Procedure

1. Transform freshly the plasmid into BL21 and wait until next day.
2. Grow up a culture from the plate at 25 until growing nicely.
3. Dilute back overnight so that they are about OD 0.5 next day. BL21 grow with about 1 hour generation time at 25C.

   IMPORTANT: Do not over grow or you can loose the plasmid.
4. Dilute into 1L LB amp in 5L flasks. This is important in order to get enough aeration. Lower aeration can lead to lower solubility.
5. Over at 25C until about OD 0.4. Assume 1 hour generation time.
6. Take 1 ml of bugs and spin in the ependorf. Pour of the sup and freeze or add 2X sample buffer and boil. Sample A
7. Add IPTG to 100mM from 1M stock.
8. Induce for 4-5 hours.
9. Take 1 ml of induced cells, spin in ependorf and freeze or add 2X sample buffer and boil. Sample B
10. Check induction by comparing sample A with sample B.
11. Harvest the rest by spinning in 1L bottles 5K 10'. Resuspend in 10mls PBS per liter of cells and spin 15K 15 mins in 2059 tubes. Pour off PBS and freeze LN2.
12. Cool down HB4
13. Thaw pellets at RT. When just thawed, add 1ml lysis buffer to each tube. The small amount of lysis buffer at this stage ensures little sonnication time is necessary.
14. Add:
    - 50mg/ml lysozyme (add as powder).
    - 1mM PMSF (-20C in Fedor's drawer 50ml conical).
    - 1/2000 LPC (optional).
    - 0.1% BME.

    Keep on Ice
15. Prepare and ice-water bath
16. Sonnicate in differenitation sonnicator, full power 1 times 30secs.
17. Careful not to move tip to surface which induces bubbles. Return the tube to ice water bath.
18. Add lysis buffer to 10mls and mix. The mixture should be not viscous at this point. If not, next time sonnicate for longer.
19. Leave 10mins on ice
20. Spin 10K 1hour HB4.
21. recover sup through cheese cloth.

22. Check for your protein in the sup and pellet.

Solutions

Lysis buffer

- PBS
- 1mM EGTA
- 1mM EDTA