Protocol for single barcode insertion (Galactose induction)

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MATERIALS:

- Yeast strain(s): e.g. SHA185
- Plasmid library in bacteria: e.g. L139
- Yeast extract + Peptone + galactose liquid media (YPG)
    - Yeast extract: 10g/L
    - Peptone: 20g/L
    - Galactose: 20g/L
- 2x YPD liquid media
    - Yeast extract: 20g/L
    - Peptone: 40g/L
    - Glucose: 40g/L
- SC-ura plates
- Salmon Sperm DNA, sheared (10 mg/mL) (ThermoFisher cat. no. AM9680)

PROCEDURE:

Plasmid barcode library preparation:

1. Inoculate the whole frozen stock (~10^9 cells) of a library into 1L of LB liquid media.
2. Incubate at 37ºC overnight.
3. Prepare several frozen stocks from the overnight culture for future library preps.
4. Use QIAGEN Maxi prep kit to extract plasmid DNA.

Yeast culture preparation:

1. Inoculate the yeast strain by picking a single colony with a sterile toothpick into 50 ml of YPD in a 250 ml flask. Incubate at 30ºC overnight on a rotary shaker.
2. Prepare 200 ml (30 x) or 400 ml (60 x) of 2x YPD broth.
3. After 12 ~16 h of growth, count cells using Coulter Count or hemacytometer and transfer the volume containing 6.25 x 10^8 cells (30 x) or 1.25 x 10^9 cells (60x) into 50 ml centrifuge tubes and pellet the cells at 3000*g* or 5 min. Resuspend the pellets in 20 ml pre-warmed 2x YPD broth and transfer to the culture flask. Add sufficient pre-warmed 2x YPD broth (30x -130 ml; 60x 280 ml) to bring the final titer to 5 x 10^6 cells/ml.
4. Incubate the flask at 30ºC and 200 rpm until the cells have undergone two divisions, which may take up to 4 h.
5. Denature single-stranded carrier DNA (30x --350µl, 60x --650µl) for 5 min in 95ºC heat block and chill in ice/water.
6. Make up appropriate volumes of transformation mix (see below) and keep in ice/water. This can be scaled up to 100x if needed.
   (For one transformation, up to 10µg plasmid library can be used)

Ingredients	30x (ml)	60x (ml)

PEG 50% w/v	7.2	14.4
LiAc 1.0 M	1.08	2.16
ssDNA (10 mg/mL)	0.3	0.6
Plasmid library + water	2.22	4.44
Total volume	10.8	21.6

7. Harvest yeast cells by centrifugation at 3000*g* for 5 min. Use a number of 50 ml disposable conical tubes. Resuspend in ½ culture volume of sterile water. Centrifuge at 3000*g* for 5 min and resuspend the pellet again in ⅛ volume of sterile water and combine the pellets into one 50 ml conical tube. Centrifuge at 3000*g* for 5 min and discard the supernatant.
8. Pipette the transformation mix from step 6 onto the cell pellet and suspend the cells by vortex mixing vigorously for 1 min to make sure there are no big chunks of the pellet left.
9. Incubate the cell suspension at 42ºC for 40~60 min depending on the yeast strain. (We usually use 50 min). Mix the contents of the tube by inversion at 5 min intervals to ensure a constant temperature throughout the mix.
10. Prepare YPG broth (30x -10 ml; 60x -20ml) by warming it to 30ºC.
11. Centrifuge cell suspension at 3000*g* for 5 min. Pour off the transformation mix supernatant and remove the remaining liquid with a micropipettor.
12. Resuspend the cells gently in pre-warmed YPG broth. (30x -10 ml; 60x -20 ml), which will bring the titer close to the saturation density (assuming 2.5 x 10^8 is the saturation density).
13. Incubate the cell suspension at 30ºC for ~16 hours for galactose-induced Cre recombination.
14. Determine the titer of the yeast culture using Coulter Count or hemacytometer. The density (***X*** x 10^8/ml) is obtained. This number is used to estimate the number of generations that cells have undergone during galactose induction.
15. Centrifuge at 3000*g* for 5 min and pour off the YPG broth.
16. Resuspend cells in SC-ura broth or sterile water (30x -10ml; 60x -20 ml).
17. Perform a serial dilution (1:10, 1:100, 1:1000) to estimate the number of colonies. Plate the rest of the cell suspension (200µl for each plate) on SC-ura plates (30x ~ 50 plates; 60x ~100 plates).
18. Incubate the plates at 30ºC for 2~3 days and count the number of colonies on serial dilution plates to estimate the total number of transformants ***Y***.
19. To estimate the complexity of the library, divide the number of colonies (***Y***) by the number of cell divisions during galactose induction (***X***/2.5) is the estimated size of the library, which is ***Y***/ (***X***/2.5) (assuming 2.5 x 10^8 is the saturation density).