Harvesting Cells and Making Noodles

This protocol is designed to harvest a yeast cell culture and prepare it for grinding.

1. Grow cell culture to at least $3.0x10^7$ cell/mL total.

2. Spin cultures down at 4000xg, for 10 minutes, 4°C.

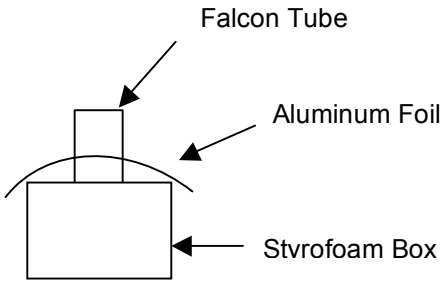
3. Wash cell pellet by resuspending pellet with 50mL ddH_2O over ice. Put resuspended solution into 50mL Falcon tube(s) and spin down at 2600xg, for 5 minutes, at 4°C. Repeat this 1x.

4. Resuspend pellet, over ice, with a volume of resuspension buffer equal to the volume of the pellet. Spin down at 2600xg, for 15 minutes. Aspirate all liquid from the pellet.

5. Spin down again (just the pellet), at 2600xg for 15 minutes to ensure all of the buffer is removed.

6. Pellet should be fairly dry and resemble a thick paste.

7. Place liquid nitrogen in a styrofoam container, top with aluminum foil and place a 50mL Falcon tube through a hole in the foil. Allow tube to cool. (See picture below.)



8. Fill the cooled 50mL falcon tube, to the very top, with liquid nitrogen.

9. With a spatula, scoop out cell paste and place into a 10mL or 20mL syringe. Press out the cell paste into the liquid nitrogen in the Falcon tube.

10. When all cell paste is gone, decant liquid nitrogen from the tube (try not to lose any noodles, can pour off liquid nitrogen by poking holes into cap of Falcon tube, screw on the cap and then turn tube upside down to pour out the liquid nitrogen.).

11. Do not tighten the tube completely, in order to allow liquid nitrogen vapor to escape. Store tubes at -80°C.

Resuspension Buffer:
1.2% PVP-40
20 mM Hepes pH 7.4

Note: Before using the resuspension buffer, add the following solutions to the volume of buffer you intend to use.

- *1:100 PIC*
- *1:100 Solution P*
- *1:1000 of DTT.*