High Efficiency Yeast Library Transformation V.1

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ABSTRACT

This is a protocol to achieve high-efficiency (10^6 transformants/replicate) when transforming yeast cells with mutational libraries.

GUIDELINES

Always work in sterile conditions: flame or microbiological hood.

DOI:
dx.doi.org/10.17504/protocols.io.j8nlkw7o6l5r/v1

Protocol Citation: Benedetta Bolognesi 2023. High Efficiency Yeast Library Transformation. protocols.io

https://dx.doi.org/10.17504/protocols.io.j8nlkw7o6l5r/v1

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Protocol status: Working

We use this protocol and it's working

Created: May 26, 2023
**MATERIALS**

Reagents per library (3 biological replicates):
- 200 ml YPDA (50 ml per replicate and some extra for the overnights on day -1)
- 80 ml SORB* (26.5 ml per replicate)
- 75 ul ssDNA (10 mg/ml) (45 ul per replicate)
- 3000 ng plasmid library (1000 ng per replicate)
- 20 ml Plate mixture* (6 ml per replicate)
- 2 ml DMSO 100% (600 ul per replicate)
- 180 ml Recovery media (YPD + 0.5M Sorbitol)* (60 ml per replicate)
- 400 ml Plasmid selection media* (60 ml per replicate for the saturation + 10ml per replicate for washes (Day 1) and 60 ml per replicate for exponential growth (Day 3)).

In case you go on with the competition: 250 ml Competition media (60 ml per replicate and extra medium for the washes)

*SORB

100 mM LiOAc, 10 mM Tris pH 8.0, 1 mM EDTA, 1 M sorbitol
1L SORB = 10g LiOAc, 182g sorbitol, 10mL Tris 1M (1000X), 2mL EDTA 0.5M (500X)

*Plate mixture:

100 mM LiOAc, 10 mM Tris-HCl pH 8 (from 1 M stock), 1 mM EDTA/NaOH (from 0.5 M stock), pH 8, 40% PEG3350
1L plate mixture = 10g LiOAc, 400g PEG3350, 10mL Tris, 2mL EDTA
0.5L plate mixture = 5g LiOAc, 200g PEG3350, 5mL Tris, 1mL EDTA
Or 400ml autoclaved PEG 50%+ others (filtered) then bring to 500ml.

*Recovery Media:

YPD + Sorbitol 0.5M
1L Recovery media = 10g Yeast Extract, 20g Peptone, 20g Glucose, 91g Sorbitol, up to 1L water

## DAY -2

1. Restreak yeast strains to transform from glycerol stock

## DAY 0 Pre - growth

2. For each biological replicate, put 1 individual yeast colony to grow in 3 mL YPDA in culture
tubes (choose three colonies that are similar in size and not too big, not too little)

3. Prepare all media (see Materials) necessaries for transformation and warm YPDA Overnight in the 30 °C incubator.

4. Measure OD of overnight cultures

5. Dilute them in 50ml of YPDA to a final OD of 0.3

<table>
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<th>OD &gt; overnight</th>
<th>Volume needed for 50ml OD 0.3</th>
<th>YPDA</th>
<th>OD &gt; dilution</th>
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6. Incubate for 03:30:00 to 04:00:00 at 30 °C

7. Prewarm reagents 30 °C Water, SORB, Plate mixture, Recovery Media and Plasmid selection media. You can also prepare the flasks with the corresponding media while the cells grow and warm the flasks directly.

8. Pre-label Falcon tubes
9. Warm up the centrifuge at $30^\circ C$. Cold centrifuges will cause your cells to die after heatshock and transformation efficiency to drop.

10. After 04:00:00 measure OD

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11. Pour cells in 50ml falcon tubes and centrifuge 5 min at 3000 x g. Remove supernatant by pouring.

12. Resuspend pellet in 10ml of H2O, then bring it up to 50ml.

13. Centrifuge 5 min at 3000 x g. Remove supernatant by pouring.

14. Resuspend in 10ml of SORB (see Materials), transfer to a new falcon, complete to 25ml of SORB.

15. Centrifuge 5 min at 3000 x g. Remove supernatant by pouring.
16 Resuspend in 1.42 ml of SORB (see Materials), transfer to a 15ml falcon

17 Incubate 30 min on a gentle orbital shaker (tape falcons to shaker)

18 In the meantime, boil 45 ul (per replicate) of 10mg/ml ssDNA for 5 min and let it sit on ice for at least 2 min

19 Prepare a labeled 50ml falcon / replicate with 6 ml of Plate Mixture (see Materials)

20 After 30 min on the orbital shaker, add 1ug of plasmid library and 45ul of ssDNA and let cells sit at RT for 5 min.

21 Turn on water bath at 42 °C

22 Incubate cells for 10 min on the orbital shaker

23 Add the 1.4 ml of cells to the 6 ml of plate mixture

24 Incubate 30 min on a gentle orbital shaker (tape falcons to shaker)
25 Add 600ul of sterile DMSO

26 Heat shock for 20 min at 42 °C in the water bath. Quickly take out tubes and shake them upside down at 30'', 1', 1'30'', 2', 2'30'', 5', 10' and 15'. Falcons 50ml must be mostly submerged but water should not reach the tube-limit level as this may cause contamination. Bring a timer with you and some weight to keep the tube-rack down and avoid floating (another rack or something heavier).

27 Dry well the top of the tubes to avoid contamination and centrifuge tubes at 3000 rpm. Remove supernatant by pouring.

28 Additional quick spin of 30 seconds. Remove remaining liquid with a pipette.

29 Resuspend cells in 10ml of Recovery Media (see Materials) and bring it up to 60mls in a 250ml flask

30 Shake 200 rpm 30 °C for 1 hour

31 Prepare 50 ml of pre-warmed selection media (e.g. -URA) for each replicate + 40mls for washes
32. Centrifuge cells 5 minutes \(3000 \times g\)

33. Resuspend cells in 10ml selection media for a wash.

34. Centrifuge cells 5 minutes \(3000 \times g\)

35. Resuspend cells in 10ml selection media and add them to the 50ml of media in the flask.

36. Plate 60 ul (out of the 60ml) on 2 separate selective plates for each replicate with sterile glass beads.

37. Measure OD before putting flasks to grow.

38. Grow cells for 48-60h \(200 \text{ rpm} \ 30 \, ^\circ \text{C}\)

**DAY 3: From saturated to exponential culture**

39. Note: This step might introduce bottlenecks. It’s a healthy practice to calculate how many cells from the saturated culture you are using to then grow the exponential culture, and if they cover your library well. Double check that the volume of cells you pipette will have enough cells to cover 100x (or more) each variant. Consider that 50% of the cells might be dead due to the saturation of the culture.
40  Measure OD 1:10

<table>
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<th>OD600 1:10</th>
<th>ul cells x 60ml OD 0.05</th>
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41  Pipette the calculated volume in flasks with 60ml pre-warmed selection media. Keep track of time. This culture will grow to exponential before protein expression/selection/competition are carried out.