General protein expression protocol.

1) In morning (around 9-10am), pick single colony of expression strain into 5 ml of LB+appropriate antibiotics and shake at 37ºC.

2) At end of day (around 6 pm) transfer 5 ml culture into 1L of LB+appropriate antibiotics and shake overnight at 37ºC.

3) The next morning check the OD600. You are aiming for 0.8-1.0, unless otherwise indicated. When culture reaches this OD600, take an **uninduced sample.**

-take 500 µl of culture and pellet in a microfuge (fullspeed, 30-60 sec)
-aspirate of supernatant
-resuspend in 100 µl of 1x protein sample buffer
-boil 5 minutes
-keep on ice

4) Induce culture with indicated amount of IPTG. Shake for 4 hours at the desired temperature. I use 30 ºC for MBP tagged proteins and 16 ºC for most other preps.

5) **Take induced sample.**

-take 500 µl of culture and pellet in a microfuge (fullspeed, 30-60 sec)
-aspirate of supernatant
-resuspend in 200 µl of 1x protein sample buffer
-boil 5 minutes
-keep on ice

6) **Take soluble sample.**

-take 10 ml of culture and pellet in centrifuge (4000 rpm, 10 min, 4ºC)
-aspirate of supernatant
-freeze until ready to process (see step 8)

7) Pellet 1 L culture in centrifuge (4000 rpm, 15 min, 4ºC). Wash with 25 mls of PBS and resuspend culture. Transfer to a 50 ml conical tube and bring volume up to 50 ml with PBS. Invert several times to mix the sample. Pellet in centrifuge (4000 rpm, 10 min, 4ºC). Freeze in liquid nitrogen and store at -20ºC.

8) Processing soluble samples. Resuspend in 1ml PBS+Complete protease inhibitor (use 1 Mini tablets per 5 ml) and transfer to a 1.5 ml Eppendorf, keeping sample on ice.

-lyse the bacteria by sonication keeping all reagents on ice during sonication: (Sonication: 2x30 sec, 15% amplitude. 1x30 sec 25% amplitude)
-pellet insoluble debris by centrifugation (14k, 15 min, 4ºC).
-make soluble input fractions by taking 100 µl of lysate and adding 30 µl of 4xSample
-store samples at -20ºC