Protein Expression Studies

Introduction

Transformation, cultivation and induction of BL21 DE3 *E. coli* to produce soluble SmChiA, VcCDA, and PpChi.

Note 1: Cannot autoclave centrifuge bottles – use Virkon.

Note 2: Samples t_0 and t_harvest are taken to test if induction succeeded – a new band will be present in t_harvest if so. Further samples will be taken to expand on this later after lysis (t_total, t_soluble, t_insoluble).

Materials

Equipment

- Biological hood, incubator, water bath, spectrophotometer (OD600), centrifuge (according to volume), shake flasks, centrifuge bottles.

Consumables

- Agar plates, inoculation loops, tips, 50mL falcon tube (not skirted), microcentrifuge tubes, cuvettes, comp BL21(DE3) *E. coli*, ddH20, ice.

Reagents

- LB medium, chloramphenicol, IPTG, lysis buffers

Procedure

Transformation

1. Add 1 µL of plasmid DNA to 25 µL of competent cells (1:25 Dilution). Mix by tapping not pipetting. (2ul and 50ul)
2. Incubate the vial(s) on ice for 30 minutes.
3. Heat shock: Incubate for exactly 30 seconds in the 42°C water bath. Do not mix or shake.
4. Remove vial(s) from the 42°C bath and quickly place on ice for 30s.
5. Add 250 µl of pre-warmed SOC medium to the vial(s).Note: SOC is a rich medium; practice good sterile technique to avoid contamination. (LB broth can also be used).
6. Incubate the vials for 1hr at 37C, 250rpm.
7. Plate pouring and cell seeding (conducted in the hood).
8. Add 50mL of LB agar into a 50mL falcon tube; add 50uL of chloramphenicol (1:1000 dilution) (34 mg/mL).

9. Pour 25 mL of solution per plate and wait 1hr for it to cool down: leave the lid half open to avoid condensation.

10. pipette 100uL of transformed cell onto each plate; spead the cell evenly with the spreader.Place the LB agar plate overnight at 37˚C. Make sure the plates are placed upside down in the incubator to prevent water disturbing the colonies, store in fridge if not using so colonies won't grow (4C), not freezer.

Making starter cultures

11. Bring under the biological hood your streaked plate (check quality of colonies), a 50 mL falcon tube, your medium, the chloramphenicol stock, tips and a pipette.

12. Add 5/10 mL LB into the falcon tube and add chloramphenicol 1× (25ug/mL) (1:1000 dilution of 1000X stock).

13. Use an inoculation loop to select a single colony from the petri dish of transformed *E. coli*. Place it into the falcon tube.

14. Place the falcon tube into the incubator at 37C at varying speeds (250rpm for SmChiA, 200rpm for VcCDA and PpChi) for overnight culture.

Preparing shake flask cultures

15. Take a shake flask, rinse it twice, add powdered LB and the corresponding amount of ddH2O (check bottle). Do not go above 50% of the maximum flask volume.

16. Plug the flask with cotton and foil and autoclave it.

17. Take the overnight starter culture, dilute it 1:10, and measure the OD600 value (blank with LB). This value (undiluted, so multiply by 10) should be used to calculate how much starter volume should go into the flask: C1V1 = C2V2 where C2 is 0.1 OD600.

C1 (OD value) V1 (unknown) = 0.1* V2 (50mL)

18. Bring the culture, flask, and chloramphenicol into the hood.

19. Add the chloramphenicol into the autoclaved (and cooled) flask at 1X final concentration. Add the calculated volume of starter culture.

20. Place the shake flask into the 37C incubator. All three enzymes are cited to undergo pre-induction shaking at varying speeds (130rpm for SmChiA, 180rpm for VcCDA, 200rpm for PpChi).

Induction

21. Measure OD600 of flasks every hour. At 0.6, the cultures are ready for induction.

22. Place the flasks in ice for 10-30 minutes to reduce their temperature - SmChiA

23. For later, take a 1mL sample, note the OD600 from before, spin it down at 13000g x 1 min, discard supernatant, and store at -20c. **This is the t_0 sample.**

24. Add IPTG to each flask to obtain a concentration of 0.5mM.

25. Place the flasks back into an incubator. SmChiA should be placed at 20c at 180rpm for 16 hours. VcCDA and PpChi should be placed at 16c at 180rpm for 18 hours. Consider doing 18 hours for both.

Harvest

26. Measure OD600. Take 1mL sample, spin it down 13000g x 1 min, discard supernatant, put at -20c. **This is the t_harvest sample.**

27. Pour the flask content into a centrifuge bottle.

28. Centrifuge at 6000g x 10 min. If 6000g isn't available, 4000g x 15 min. If also not available, 2500g x 30 mi. Ideally do this at 4c. Note that each enzyme is spun at these rates differently in the papers (first is SmChiA, second is PpChi, third is VcCDA) but it shouldn't make a difference. **Should probably spin fragile VcCDA at 2500g just in case.**

29. Decant supernatant into autoclavable waste.

30. If not working on cells immediately after, freeze in -20c as a bare pellet.

31. Resuspend cell pellet in a 50mL falcon tube using buffer at 10mL/g wet cell weight. This buffer should be: 50mM sodium phosphate, pH7.5, 300mM NaCl, 20mM imidazole + single tablet of EDTA-free protease inhibitor (must all be at 4c). **Alternatively just use Tris.** This is the first step of sonication (rest not in protocol).

32. **Sonication sample, spun down debris, supernatant - all sampels**

33. All samples run on SDS-PAGE