Protein Expression and Lysis

● Protein Expression

1. Plate the *E.coli* glycerol stock with plasmid pET-28a-DME-C in solid LB (30 µg/ml of kanamycin). Incubate for 16h at 37ºC.
2. Inoculate a colony in 5ml of LB containing 30 µg/ml of kanamycin and grow at 30ºC, 200 rpm, 18h.
3. Measure the optical density of the pre-inoculum (OD) at 580nm
4. Use the pre-inoculum to prepare a 50 mL (for initial expression tests) or 500 mL (for expression in larger volume) inoculum of LB (30 µg/ml kanamycin)
5. Measure OD (start at OD = 0.2).
6. Incubate the culture at 37°C, 200 rpm, until it reaches OD = 0.7.
7. When reaching the logarithmic phase (DO of 0.7), separate an 1 mL (for initial expression tests) or 5 mL (for expression in larger volume) aliquot as a non-induced sample and induce the rest of the culture for 4h with 1mM (final concentration) of IPTG.
8. Collect 1 aliquot after 1h, 2h, 3h and 4h of induction.
9. Centrifuge the induced and non-induced aliquots for 20 minutes at 6000 × g at 4 °C and store the pellet at -20 °C.

● Lysis

We tested some different lysis conditions throughout the project, which are described below:

- ○ <u>For initial expression tests:</u>

1. Centrifuge cells at 6000 × g, 4 °C, for 20 minutes
2. Resuspended in native lysis buffer (50 mM sodium phosphate and 300 mM NaCl) or denaturant (50 mM sodium phosphate, 300 mM NaCl and 8M Urea)
3. Lyse by sonication, using a 10% waveform amplitude, with 3 pulses of 30 seconds, interspersed with an ice bath.

4. Centrifuge the lysate at 6000 x g, 4ºC, for 15 minutes, to obtain the soluble (supernatant) and insoluble (pellet) fractions.
5. Ressuspend pellets in their respective lysis buffers before storage at -20ºC.

- <u>For higher volume expression:</u>

1. Centrifuge cells at 6000 × g, 4 °C, for 20 minutes
2. Add lysis buffer (50 mM Na_2HPO_4, 300 mM NaCl, protease inhibitor tablet) with or without Cellytic (proportion 1 mL Cellytic for 4 mL final volume).
3. Sonicate at 10% waveform amplitude, during 20 min, 5 pulse, 15 sec ice bath pause.*
4. Centrifuge the lysate at 6000 x g, 4ºC, for 15 minutes, to obtain the soluble (supernatant) and insoluble (pellet) fractions.
5. Ressuspend pellets in their respective lysis buffers before storage at -20ºC.