P01003
DSY Yeast Transformation Kit

Contents
50% PEG solution (50 ml)
10x TE buffer (12 ml)
1M LiOAc solution (12 ml)
Single stranded carrier DNA, 2 mg/ml (2 ml)
Sufficient for 5 library scale transformations or 80 small scale transformations

Storage
Store 50% PEG solution, 10x TE buffer and 1M LiOAc solution at room temperature.

Store single stranded carrier DNA at -20°C.

Before you start
Thaw single stranded DNA on ice. Incubate at 95°C for 5 minutes and immediately place in an ice/water bath for 5 minutes. Repeat and store the denatured single stranded DNA at -20°C. The single stranded carrier DNA is now ready to use for small scale transformation (Protocol 1). For library scale transformation, a separate denaturation step is needed (see Protocol 2).

Background

Lithium acetate (LiOAc)-mediated yeast transformation is the most widely used method for introducing DNA into yeast cells. Among other parameters, the size of the carrier DNA fragments is essential to achieve high transformation efficiencies using the LiOAc method[1,2]. The carefully optimized components, together with large-fragment carrier DNA, ensure that you will achieve the highest possible transformation efficiencies using the DS Yeast Transformation kit.

Protocol 1: Small scale transformation

The following protocol can be used to transform a single plasmid or combinations of 2-3 different plamids into yeast. It is also suitable for transforming a linearized vector and a PCR fragment into yeast for *in vivo* recombination. Typical transformation effciencies range from $1x10^3$ to $1x10^4$ cfu/µg plasmid.

1. Inoculate several fresh yeast colonies into 20 ml liquid YPAD medium and grow overnight at 30°C with shaking.
2. Pellet the culture for 5 minutes at 700x g and resuspend the cells in 1 ml sterile deonized water.
3. Prepare the PEG/LiOAc master mix:

PEG/LiOAc mix	
Reagent	**Amount for 10 transformations**
50% PEG	2.4 ml
1 M LiOAc	360 µl
ss-DNA	250 µl

4. Set up the transformation mixes:

Transformation setup		
Plasmids	-	1.5 µg plasmid
In vivo recombination	1.5 µg PCR product	1.0 µg digested vector

5. Add 300 µl PEG/LiOAc master mix to each tube and vortex briefly.
6. Add 100 µl resuspended yeast cells from step 2 to each tube, vortex 1 minute to thoroughly mix all components.
7. Incubate in a 42°C water bath for 45 minutes.
8. Pellet the reactions for 5 minutes at 700x g.
9. Dissolve each pellet in 100 µl 0.9% NaCl and plate each transformation onto one 10 cm diameter selective SD plate .
10. Seal plates with parafilm and incubate for 2-3 days at 30°C.

Protocol 2: High-efficiency library scale transformation

The following protocol is used to transform a yeast strain already bearing a bait plasmid with a cDNA library for a yeast two-hybrid, DUALmembrane or DUALhunter interaction screen. However, the protocol may also be adapted for general high efficiency transformation by using an untransformed yeast strain and replacing the selective SD medium with YPAD medium. The protocol requires 28 µg of plasmid DNA and should yield transformation efficiencies in the range of $5x10^5$ to $2x10^6$ clones/µg DNA.

1. Inoculate several fresh yeast colonies into 20 ml appropriate selective SD medium and grow overnight at 30°C with shaking.
2. Inoculate 100 ml of the same SD medium with the entire culture from step 1 and grow overnight at 30°C with shaking.
3. Prepare a 1:10 dilution of the overnight culture in water by diluting 100 µl culture in 900 µl water. Prepare a blank by diluting 100 µl SD medium in 900 µl water.
4. Measure the OD_{546} of the 1:10 dilution and calculate the OD_{546} of the undiluted culture by multiplying with 10.
5. Calculate the amount of culture needed for 30 OD units (e.g. if your culture has an OD_{546} of 0.6 you need 30 OD units / 0.6 OD per ml culture = 50 ml). Aliquot this amount of the overnight culture into 50 ml Falcon tubes and spin down at 700x g for 5 minutes.
6. Resuspend the pellet in 200 ml 2x YPAD (pre-warmed to 30°C) and transfer to a 1 liter shaker flask.
7. Grow the cells at 30°C with vigorous shaking to an OD_{546} of 0.6 (two cell divisions).

   Note

   Optimal transformation efficiencies are achieved only if the majority of your cells have undergone two cell divisions. Make sure to accurately adjust your 2x YPAD culture to an OD_{546} of 0.15 and

only proceed to the next step once the culture has reached an OD_{546} of 0.6. This should take 3-5 hours but it may take longer if your bait is slows down the growth of yeast.

8. Prepare the single stranded carrier DNA: thaw carrier DNA and incubate the amount to be used for transformation for 5 minutes at 95°C, then place immediately in an ice/water bath. Repeat once. Your carrier DNA is now ready for use.
9. Prepare the LiOAc/TE mix:

LiOAc/TE mix	
Reagent	**Amount for 1 transformation (8 ml)**
1 M LiOAc	0.88 ml
10x TE pH 7.5	0.88 ml
ddH2O	6.24 ml

10. Prepare the PEG/LiOAc master mix:

PEG/LiOAc mix	
Reagent	**Amount for 1 transformation (12 ml)**
1 M LiOAc	1.2 ml
10x TE pH 7.5	1.2 ml
50% PEG	9.6 ml

11. Divide the 200 ml culture into four 50 ml Falcon tubes.
12. Centrifuge at 700x g for 5 minutes.
13. Resuspend each pellet in 30 ml of sterile water by vortexing.
14. Centrifuge at 700x g for 5 minutes.
15. Remove the supernatant, resuspend each pellet in 1 ml LiOAc/TE mix and transfer to an eppendorf tube.
16. Centrifuge at 700x g for 5 minutes.
17. Remove the supernatant and resuspend each pellet in 600 µl of LiOAc/TE mix.
18. Set up four 50 ml Falcon tubes and add 7 µg of the cDNA library to each tube.
19. Add 100 µl denatured single stranded carrier DNA to each tube.
20. Add 600 µl yeast cells from step 17 to each tube.
21. Vortex briefly to mix.
22. Add 2.5 ml PEG mix to each tube.
23. Vortex for 1 minute to thoroughly mix all components.
24. Incubate at 30°C for 45 minutes, mix briefly every 15 minutes.
25. Add 160 µl DMSO to each tube, mix immediately by shaking.
26. Incubate at 42°C for 20 minutes.
27. Pellet cells at 700x g for 5 minutes.

28. Resuspend each pellet in 3 ml 2x YPAD.
29. Let the cells recover at 30°C for 90 minutes with shaking.
30. Pellet the cells at 700x *g* for 5 minutes
31. Resuspend each pellet in 1.2 ml 0.9% NaCl, pool into one tube
32. Plate onto the prepared selective plates: plate 300 µl resuspended cells per 15 cm plate
33. Use the remaining resuspended cells to prepare 1:100, 1:1000 and 1:10'000 dilutions in 0.9% NaCl and plate 100 µl of each dilution onto an SD plate where selective pressure is applied only to the bait and prey vectors (e.g. SD-trp-leu for the DUALhybrid, DUALmembrane or DUALhunter systems). These plates are used later to calculate the transformation efficiency.
34. Seal all plates with parafilm and incubate at 30°C for 3-4 days.

Troubleshooting

- For highest transformation efficiencies, single stranded carrier DNA should be denatured two times at 95°C for 5 minutes.
- Always store single stranded carrier DNA at -20 °C.
- Always close the bottle containing PEG solution tightly to avoid evaporation.
- During incubation in 2x YPAD medium, make sure the cells have undergone two cell divisions. Start with an OD_{546} of 0.15 and grow the cells to an OD_{546} of 0.6.
- Make sure the media were made correctly. If you suspect that the media or amino acid stock solutions have been over-autoclaved, make fresh solutions and either filter sterilize them or adjust the autoclave settings.
- Try re-growing yeast from the original glycerol stock.
- The protein encoded by the plasmid may be toxic.Try using a vector that expresses lower levels of the protein or use a vector with an inducible promoter.

Literature

1. Gietz RD, Woods RA.Genetic transformation of yeast (2001) *Biotechniques* **30**(4):816-20, 822-6, 828 passim)
2. Gietz, R.D. and R.A. Woods. (2002) Transformation of yeast by the LiAc/SS carrier DNA/PEG method. *Methods Enzymol* **350**: 87-96.

Support

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