E. coli Lysis by Sonication for Protein Purification

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Background

To lyse *E. coli* cells without damaging desired proteins, we employ sonication for cell disruption. It's crucial to maintain a low temperature in the cell suspension to prevent protein denaturation due to heat. Throughout the process, the **cell suspension needs to be kept chilled on ice.**

IMPORTANT: Wear earplugs and eye protection when using the sonicator.

Materials:

- Cell pellet (e.g. from 50–200 mL culture)
- Cell-washing Buffer
    - 20 mM sodium phosphate, 500 mM NaCl, pH 7.4
- Lysis Buffer
    - 20 mM sodium phosphate, 500 mM NaCl, 30 mM imidazole, pH 7.4
- Ice
- Sonicator with microtip or probe
- Centrifuge (refrigerated 4˚C)
- 70% Ethanol
- MQ water
- Very small beakers (could use Falcon tubes also)

Steps:

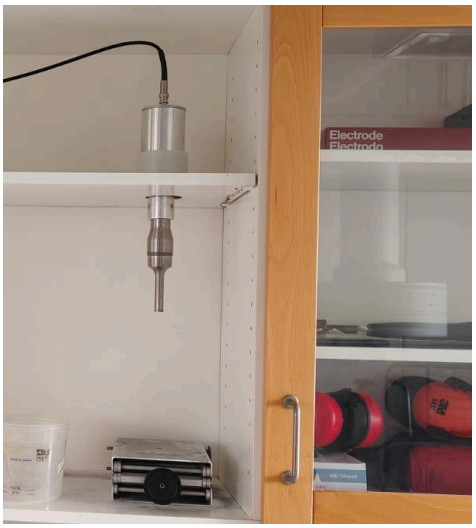
1. **Measure the OD before cell pelleting.**

2. **Pellet the cells:**
    - Pellet 50-100 mL of expression media through centrifugation, 10,000 x g for 1 min. Weigh the tubes before centrifugation; they can differ by a maximum of 0.05 g, but preferably by 0 g.

3. **Wash the cells:**
    - Add 10 mL of Cell-Wash Buffer, resuspend the pellet, then centrifuge again at same conditions

4. **Resuspend the pellet:**
    - Add Lysis Buffer to an OD 10 (Calculate the amount needed.)
    - Pipette or vortex to fully resuspend the pellet.
    - Keep the tube **on ice in an ice box at all times** to prevent overheating.

5. **Setting up the Sonicator**





Left: Sonicator needle, the jack (below)
Right: Sonicator controls, Spanner, screw-on knob, 4420 tip and 4417 tip

- Using the ethanol, wipe the tips and the needle.
- Depending on the volume used, select the needle. (If volume less than 1 mL, use 4417; if less than 30 mL, use 4420, if greater than 30, use the kob)
- Using the spanner, untighten the knob (or not) and screw on the required tip to use. Tighten it with the spanner.
- Turn on the sonicator (| symbol) and adjust the settings. The amplitude for the 4420 tip may not go beyond 40%, so keep it at 35. If using the knob for volume greater than 30 mL, use amplitude 60%.

For ci2350:

- **Sonicate (we use)**: 20% amplitude, 3 s on/off pulses, 2 min on time, repeat 3 times for a total of 4 min. 1 min between repeats to let them cool off.

For AKR1D1:

- **Sonicate (we use)**: 30% amplitude, 3 s on/off pulses, 2 min on time, repeat 3 times for a total of 4 min. 1 min between repeats to let them cool off.

For atcABC:

- **Sonicate**: used ci2350 settings; article used French press

- After finishing, ensure the knob is screwed back on post cleaning.

IMPORTANT POINT: (a) Before and after using the sonic probe, clean it with 70% ethanol and then with MQ water. (b) Make sure that the probe does not touch the beaker/Falcon tube, but only keeps in contact with the cell suspension; the cell will not be able to lyse otherwise. Keep the beaker/Falcon tube straightened, and always ensure enough ice is present.

6. Centrifuge:

Take a sample in a Falcon tube (either 50 mL or 15 mL (use adapters)) that would be used to check the inclusion bodies.

- Spin at 20,147 × g for 1h 30 min at 4 °C.
- This pellets cell debris and inclusion bodies, leaving soluble protein in the supernatant.

Proceed with purification: Collect the supernatant for further downstream processes and keep it cold.