YEAST LYSIS WITH MORTAR AND PESTLE

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Materials:

Liquid N_2, Mortar, Pestle, Lysis buffer of choice for protein prep, Protease Inhibitors, 60mL syringe, ultracentrifuge and tubes, 10mL syringe and 18-gauge needle.

Cell prep (making noodles):

Oversaturated cultures that have been sitting at OD_{600}~3 for a while have thicker cell walls and as a result are much harder to pop. Also, resuspending in too much lysis buffer decreases grinding efficiency.

1. **Let cells grow until OD_{600}=2.0-3.0.**

   Typical prep size is 6L culture.

2. **Collect cells by centrifugation in 1L plastic bottles at 3K rpm for 15min.**

   (For cells grown in minimal media, dilute culture 1:1 with cold diH_2O to aid in pelleting, spin 25min).

3. **Resuspend and pool cell pellets during a wash with cold diH_2O.**

   Spin again as before, but this time at 4^oC.

PAST THIS POINT, KEEP EVERYTHING COLD.

4. **Resuspend cell pellet in 5mL of cold lysis buffer + protease inhibitors.**

   (5mL per 6L saturated culture, or 1.2mL per L).

5. **Freeze cells by dropping them into liquid nitrogen.**

   Half-fill a 1L plastic beaker with liquid nitrogen. Remove the 60mL syringe's plunger, parafilm the exit of the syringe, then transfer cells into the syringe (NOTE: no needle). While holding the syringe over N_2, remove the parafilm and reinsert the plunger. Drip cell suspension from the syringe into the liquid nitrogen. Add more N_2 as necessary. After all are done and frozen, scoop yeast noodles out into 50mL Falcons (cooled w/ liquid N_2 during cell transfer) at -80^oC.

Lysis step:

6. **Prepare mortar and pestle for grinding.**

   Wash and dry a mortar and pestle. Cover it with foil, and store at -80^oC for at least one hour (typically overnight). Get a full bottle of liquid N_2. Get a foam ice bucket and put some dry ice in it (not too much dry ice, as the mortar must sit stably inside). Retrieve the chilled mortar and place it into the dry ice bucket such that the ice contacts it maximally. Cover the bottom of the mortar basin with liquid N_2 to chill.

7. **Add the yeast noodles. Grind with pestle for 60min or until ~50% lysis is achieved.**

   The technique I've had the most success with is grinding noodles submerged in liquid N_2 until they become a fine powder, to keep the noodles from cracking and jumping out of the mortar, and then grinding the fine powder dry, only adding N_2 to chill the powder and keep it frozen. I favor using a circular, stirring motion for grinding.

   Monitor lysis by taking a pipette tip, scraping up a small amount of powder, and transferring that powder to an eppendorf tube with ~20uL H_2O. Then, resuspend the cells and then score lysis under a microscope. Popped yeast are dark, intact yeast are bright. Again, aim for 50% lysis.

   Do not let the pellet thaw—add liquid N_2 to the mortar from time to time to keep the cells frozen, and swirl the liquid to collect yeast from the mortar walls.

8. **Transfer popped cell powder with a clean scoop to 50mL falcon tubes and thaw @ RT.**

   This transfer is most efficient if barely enough N_2 is added to the powder to make it congeal into a paste).

9. **Once the powder at the edges has thawed, add 40mL lysis buffer with protease inhibitors, and continue thawing on a nutator at room temperature.**

   Split 40mL between all falcon tubes.

10. **Once thawing is totally complete, incubate on ice for 30min.**

11. **Pre-spin crude lysate at 3K rpm for 15' in a J-6B at 4^{o}C, then transfer the supernatant to a clean ultracentrifuge tube.**

12. **Spin the supernatant at 40K rpm for 60' at 4^{o}C in a Ti-45 rotor.**

    Please balance ultracentrifuge tubes perfectly using our scales. Each tube should hold up to ~70mL. Also, spinning less than 35mL volume may stress the tubes and deform them, making removal of the tube from the rotor impossible except by autoclaving—this kills the sample. Also, every so often a centrifuge tube will break and leak. If that happens, throw the broken centrifuge tube away.

13. **VERY CAREFULLY remove rotor lid and remove tubes.**

    Shaking the tubes mixes the top lipid layer with the clarified supernatant underneath. In fact, you should probably do this transfer in the centrifuge room.

14. **Collect clear supernatant with a 10mL luer-lok syringe attached to a pink-needle (18 gauge). Transfer into chilled 50mL Falcons.**

    Care is taken to avoid disturbing the top lipid layer in order to prevent sample contamination.

You now have clarified cell lysate for use in purifications; see my FLAG or TAP purification protocols for further steps.