

Synthetic Lethality Analysis by Microarray

Sporulation Protocol for Production dSLAM

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

Day 1 (Day 4 of Production Schedule)

1. For each query gene transformation, inoculate **25 ODU of the *yfgΔ::URA3/YFG xxxΔ:kanMX/XXX* heterozygous diploid pool** into 50 ml YPD (use a 250 ml flask). Incubate culture for 2 hr at 30°C with shaking.
2. After 2 hr, transfer culture to a sterile 50 ml conical tube. Pellet cells by centrifugation in swinging bucket rotor (**3000 rpm, 4 min, RT**).
3. Decant supernatant, vortex tube to loosen cell pellet, then resuspend cells in 25 ml sterile water to wash.
4. Pellet cells as in Step 2 above, decant supernatant, and vortex tube to loosen cell pellet.
5. Resuspend cells in **50 ml Sporulation Medium**, then decant cells into a sterile 250 ml Corning flask with orange screw top.

Sporulation Medium

1% KOAc

0.005% ZnOAc

300 μM L-Histidine-HCl

For 1 liter

100 ml 10% KOAc

10 ml 0.5% ZnOAc

3 ml 100 mM L-Histidine-HCl

887 ml sterile water

6. Incubate cultures for 5 days at 25°C with shaking.

Day 5 (Day 9 of Production Schedule)

7. After FIVE full days, transfer cultures to 50 ml conical tubes. As needed, adjust volume up to 50 ml with fresh Sporulation Medium. This will simplify calculations later (see below).
8. Place 10 μl of the sporulated culture on a microscope slide and assess sporulation efficiency microscopically. Score ~ 100-200 cell bodies. To avoid bias, score all cell bodies in each of several randomly chosen fields. Score dyads, triads, and tetrads as Spo⁺. The frequency of Spo⁺ cells can range from 15% to 50%.

9. Transfer 500 μ l of the culture to a 1 ml cuvette containing 500 μ l water (1:1 dilution). Mix well, avoiding air bubbles. Measure OD₆₀₀.
10. Calculate the Total ODU's for each culture. (i.e. OD₆₀₀ x 2 x 50). Pellet cells by centrifugation in swinging bucket rotor (**3000 rpm, 4 min, RT**) and resuspend in sterile water to give ~ 10 ODU/ml. NOTE: Simply multiply the OD₆₀₀ of the 1:1 dilution by 10 to calculate the appropriate volume.
11. If you have not already done so, add **sterile glass beads** to small (100-mm) and large (150-mm) haploid selection plates. Store plates upside-down until the transformants are plated so that the beads won't dent the surface of the medium.
12. For each sporulated culture, plate ~ 4 ODU (i.e. 400 μ l) onto:
 - **ONE 150-mm EXPERIMENTAL** haploid selection plate (aka "Magic Medium-URA"). This plate contains **SC-URA-HIS-ARG+CAN+G418** medium and will permit growth of only *MATa ura3 Δ yfg Δ ::URA3 xxx Δ ::kanMX* double mutant haploids.
 - **ONE 150-mm CONTROL** haploid selection plate (aka "Magic Medium+URA"). This plate contains **SC+URA-HIS-ARG+CAN+G418** medium that will permit growth of all *MATa ura3 Δ xxx Δ ::kanMX* haploids regardless of whether the query gene, *YFG*, is disrupted by *URA3*.

Swirl beads to distribute cells evenly on each plate. Once liquid has dried in, turn plates upside, but retain glass beads in the lid. They will be used later to "scrape" off the haploid pools.

NOTE: A 5-FOA-containing haploid selection plate (aka "Magic Medium+URA+FOA") containing **SC+URA-HIS-ARG+CAN+G418+5-FOA** medium can be used as an alternative **CONTROL**. This medium will specifically select for *MATa ura3 Δ YFG⁺ xxx Δ ::kanMX* haploid single mutants.

13. To determine the frequency of *URA3⁺ G418^R* double mutant haploids and of *G418^R* haploids overall, prepare a 1:2000 dilution of each sporulated culture. Typically, we first prepare a 1:100 dilution first (10 μ l into 1 ml), then dilute this 20-fold (20 μ l 1:100 into 400 μ l). Plate 100 μ l of the 1:2000 dilution onto:
 - ONE 100-mm **MM-URA** haploid selection plate
 - ONE 100-mm **MM+URA** haploid selection plate
 - ONE 100-mm **MM+URA+5-FOA** haploid selection plate, as necessary

Swirl beads to distribute cells evenly on each plate. Once liquid has dried in, invert plates and discard glass beads

14. Incubate all plates at 30°C for 2 days.

Day 7 (Day 11 of Production Schedule)

15. Photograph or scan each small plate and make `<fn>.jpeg` annotated files to be uploaded into the database (SL-db). Count the colonies on the small plates, then multiply by 8000 to determine the approximate number of cells spread onto the large plates. The Bio-Rad GelDoc can be used for this purpose (Sensitivity set at 1.5; Bin 3 or 5). Also note colony size and morphology.

Ideally, the number of Ura⁺ haploid colonies (**MM-URA**) should be half of the total number of colonies plated (**MM+URA**) or equal to the number of Ura⁻ colonies (**MM+FOA**). However, the proportion of Ura⁺ colonies can be less than expected, if for example, there is non-random spore inviability associated with the *yfgΔ::URA3* query gene disruption.

16. Scrape cells from each **MM-URA** and **MM+URA** 150-mm plate using 10-11 ml sterile water. Transfer each individual “scrape” to a **15 ml conical tube**. There is no need to use a second addition of water here.
17. Determine the OD₆₀₀ of each haploid pool. For this, mix 10 μl of cells with 990 μl H₂O in a 1 ml plastic cuvette (1:100 dilution).
18. For each sample, freeze TWO to FOUR 25 ODU aliquots in Matrix Latch Rack tubes (1.4 ml round-bottomed) at the position corresponding to the query gene’s original plate and well location. For **MM+URA**-selected haploids (**Control**), label latchracks with **XY barcodes**. For **MM-URA**-selected haploids (**Experimental**), label latchracks with **YY barcodes**. Store all latchracks at -80°C.
19. For each query gene used for dSLAM transformation, prepare high quality genomic DNA (gDNA) from both the Control and Experimental haploid pools. For this purpose, we currently use a **MasterPure™ Yeast DNA Purification Kit** (Epicentre) followed by a Qiagen **QIAamp MinElute Column**. See protocol for additional information.
20. For those experiments in which a subset of primary Ura⁺ transformants appeared 5-FOA^R, replica print **MM-URA**-selected haploids to **MM+URA+FOA** plates to assess the stability of the *URA3* marker. Usually, but not always, unstable transformants are lost during the sporulation process.