

## Yeast Transformation

### I. Protocol

A. Yeast cell preparation and transformation.

#### **Three to four days prior to the next step:**

1. Streak out (from the  $-80^{\circ}\text{C}$ ) the yeast host strains onto YPAD plates. (It generally takes 3-4 days to get good-sized yeast colonies.)

#### **Day 1:**

2. Pick a single colony of each into 5 ml YPAD. Grow overnight.

#### **Day 2:**

3. Inoculate 250 ml YPAD with the 5 ml overnight culture. Grow overnight.

(Note: The 250 ml overnight culture is sufficient for numerous transformations.)

#### **Day 3:**

4. Spin cells down at 4000 x g.

5. Resuspend cells in 250 ml fresh media.

6. Grow cells for approximately two additional generations (3 – 4 hours).

Note: steps 5 and 6 are optional but when performed result in a significant increase in yeast transformation efficiency.

7. Spin cells down at 4000 x g.

8. Resuspend cells in 10 ml sterile  $\text{dH}_2\text{O}$  (this is a washing step).

9. Transfer cells to a 50 ml Oak ridge tube.

10. Spin cells down at 6000 x g.

11. Resuspend cells in 0.5ml ml freshly made 1X TE / 1X LiAc (~1-2ml of a very dense yeast suspension should be obtained).

12. Incubate ~ 60 min at  $30^{\circ}\text{C}$  with gentle shaking/agitation.

13. Meanwhile prepare a mixture of 10uL carrier DNA and 10uL transforming DNA.

14. At the conclusion of the 60-minute incubation, add 200uL of the yeast cell suspension to the 20uL volume of carrier and transforming DNA.

15. Incubate ~ 30 min at 30°C with gentle shaking/agitation.

16. To the yeast/DNA mixture add 1.2 ml 1X TE / 1X LiAc /40% PEG solution.  
Mix well.

<u>1X TE / 1X LiAc / 40% PEG:</u>	<u>1.2ml</u>
1) 10X TE	120µL
2) 10X LiAc	120µL
3) 50% PEG	980µl

17. Incubate ~ 30 min at 30°C with gentle shaking/agitation.

18. Heat shock yeast DNA mixture for 15 min at 42°C in a water bath.

19. Quick spin cells in microfuge (~10-12 sec; if you spin too long, the next step of resuspending the cells is much more difficult).

20. Resuspend cells in 500uL T<sub>10</sub>E<sub>0.1</sub> pH 7.5

21. Quick spin in microfuge.

22. Decant off s/n.

23. Repeat yeast cell T<sub>10</sub>E<sub>0.1</sub> pH 7.5 washing (steps 19- 21).

24. Resuspend with 200uL T<sub>10</sub>E<sub>0.1</sub> pH 7.5.

25. Plate serial dilutions (10<sup>0</sup>, 10<sup>-1</sup>, 10<sup>-2</sup> and 10<sup>-3</sup>; i.e., the equivalent of 100uL, 10uL, 1µL and 0.1µL)

The yeast should be allowed to grow ~ 3-4 days.

## B. DNA preparation

### 1. Carrier DNA (herring sperm (HS), 2-8 kb, single stranded)

- a) Dissolve 25 mg of HS DNA in 2,250 ml H<sub>2</sub>O.  
Gentle heat, vortexing and patience helps.
- b) Add 250  $\mu$ L 10 NEB *Hae* III buffer.
- c) Partition into 5-500  $\mu$ L aliquots.
- d) Digest each aliquot with a decreasing quantity of *Hae* III.  
Suggested Units/ $\mu$ g of HS DNA:
  - 1) 0.0625 U/ $\mu$ g
  - 2) 0.025 U/ $\mu$ g
  - 3) 0.005 U/ $\mu$ g
  - 4) 0.001 U/ $\mu$ g
  - 5) Uncut control

(It is best to dilute the enzyme first in 1X buffer due to the difficulty of pipetting such small volumes of enzyme into the larger volume of viscous HS DNA.)

- e) Run an aliquot on a gel to determine the fraction that contains the DNA of the appropriate size. Pool samples if there are more than one that has the correctly sized DNA.
- f) Phenol, phenol/chloroform, chloroform extract the HS DNA. EtOH precipitate the DNA.
- g) Resuspend such that the final concentration is 20  $\mu$ g per  $\mu$ L.
- h) Boil 10 min to denature.
- i) Place on ice; it is now ready for use. Store at  $-20^{\circ}\text{C}$ .

**A very rapid and effective alternative to the *Hae*III digestion is to simply put the DNA in a syringe and pass the resuspended DNA up and down 10-20 times through a 25g needle.**

### 2. Transforming DNA

- a)  $\sim$ 1 $\mu$ g in 10 $\mu$ L TE
- b) CsCl, Qiagen (or some other Company's kit clean) or phenol extracted mini-prep DNA all work well.

## II. Materials

### A. Stuff

1. YPAD plates
2. 2 X 500 ml YPAD
3. 10X TE
4. 10X LiAc
5. 50 % PEG 3,350
6. 1X TE
7. Plates synthetic complete (SC) minus appropriate prototrophic selection marker.
8. Sterile centrifuge bottles
9. Sterile Oak Ridge tubes

## B. Recipes

1. YPAD 1L
  - a) Yeast Extract 10g
  - b) Peptone 20g
  - c) Dextrose\* 20g
  - d) Adenine sulfate 40mg
  - e) dH<sub>2</sub>O 1L
  - f) Bacto agar 20g
  
2. 10X TE<sub>(7.5)</sub> 100ml
  - a) 1M Tris (pH 7.5) 10ml
  - b) 0.5M EDTA 2.0ml
  - c) dH<sub>2</sub>O to 100ml
  - d) A/C
  
3. 10X LiAc 100ml
  - a) LiAc 10g
  - b) pH w/ acetic acid to ~ 7.5
  - c) dH<sub>2</sub>O to 100ml
  - d) (f), A/C
  
4. 50% PEG 3,350 100ml
  - a) PEG 3,350 50g
  - b) dH<sub>2</sub>O to 100ml (heating helps dissolve PEG)
  - c) (f), A/C
  
5. T<sub>10</sub>E<sub>0.1</sub> pH 7.5 200ml
  - a) 1M Tris pH 7.5 2.0ml
  - b) 0.5M EDTA 40μL
  - c) dH<sub>2</sub>O 198 ml
  
6. Synthetic complete (SC) media<sup>#</sup>
  1. 10X SD<sup>^</sup> plus Dextrose 100ml
    - a) YNB 6.7g
    - b) Dextrose 20g
    - c) dH<sub>2</sub>O to 100ml
    - d) A/C<sup>\*\*</sup> or f/s
  
  2. 100X ΔLEU, ΔTRP amino acid stock (yeast strain CG1945)
    - a) ADE 200mg
    - b) HIS 200mg
    - c) LYS 300mg

- d) MET 200mg
- e) URA 200mg
- f) dH<sub>2</sub>O to 100ml
- g) A/C
- h) Store at RT (otherwise ADE & URA will fall out of soln)

Add the amino acids to just under 100ml dH<sub>2</sub>O. The pH will need to be adjusted to ~10 with NaOH in order for the amino acids to all go into solution. Bring the final volume to 100ml. Filter sterilize or autoclave. Store in a dark bottle at RT

Note: A very useful alternative is to prepare a dry mixture of each of the amino acids and then add the appropriate quantity directly to the medium (e.g., the SD ΔLEU below) to be autoclaved.

### 3. 100X LEU

- a) LEU 300mg
- b) dH<sub>2</sub>O to 100ml
- c) A/C
- d) Store at 4°C or RT

### 4. 100X TRP

- a) TRP 200mg
- b) dH<sub>2</sub>O to 100ml
- c) A/C or f/s (Soren once told me that TRP should not be A/C)
- d) Store at 4°C or RT in the dark

### 5. SD ΔLEU 1L (~40 plates)

- a) dH<sub>2</sub>O 880ml
- b) Bacto-agar 20g
- c) A/C
- d) 10 YNB/Dextrose 100ml
- e) 100X SC (ΔLEU, ΔTRP) stock 10ml
- f) 100X TRP 10ml

### 5. SD ΔTRP 1L (~40 plates)

- a) dH<sub>2</sub>O 880ml
- b) Bacto-agar 20g
- c) A/C
- d) 10 YNB/Dextrose 100ml
- e) 100X SC (ΔLEU, ΔTRP) stock 10ml
- f) 100X LEU 10ml

\*The Dextrose is often prepared separately (but it is not critical to do so) and added to the other components after autoclaving because it causes caramelization of the media when autoclaved together with the other components.

#These are examples of selectable SD media; of course the genotype of the yeast and the marker on the plasmid will ultimately determine the required composition of the selectable media.

^SD = Difco's yeast nitrogen base (YNB) without amino acids.

\*\* Again, autoclaving causes caramelization of the dextrose in conjunction with other media components.

**Note:** This is for a large-scale transformation e.g., multiple plasmids or libraries etc. The protocol may be scaled up or down depending upon the specific need. I have also successfully transformed a single colony picked off of a plate when I was in a hurry (transformation efficiency was low but then all I needed was one colony).

**Reference** (read this, it is great because it explains everything): Robert H. Schiestl and R. Daniel Gietz (1989) High efficiency transformation of intact yeast cells using single stranded nucleic acids as a carrier. *Curr. Genet.* 16:339-346.