PROTEIN OVEREXPRESSION/EXTRACT PREPARATION

1. Inoculate LB + Amp (100 µg/ml) with overnight culture (10 ml/liter) grown at 30° or 37°C
    A. Incubate at 30°C or 37°C for 3-4 hours (A_{595}=0.95)

2. Remove 1 ml aliquot of "uninduced" cells, spin down, and reconstitute in 150 µl of 1x SDS sample buffer (use 15 µl for gel)
    A. Induce cells by addition of IPTG to a final concentration of 1.0 mM and incubate for an additional 3-4 hours at 30° or 37°C

3. Measure A_{595} and remove an aliquot of cells normalized to the previous sample (i.e. same number of cells as in previous sample according to the O.D. reading)
    A. spin down sample of cells and reconstitute in 100 µl of 1x SDS sample buffer (use 15 µl for gel)

4. Spin down remaining cells at 5000 rpm for 5 minutes in a preweighed bottle. The preweighed bottle will be used to determine the weight of the wet cells

5. Wash the cells in 20 mM Tris pH 8.0, 0.1 mM EDTA and 50 mM NaCl (TEN_{50}), pelleted the cells and determine the weight of the wet cells

6. Determine volume of lysis buffer (TEN_{50}) required (3 ml/gram of cells) and reconstitute the cells in half of the required lysis buffer. Dissolve lysozyme to 1.0 mg/ml in remaining lysis buffer

7. Gently transfer cells to a 50 ml screw cap conical tube, slowly add lysis buffer + lysozyme, mix by inversion being careful not to create air bubbles and incubate on ice for 30 minutes (gently invert several times every 10 minutes)

8. Add protease inhibitors, leupeptin and pepstatin A, to final concentration of 1 µg/ml and gently mix by inversions

9. Freeze/thaw cells four times in either liquid nitrogen or MeOH/dry ice and 37° C baths
    A. do not completely thaw cells in 37° C bath; only thaw until small piece of frozen cells remains and then completely thaw by inverting at r.t.

10. If you cannot complete the preparation of the extract and resolve it over the DEAE column then keep the extract frozen on the fourth freezing and store it at -80°C until you can complete it.

11. Sonicate extract until solution becomes non-viscous
    A. Keep extract on ice between short sonications to keep cold

12. Transfer extract to polypropylene tube and spin at 15,000xg for 30 minutes at 4° C

13. Pour supernatant into an ultracentrifuge tube and spin at 123,000xg for 1 hour at 4° C in the Ti60 rotor

14. Dilute 2 µl of above supernatant sample with 58 µl of 1x SDS sample buffer and resolve 15 µl of this sample and 15 µl of the "uninduced" and "induced" samples on a 10% SDS-PAGE

    If crude extract contains an adequate amount of protein then continue to the DEAE Sepharose column