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Version 1

High Efficiency Yeast Library Transformation V.1

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Atlas of Variant Effects ...



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Protocol status: Working

We use this protocol and it's working

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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.



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

2d

- 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) necessities for transformation and warm YPDA
🕒 Overnight in the 🌡️ 30 °C incubator.

DAY 1 Transformation and recovery

7h 30m

- 4 Measure OD of overnight cultures
- 5 Dilute them in 50ml of YPDA to a final OD of 0.3

	OD >overnight	Volume needed for 50ml OD 0.3	YPDA	OD > dilution

- 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

7h 30m



9 Warm up the centrifuge at  30 °C . Cold centrifuges will cause your cells to die after heatshock and transformation efficiency to drop.

10 After  04:00:00 measure OD

4h

	OD600

11 Pour cells in 5 0ml falcon tubes and centrifuge 5 min  3000 x g . Remove supernatant by pouring.

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

13 Centrifuge 5 min  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  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 labelled 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 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 x g
- 33 Resuspend cells in 10ml selection media for a wash.
- 34 Centrifuge cells 5 minutes  3000 x 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 rpm  30 °C

DAY 3: From saturated to exponential culture

- 39 Note: This step might introduce bottlenecking. 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

	OD600 1:10	ul cells x 60ml OD 0.05



- 41 Pipette the calculated volume in flasks with 60ml pre-warmed selection media. Keep track of time. This culture with grow to exponential before protein expression/selection/competition are carried out.