (SC) selection medium[12] adjusted to pH 5.6. The duration of incubation at 42° is the most important variable in the transformation reaction. For stationary phase and agar grown cells the yield of transformants with some strains is increased from $2–4 \times 10^3/\mu g$ plasmid DNA after 20 min at 42° to $>1 \times 10^6$ if the incubation is extended to 180 min. Log-phase cells show optimal transformation, 5×10^6 to $1 \times 10^7/\mu g$ plasmid DNA, after 40 to 60 min at 42°.

Reagents and Solutions

The following reagents and solutions are required for all four LiAc/SS-DNA/PEG protocols.

Lithium Acetate (1.0 M). Dissolve 5.1 g of lithium acetate dihydrate (Sigma Chemical Co. Ltd., St. Louis, MO) in 50 ml of water, sterilize by autoclaving for 15 min, and store at room temperature.

Polyethylene Glycol 3350 (50%, w/v). Dissolve 50 g of PEG 3350 (Sigma) in 30 ml of distilled, deionized water in a 150-ml beaker on a stirring hot plate. When the solution has cooled to room temperature, make the volume up to 100-ml in a 100-ml measuring cylinder and mix thoroughly by inversion. Pour the solution in to a suitable glass bottle and autoclave for 15 min. Store, securely capped, at room temperature. Evaporation of water from the solution will increase the concentration of PEG and severely reduce the yield of transformants.

Single-Stranded Carrier DNA (2.0 mg/ml). Dissolve 200 mg of salmon sperm DNA (Sigma) in 100 ml of TE buffer (10 m*M* Tris-HCl, 1 m*M* Na_2 EDTA, pH 8.0) by stirring at 4° for 1–2 hr. Store 1.0-ml samples at −20°. Denature the carrier DNA in a boiling water bath for 5 min and chill in ice/water before use. Boiled samples, stored at −20°, can be reused or reboiled three times without loss of activity.

[12] M. D. Rose, *Methods Enzymol.* **152,** 481 (1987).

Yeast Growth Media. Yeast strains are grown up on plates of YPAD agar (YPD[13] supplemented with 100 mg adenine hemisulfate per liter). The yeast cells to be transformed are usually regrown in liquid 2× YPAD medium[3] (2% Bacto-yeast extract, 4% Bacto-peptone, 4% glucose and adenine hemisulfate, 100 mg/liter). SC selection medium[12] is adjusted to pH 5.6 with 1.0 *N* NaOH and autoclaved. Commercial formulations of YPD agar (Bacto YPD agar) and broth (Bacto YPD broth) media are available from Becton Dickinson Microbiology Systems (Becton Dickinson, Sparks, MD; www.bd.com). These media should be supplemented with adenine hemisulfate as above.

Rapid Transformation Protocol

Day 1. Inoculate the yeast strain in a 2-cm^2 patch onto YPAD agar and incubate overnight at 30°. Alternatively, the yeast strain can be inoculated into 5 ml of liquid medium (2× YPAD or SC selection medium) and incubated on a shaker at 30° and 200 rpm.

Day 2

1. Heat a tube of carrier DNA in a boiling water bath for 5 min and then chill in ice/water.
2. Scrape a 50-μl portion of yeast from the YPAD plate and suspend the cells in 1 ml of sterile water in a 1.5 ml microcentrifuge tube. The suspension will contain about 5×10^8 cells. Cells grown overnight in 2× YPAD broth will reach a titer between 1 and 2×10^8/ml; the titer in SC medium will be about 5×10^7/ml. Harvest 2 ml of a YPAD culture and 5 ml of an SC culture. Note: Cells in log phase growth on agar or in liquid medium will transform with high efficiency.
3. Pellet the cells at 13,000 rpm at room temperature in a microcentrifuge for 30 sec and discard the supernatant.
4. Add the following components of the Transformation Mix (T Mix) to the cell pellet in the order listed:

Component	Volume (μl)
PEG 3350 (50%, w/v)	240
Lithium acetate 1.0 *M*	36
Boiled SS–Carrier DNA (2 mg/ml)	50
Plasmid DNA (0.1 to 1 μg) plus water	34
Total volume	360

Be sure to vortex mix the carrier DNA before pipetting it.

[13] F. Sherman, *Methods Enzymol.* **194,** 3 (1991).

5. Incubate the tube in a water bath at 42° for 40 to 60 min. Many laboratory strains will yield up to 1×10^5 transformants/μg plasmid after 60 min incubation. Extending the time at 42° to 180 min increases the yield to $>1 \times 10^6/\mu$g with some strains.
6. Microcentrifuge at 13,000 rpm at room temperature for 30 sec and remove the T Mix with a micropipettor.
7. Pipette 1.0 ml of sterile water into the tube and resuspend the cells by stirring with a micropipette tip and then vortex mixing vigorously.
8. Pipette 10- and 100-μl samples onto plates of appropriate SC selection medium, incubate at 30° for 3–4 days, and isolate transformants. The 10-μl samples should be pipetted into 100-μl puddles of sterile water.

This protocol can be used with cultures that have been stored at room temperature or in a refrigerator. The yield will be reduced with older cultures but will generally be sufficient to isolate a number of transformants of the desired genotype.

High-Efficiency Transformation Protocol

This protocol can be used to generate sufficient transformants in a single reaction to screen multiple yeast genome equivalents for plasmids that complement a specific mutation. It can also be used to transform integrating plasmids, DNA fragments and oligonucleotides[14] for yeast genome manipulation. Finally, it is used to optimize the conditions for transformation of a particular yeast strain, for example, the transformation of a plasmid library into a two-hybrid yeast strain transformed with a bait plasmid by the rapid transformation protocol. The high efficiency protocol can also be employed to transform a yeast strain simultaneously with two different plasmids, such as the two-hybrid bait and prey plasmids.

Day 1. Inoculate the yeast strain into 5 ml of liquid medium (2× YPAD or SC selection medium) and incubate overnight on a rotary shaker at 200 rpm and 30°. Place a bottle of 2× YPAD and a 250-ml culture flask in the incubator as well.

Day 2

1. Determine the titer of the yeast culture by pipetting 10 μl of cells into 1.0 ml of water in a spectrophotometer cuvette and measuring the OD at 600 nm. For many yeast strains a suspension containing 1×10^6 cells/ml will give an OD_{600} of 1.0. Alternatively, titer the culture using a hemocytometer.
2. Transfer 50 ml of the prewarmed 2× YPAD to the prewarmed culture flask and add 2.5×10^8 cells to give 5×10^6 cells/ml.
3. Incubate the flask on a rotary or reciprocating shaker at 30° and 200 rpm.
4. When the cell titer is at least 2×10^7 cells/ml, which should take about

[14] L. I. Linske-O'Connell, F. Sherman, and G. McLendon, *Biochemistry* **34,** 7094 (1995).

4 hr, harvest the cells by centrifugation at 3000*g* at room temperature for 5 min, wash the cells in 25 ml of sterile water and resuspend in 1 ml of sterile water.

5. Boil a 1.0-ml sample of carrier DNA for 5 min and chill in an ice/water bath while harvesting the cells.
6. Transfer the cell suspension to a 1.5-ml microcentrifuge tube, centrifuge for 30 sec, and discard the supernatant.
7. Add water to a final volume of 1.0 ml and vortex-mix vigorously to resuspend the cells.
8. Pipette 100-μl samples (ca. 10^8 cells) into 1.5 ml microfuge tubes, one for each transformation, centrifuge at 13,000 rpm at room temperature for 30 sec, and remove the supernatant.
9. Make up sufficient T Mix for the planned number of transformations plus one extra. Keep the T Mix in ice/water.

Reagents	Number of transformations planned		
	1	5 (6×)	10 (11×)
PEG 3350 (50%, w/v)	240 μl	1440 μl	2640 μl
Lithium acetate 1.0 *M*	36 μl	216 μl	396 μl
Boiled SS-Carrier DNA (2 mg/ml)	50 μl	300 μl	550 μl
Plasmid DNA plus water	34 μl	204 μl	374 μl
Total volume	360 μl	2160 μl	3960 μl

10. Add 360 μl of T Mix to each transformation tube and resuspend the cells by vortex mixing vigorously.
11. Incubate the tubes in a 42° water bath for 40 min.
12. Microcentrifuge at 13,000 rpm at room temperature for 30 sec and remove the T Mix with a micropipettor.
13. Pipette 1.0 ml of sterile water into each tube; stir the pellet with a micropipette tip and vortex vigorously.
14. Plate appropriate dilutions of the cell suspension onto SC selection medium. For transformation with an integrating plasmid (YIp), linear construct, or oligonucleotide, plate 200 μl onto each of five plates; for a YEp, YRp, or YCp library plasmid dilute 10 μl of the suspension into 1.0 ml of water and plate 10- and 100-μl samples onto two plates each. The 10-μl samples should be pipetted directly into 100-μl puddles of sterile water on the SC selection medium.
15. Incubate the plates at 30° for 3 to 4 days and count the number of transformants.

The transformation efficiency (transformants/1 μg plasmid/10^8 cells) can be determined by calculating the number of transformants in 1.0 ml of resuspended cells per 1.0 μg plasmid per 10^8 cells. For example, if the transformation of 1.0×10^8 cells with 100 ng plasmid resulted in 500 colonies on a plate of SC selection medium spread with 1 μl of suspension:

$$\text{Transformation efficiency} = 500 \times 1000 \text{ (plating factor)} \times 10 \text{ (plasmid factor)} \times 1 \text{ (cells/transformation} \times 10^8\text{)}$$

$$\text{Transformation efficiency} = 5 \times 10^6 \text{ transformants/1.0 } \mu\text{g plasmid/}10^8 \text{ cells}$$

Transformation efficiency declines as plasmid concentration is increased[1] but the actual yield of transformants per transformation increases. For example, 100 ng of plasmid in a transformation might give a transformation efficiency of 5×10^6 and a yield of 5×10^5 transformants, whereas with 1 μg of plasmid the transformation efficiency might be 2×10^6 and the yield 2×10^6 per transformation. In order to obtain 5×10^6 transformants it is simpler to set up two or three transformations with 1 μg of plasmid DNA, or a single 3-fold scaled up transformation, than to carry out 10 reactions with 100 ng of plasmid in each.

Large-Scale Transformation Protocol

The high efficiency transformation protocol can be scaled up 10- to 120-fold to generate the large numbers of transformants required for systems such as a two-hybrid screen.[3,11,15] It is best to scale up a transformation reaction to obtain a higher transformation yield rather than just increasing the amount of plasmid DNA. For example, for a 10-fold scale-up, use all of the cells from a 50-ml regrown culture ($\sim 1 \times 10^9$ cells) with 10 μg plasmid DNA in 3.6 ml of T Mix. The incubation at 42° should be extended to at least 60 min to allow for temperature equilibration.

Microtiter Plate Transformation

The following protocols can be used to accomplish a large number of transformation reactions in round-bottom 96-well microtiter plates. The agar plate protocol can be used to transform a plasmid into many different yeast strains and the liquid culture protocol can be used to introduce many different plasmids or constructs into a single strain. The liquid culture protocol can be used to optimize the conditions for the transformation of a specific yeast strain as suggested above. Both of these protocols require a microtiter plate centrifuge rotor, a 96-prong replicator (Fisher Scientific, Nepean, Ontario, Canada, www.fishersci.ca), 150-mm petri plates, an eight-channel micropipettor (Eppendorf or Titer Tek), and sterile troughs. We have found that an 8×8 well custom-made replicator (lacking the four corner prongs)

[15] R. A. Woods and R. D. Gietz, *in* "Methods in Molecular Biology, Vol. 177," (P. N. MacDonald, ed.), p. 85. Humana Press, Totowa, NJ.

that can be used with 100 x 15 mm regular petri dishes is often more convenient for up to 60 transformations than the full-size replicator.

Agar Plate Protocol

Day 1

1. Sterilize the replicator by dipping the prongs into a dish of 95% (v/v) ethanol and passing them through a bunsen flame.
2. Rest the replicator "prongs up" in a beaker and lower a plate of YPAD onto the prongs to make an imprint on the agar.
3. Use toothpicks or an inoculating loop to patch the yeast strains onto the imprints. Make an orientation mark on the bottom of the plate and incubate the plate overnight at 30°.

Day 2

1. Dispense 150-μl samples of sterile water into the wells of a microtiter plate.
2. Sterilize the replicator and rest it "prongs up" in a beaker.
3. Invert the YPAD plate over the replicator and align the patches of yeast with the tips of the prongs. Lower the plate onto the replicator, ensuring that all of the patches of yeast make contact, and move the plate gently in very small circles to transfer cells to the replicator. Remove the plate and inspect the prongs; use a toothpick or inoculating loop to add cells if necessary.
4. Lower the replicator into the microtiter plate wells and agitate to suspend the cells. The average number of cells/well will be $\sim 1 \times 10^7$; a second transfer will double the number. Mark the orientation of the microtiter plate.
5. Centrifuge at 3500 rpm at room temperature for 10 min using a microtiter plate rotor with an appropriate balance plate (if necessary).
6. Remove the medium by aspiration with a sterile micropipette tip attached to a vacuum line. Be careful not to touch the cell pellet with the tip. Alternatively, shake the water out of the wells into a sink. This takes practice, but is much faster than aspiration!
7. Boil carrier DNA (2 mg/μl) for 5 min and chill in ice/water.
8. Prepare T Mix minus PEG. The volumes below are for a single well and 96 wells (allowing 4 extra). Keep the T Mix minus PEG in ice/water.

Component	1 well	96 wells
Lithium acetate 1.0 *M*	15.0 μl	1.5 ml
Carrier DNA (2 mg/ml)	20.0 μl	2.0 ml
Plasmid DNA + water	15.0 μl	1.5 ml
Total volume	50.0 μl	5.0 ml

Note: We use 20 ng plasmid DNA per well; however, more can be added.

9. Pipette 50 μl T Mix minus PEG to each well. Clamp the plate on a rotary shaker and agitate at 400 rpm for 2 min to resuspend the cell pellets. The cells resuspend readily in T Mix minus PEG but not in T Mix.
10. Pipette 100 μl PEG 3350 (50% w/v) into each well. Clamp the plate on the rotary shaker at 400 rpm for 5 min to ensure that the cell suspension is homogeneous.
11. Place the microtiter plate in a ZipLoc sandwich bag or seal it with Parafilm and incubate at 42° for 3–4 hr.
12. Centrifuge the microtiter plate as before and remove the T Mix by aspiration.
13. The transformation reactions can be sampled as follows:
    (a) *Quantitative samples:* Pipette 100 μl of water into the wells. Clamp the plate on the rotary shaker at 400 rpm for 5 min to resuspend the cells. Pipette 5 μl samples into 100-μl puddles on regular plates of SC selection medium.
    (b) *Qualitative samples:* Pipette 50 μl of water into the wells. Resuspend the cells and use the sterile replicator "prongs down" to print onto plates of SC selection medium. The transfer volume is approximately 10 μl. Additional samples can be overlaid with care if required.
14. Incubate the plates at 30° for 2 to 4 days and recover transformants.

We have obtained up to 8000 transformants/well with 1×10^7 cells/well, 20 ng plasmid, and 4 hr incubation at 42°.

Liquid Culture Protocol. This protocol is used when transforming a single strain with multiple plasmids or DNA constructs. The yeast culture is grown overnight and regrown for two divisions as in the high efficiency transformation protocol. A complete microtiter plate (96 wells) with 4×10^7 cells/well will require 200 ml of regrown culture and 8 μg plasmid. The cells of the regrown culture should be harvested, washed, and resuspended in water at 4×10^8 cells/ml as described in the high efficiency transformation protocol.

Day 1. As in high-efficiency transformation protocol.

Day 2

1. Dispense 100-μl samples (4×10^7 cells) of the suspension of regrown cells into the wells of the microtiter plate. Centrifuge and remove the supernatants.
2. Continue from step 9 of the agar plate protocol with the following changes:
   (a) Increase the amount of plasmid in the T Mix minus PEG accordingly.
   (b) Incubate the plates at 42° for 60 min.
3. Sample the wells by plating or replica plating onto SC selection medium.

Frequently Asked Questions

For several years we have maintained a Web site[16] devoted to yeast transformation by the LiAc/SS-DNA/PEG protocol. The most frequently asked questions are concerned with the following:

(a) *A very low number of transformants.* Many factors can result in low numbers of transformants. (i) Check the dilution factor and plate a larger sample if necessary: we regularly plate 200-, 20-, and 2-μl samples when using a new yeast strain or plasmid. (ii) Check the integrity of the plasmid preparation prior to use. Plasmid degradation severely reduces the recovery of transformants. (iii) PEG concentration is critical for good transformation. Make fresh 50% (w/v) PEG 3350 reagent with care to ensure the proper concentration. PEG at this concentration is a viscous solution and is difficult to mix and pour. Use a plastic measuring cylinder and beaker to make the solution and carefully pour into a securely capped container. Evaporation of water from the PEG solution over time will increase the concentration and reduce the recovery of transformants. (iv) Other possible factors may include lithium acetate or temperature sensitivity of the yeast strain.

(b) A very large number of transformants. Check that the genetic markers in the yeast strain and plasmid correspond to the medium used to select for transformants. For example, if the medium selects for uracil prototrophy and the yeast strain is *URA3*, all the cells plated will grow.

(c) Purity of plasmid DNA. Plasmid DNA does not have to be extensively purified to be used for transformation by these protocols. Plasmid DNA isolated using the classic mini-preparation procedure usually transforms better than more highly purified plasmid DNA.

Optimization of Transformation for Specific Yeast Strains

Yeast strains vary tremendously in their transformation characteristics; some transform well and others poorly. The protocols described above will work well for most strains. If a strain transforms poorly it is best to obtain one with good transformation characteristics from a reputable source. When using a particular yeast strain, optimize the protocol(s) by investigating the following parameters in the order listed.

1. Duration of 42° incubation
   (a) Rapid and agar plate (microtiter plate) transformation protocols (60–300 min)
   (b) High efficiency and liquid culture (microtiter plate) protocols (20 to 80 min)

[16] R. D. Gietz, Web site: http//www.umanitoba.ca/faculties/medicine/units/biochem/gietz

2. Amount of carrier DNA (25–75 μl)
3. Amount of plasmid DNA (0.1–5 μg)
4. Number of cells (0.5–4 $\times$ 10^8)
5. Lithium acetate concentration (18–54 μl)
6. PEG concentration (220–270 μl)

Several workers have reported that the addition of dimethyl sulfoxide (DMSO) and/or ethanol to the Transformation Mix increases the recovery of transformants significantly.[17–21] In our experience these additions at best double or triple the yield if other conditions, particularly the duration of incubation at 42°, have been optimized. Our findings for the strain DY2389 are summarized below:

Protocol	Additive	Min at 42°	Time of addition	Increase in yield
Rapid	DMSO, 5%	180	With T Mix	3.3×
Rapid	Ethanol, 5%	180	After 60 min at 42°	2.8×
High efficiency	DMSO, 1%	40	With T Mix	1.7×
High efficiency	Ethanol, 2.5%	40	After 10 min at 42°	1.9×

Some strains do show a marked response to additives. For example, Y190, which transforms well using the high efficiency protocol, 5×10^6 transformants/μg,[5] transforms poorly by the rapid protocol. Only 16,800 transformants/μg were recovered after incubation at 42° for 180 min; the addition of 5% DMSO to the T Mix increased the yield nearly 14-fold to 231,100 transformants/μg.

Summary

In this chapter we have provided instructions for transforming yeast by a number of variations of the LiAc/SS-DNA/PEG method for a number of different applications. The rapid transformation protocol is used when small numbers of transformants are required. The high efficiency transformation protocol is used to generate large numbers of transformants or to deliver DNA constructs or oligonucleotides into the yeast cell. The large-scale transformation protocol is primarily applicable to the analysis of complex plasmid DNA libraries, such as those required for the yeast two-hybrid system. The microtiter plate versions of the rapid and high efficiency transformation protocols can be applied to high-throughput screening technologies.

[17] V. Lauermann, *Curr. Genet.* **20,** 1 (1991).
[18] J. Hill, K. A. Ian, G. Donald, and D. E. Griffiths, *Nucleic Acids Res.* **19,** 5791 (1991).
[19] R. Soni, J. P. Carmichael, and J. A. Murray, *Curr. Genet.* **24,** 455 (1993).
[20] P. L. Bartel and S. Fields, *Methods Enzymol.* **254,** 241 (1995).
[21] G. Cagney, P. Uetz, and S. Fields, *Methods Enzymol.* **328,** 3 (2001).