Yeast Extract Protocol

1. Grow cells in YEPD overnight with appropriate antibiotics (to get ~0.5g of powder grow at least 500mL to a **dense** OD)
2. Centrifuge at 3000-4000 rpm for 5min to pellet. Discard supernatant.
3. Add 1mL **ice cold** sterile water per pellet from 1L overnight. Vortex to resuspend.
4. Remove the plunger from a sterile syringe and parafilm the tip, and transfer resuspended pellet to the syringe (10mL is normally more than adaqueate).
5. Get a 50mL centrifuge tube per sample, label.
6. Add liquid nitrogen to a coffee grinder and the lid. ***from this point on work *quickly*!**
7. Drop in the yeast from syringe in small drops to assist in disruption.
8. Once yeast is in liquid nitrogen, grind for ~2min or until a fine powder is achieved.
9. Transfer pellet to appropriate 50mL conical. Add liquid nitrogen to keep cold.
10. Cap and put in the -80.

***Note: Depending how much powder you use at a time do not store too many experiments worth of powder in one tube. The freeze thaw of removing the powder from the -80 and getting back into the -80 takes a huge toll on the viability of the powder.**

1. When you are ready to use the powder. Remove powder from 50mL conical by pouring it into a 1.5mL microcentrifuge tube. (**1 full tube = ~0.5g**)
2. Add appropriate proportion of appropriate buffer (**to 0.Xg of powder, add X fraction of 100uL of buffer**; i.e to 0.5g of powder, add 50uL of buffer; to 0.3g of powder, add 30uL buffer, etc)
3. Once buffer is added, vortex sample (while periodically icing) until a homogeneous resuspension is achieved, this may take a few vortexes until the full powder thaws over ice.
4. Centrifuge at 13.2rpm (max speed) for 20min.
5. Remove supernatant to a clean tube and dispose of pellet.
6. Supernatant will be good fro ~1hr to ~1.5hrs of work before proteins start denaturing.