# Whole-cell extract prep

(Ray Chan, Meyer lab)

All steps are performed at 4 °C on ice.

1. Thaw embryos frozen in homogenization buffer (embryo pellet and equal volume of buffer). If embryos are frozen without buffer, add an equal volume of buffer. Add fresh protease inhibitors (500x aprotinin, pepstatin A, leupeptin, PMSF) and 2-βME to 5mM (0.7 λ to 1 mL).

2. Place the tube on ice and sonicate it with ten 30-second bursts at 10% power or about 6W (Program 2). Wait 1 minute in between bursts for cooling. Place tube on ice in glass beaker while sonicating.

3. Pellet debris by centrifugation at 5,000 x g for 10 minutes in a refrigerated centrifuge (7,300 rpm in microcentrifuge). Collect the supernatant.

4. Sonicate as described in step 2 to shear the DNA.

5. Pellet debris by centrifugation at 25,000 x g in a refrigerated centrifuge for 10-20 minutes (or full speed in microcentrifuge). Collect and quick freeze the supernatant in aliquots.

6. Measure concentration using Pierce Commassie Kit microassay. Use 5, 10, 15, 20, 25 ug BSA (2.5, 5, 7.5, 10, 12.5 uL of 2mg/mL stock) in 1 mL water for standards, and 0.1, 0.5, 1, 2, 5 uL of protein extract in 1 mL water. Prepare samples in cuvettes or glass tubes. Add 1 mL dye, mix, and measure $A_{595}$ within 30-45 minutes. Typical concentration range is 5-20 mg/ml.

**SOLUTIONS**

**Homogenization Buffer**
50 mM HEPES-KOH, pH 7.6
1 mM EDTA
140 mM KCl
0.5% NP-40
10% glycerol

**Protease inhibitors, 500X**
Calbiochem/EMD Biosciences, #539134

**Coomassie Protein Assay Kit**
Pierce, #23200