Making Yeast Competent Cells and Yeast Cell Transformation

Yongxian Lu

Carnegie Institution for Science, Stanford University, Stanford, USA

[Abstract] This is a simple but reliable protocol to make very high transformation efficiency yeast competent cells. By expressing your gene of interest, protein function can be studied in yeast cells.

Materials and Reagents

1. Bacto-Yeast extract (Thermo Fisher Scientific)
2. Bacto-peptone (Thermo Fisher Scientific)
3. Glucose (dextrose) (Thermo Fisher Scientific)
4. Bacto-agar (Thermo Fisher Scientific)
5. Deionized H_2O
6. Glycerol (Sigma-Aldrich)
7. Dimethyl sulfoxide (DMSO) (Sigma-Aldrich)
8. PEG 3350 (Sigma-Aldrich)
9. Lithium acetate dihydrate (LiAc) (Sigma-Aldrich)
10. Salmon sperm DNA (Life Technologies, Invitrogen™)
11. Yeast nitrogen base (YNB) (Sigma-Aldrich)
12. 2-(N-morpholino) ethanesulfonic acid (MES)
13. ADE (Sigma-Aldrich)
14. Agar
15. YPAD plate & liquid medium (see Recipes)
16. Transformation solution (see Recipes)
17. YNB + MA plate (see Recipes)

Equipment

1. Water bath (VWR International)
2. Centrifuges (Eppendorf)
3. 30 °C shaker and incubator (VWR International)
4. Standard petri dishes (VWR International)
5. 1.5 ml centrifuge tubes (Eppendorf)

Procedure

A. Make yeast competent cells (Modified from Gietz & Schiestl, 2007)

1. Obtain yeast strains of interest and streak on YPAD plates. Let cells grow 2 d before inoculation.
2. 1st Inoculation: Inoculate one colony into 25 ml YPAD liquid medium. Grow cells overnight at 30 °C with shaking speed around 200 rpm.
3. 2nd inoculation. Transfer the 25 ml cell culture into 75 ml YPAD medium. Grow cells at 30 °C for 4 h.
4. Harvest cells by centrifugation at 3,000 *x g* for 5 min, wash cells with 0.5 volumes of sterile H_2O.
5. Centrifuge again with 3,000 *x g* for 5 min.
6. Re-suspend cells in 0.01 volumes of sterile H_2O, transfer to a suitable centrifuge tube and pellet at 3,000 *x g* for 5 min at 20 °C.
7. Re-suspend cell pellet in 0.01 volumes of filter sterilized frozen competent cell solution (5% v/v glycerol, 10% v/v DMSO).
8. Dispense 50 µl cells into 1.5 ml Eppendorf tubes.
9. Place the tubes into a box with Styrofoam or cardboard (slow freezing is essential for good survival rates).
10. Store the box in a -80 °C freezer (cells can be kept at this condition for up to one year).

B. Yeast cell transformation

1. If using competent cells stored at -80 °C, thaw cells at 37 °C water bath for 15-30 sec. if using freshly made competent cells, go to step 2).
2. Centrifuge at 13,000 *x g* for 2 min to remove the supernatant.
3. Make the transformation solution for the planned number of transformations plus one extra (negative control) (see the recipes).
4. Add the solution to the cell pellet, vortex to re-suspend the cells.
5. Incubate in a 42 °C water bath for 40 min.
6. Centrifuge at 13,000 *x g* for 30 sec and remove the supernatant.
7. Pipette 1 ml of sterile H_2O into the transformation tube to re-suspend the pellet.
8. Plate 200 µl of the cell suspension onto the YNB + MA plate growth plate with your selection marker.
9. Incubate plates at 30 °C for 2~4 d.

Recipes

1. Make YPAD plate & liquid medium
   1% Bacto-yeast extract - 10 g/L
   2% Bacto-peptone -20 g/L
   2% Glucose (Dextrose) - 20 g/L
   If making YPD plates, add 20 g Bacto-agar.
   Fill up to 1 L with deionized H_2O.
   NO pH adjustments.
   Note: Autoclave agar and glucose separately, to avoid caramelization.
2. Transformation solution
   PEG 3350 [50% (w/v)] 260 µl
   1 M LiAc 36 µl
   Salmon sperm DNA (10 mg/ml) 10 µl
   Plasmid 3 µl
   Sterile H_2O 51 µl
   Total 360 µl
3. YNB+ MA plate (100 ml)
   YNB 0.67 g
   20 mM MES 0.39 g
   ADE 0.01 g
   Agar 2 g
   Glucose 2 g

References

1. Gietz, R. D. and Schiestl, R. H. (2007). Frozen competent yeast cells that can be transformed with high efficiency using the LiAc/SS carrier DNA/PEG method. *Nat Protoc* 2(1): 1-4.
2. Lu, Y., Chanroj, S., Zulkifli, L., Johnson, M. A., Uozumi, N., Cheung, A. and Sze, H. (2011). Pollen tubes lacking a pair of K^+ transporters fail to target ovules in *Arabidopsis*. *Plant Cell* 23(1): 81-93.