Synthetic Lethality Analysis by Microarray

High Efficiency Yeast Transformation Protocol for Production

REFERENCE: Pan X, Yuan DS, Xiang D, Wang X, Sookhai-Mahadeo S, Bader JS, Hieter P, Spencer F, Boeke JD. (2004). A robust toolkit for functional profiling of the yeast genome. Mol Cell. 16(3):487-496.
Modified for Production by PBM & CT

For 20 + 2 control transformations (1 liter of culture)

Day 1

1. Remove **TWO** 1-ml aliquots of the ***xxx*Δ*::kanMX/XXX*** **heterozygous diploid deletion POOL** from the –80°C freezer, thaw at room temperature, and invert several times to resuspend cells. Inoculate 500 µl into each of 4 1L flasks containing 250 ml of YPD-rich. Optional: Measure starting OD_{600} (should be ~ 0.15/ml). Reserve a small amount of YPD liquid to use as blank for OD_{600}.

2. Grow yeast on a shaker at 30°C for 5-6 h, or until OD_{600} triples to ~ 0.5 ODU/ml. This will give ~250 ODU or 5e9 cells.

   NOTE: For cultures to be ready to go in the morning, pipet cell inoculum into a sterile metal test tube cap along with enough YPD liquid to "sink" the cap (i.e. mix 2 ml cells + 3 ml YPD → 1.25 ml per flask). Use long sterile forceps to carefully place cap into flask containing YPD, resting it upright on the bottom of the flask. Set water bath on a timer to start shaking at **~ 4:00 am**. The cap will be knocked over once shaker starts.

Day 2

3. Transfer cells to sterile Corning® 250 mL polypropylene (PP) centrifuge tubes. Harvest cells by centrifugation in **Sorvall RC-5C using SLA1500 rotor (3K rpm, 6 min, RT)**. Decant medium. Loosen cell pellets by vortexing, then resuspend in **sterile water** to wash. Combine all cells in one bottle at this stage and re-centrifuge. Decant liquid. Loosen pellet and resuspend in **0.1 M LiOAc** to wash. Loosen pellet and resuspend in **~19 ml 0.1 M LiOAc**. This should give **~22 ml cell suspension**, final volume.

4. Aliquot 1 ml cells to each of 22 microfuge tubes. Spin cells in microfuge at 3000 rpm for 30 sec. Flick away residual liquid, vortex or shake to loosen cell pellets and store tubes at room temperature.

5. Prepare **PEG/LiOAc/ssDNA mixture** containing the following amounts of reagents per transformation. Adjust amounts for the number of transformations to be done plus 1 or 2 extra (e.g. for 6 txn's add 4.34 ml (≈ 7 x 620 µl) 50% PEG, etc.).

Single Txn		22 + 2 = 24 Txn's	
620 µl	50% PEG-3350*	14.88 ml	☐
90 µl	1 M LiOAc	2.16 ml	☐
40 µl	10 mg/ml ss DNA‡	960 µl	☐
750 µl		18.00 ml	

* Prepare 50% PEG-3350 stock with sterile water, vacuum filter and cap tightly for storage. Aliquots can be frozen at -20°C for future use.
‡ First heat salmon sperm DNA at 100°C for 5 min, then transfer tube to an ice bath. DO NOT use leftover (i.e. once-heated) salmon sperm DNA for SLAM. *NOTE: For production SLAM, we purchase sheared salmon sperm DNA from Eppendorf AG (Cat. No. 0032 006.957 or 955-15-562-9).*

6. Add ***yfgΔ::URA3*** **PCR-amplified DNA** (~100-150 µl) to each ~100 µl cell aliquot. Vortex to resuspend cells *thoroughly*.

7. Add **750 µl PEG/LiOAc/ssDNA** mixture to each microfuge tube. Immediately invert microfuge tube several times to "pre-mix." Vortex all samples vigorously for 20-30 seconds to thoroughly mix (using multi-tube shaker).

8. Incubate cells on the roller drum for 30 min at 30°C.

9. If you have not already done so, add **sterile glass beads** (10-20) to ONE 150-mm and TWO 100-mm round plates containing SC-uracil/leucine solid medium (SC-URA-LEU). Store plates upside down until the transformants are plated so that the beads won't dent the surface of the medium.

10. After the 30°C incubation, add **100 µl DMSO** to each transformation and mix immediately.

11. Heat shock cells at 42°C for 14 min (use aluminum heating block). Invert tubes once or twice during heat shock period to promote even heating. Centrifuge to pellet cells, ≤3000 rpm for 30 sec.

12. Aspirate PEG/DMSO supernatant, switching tips for each sample. Add **1 ml 5 mM $CaCl_2$** and resuspend cells thoroughly by gently pipeting up and down (~1200 µl final volume).

13. Let tubes stand for at least 5 min (*but not longer than 15 min!*) at room temperature.

14. ***Titer transformation efficiency.*** Transfer 2 µl of the resuspended transformed cells to a second microfuge tube containing **198 µl 5 mM $CaCl_2$** (1:100 dilution). Plate 100 µl and 10

µl of this dilution onto **100-mm SC-URA-LEU plates** (for the 10 µl aliquot, pipet 100 µl of 5 mM $CaCl_2$ onto plate first). Swirl glass beads to spread the transformed cells evenly. Do platings at the same time, working quickly. Turn plates upside down and discard the glass beads.

15. Return transformation microfuge tubes to the centrifuge and spin at ≤3000 rpm for 30 sec.

16. Remove ~500 µl of supernatant. Resuspend cells in the remaining liquid and transfer to the **150-mm SC-URA-LEU plate**. Swirl beads to spread the cells evenly. Once cells dry in, turn plates upside, but retain glass beads in the lid. They will be used later to "scrape" transformants off the plate.

17. Incubate all plates at 30°C for 2 full days (48 hr).

Day 4

18. Photograph or scan each 100-mm SC-URA-LEU plate and make <fn>.jpeg annotated files to be uploaded into the database (SL-db). Also count the number colonies on the 100-mm plates. The Bio-Rad GelDoc can be used for this purpose (Sensitivity set at 1.5; Bin 3). Also note colony size and morphology. Ideally, there should be **≥ 30 colonies on the 10 *µ*l plate**—this corresponds to ≥ 300,000 (3 x 10e5) total Ura^+ transformants. *Calculation*: Total Ura^+ transformants ≈ # colonies in 100 *µ*l x 1200 (i.e. # colonies in 100 *µ*l/100 *µ*l x 200 *µ*l/2 *µ*l x 1200 *µ*l). If this minimal number of transformants is not obtained, the experiment should be discarded.

19. Provided the desired number of transformants is obtained, scrape cells from each 150-mm SC-URA plate using 2 additions of sterile water (7 ml, then ~3-5 ml) and swirling the glass beads to scrape cells from media surface. Transfer both "scrapes" to a single **15 ml conical tube containing 2.5 ml sterile 80% glycerol**. Recover **only 10 ml** of scraped cells in total, so as to bring the final volume to 12.5 ml (~16% glycerol). Mix well.

20. Determine the OD_{600} of each transformed pool. For this, mix 10 µl of cells with 990 µl sterile H_2O in a 1-ml plastic cuvette (1:100 dilution).

21. Proceed to sporulation and/or freeze FIVE 25 ODU aliquots at –80°C in Matrix Latch Racks (1.4 ml round-bottomed tubes) for future use.

22. For each transformation, replica print one of the SC-URA-LEU plates used to titer transformation efficiency to an SC-LEU+5-FOA plate. Patch stable *URA3*$^+$ and *ura3*Δ strains on some of the plates as controls. If 5-FOA^R colonies arise with high frequency, the Ura^+ transformants are not stable, perhaps owing to the presence of an ARS on the query gene fragment. In this case, it is important to also test the stability of Ura^+ haploids selected after sporulation.