Small Scale Bacteria Lysis
SDS PAGE Gel Migration

Aim

To verify the synthesis and production of proteins from liquid culture of bacteria.

Equipment

- Lysis solution B-PER
- Lysis buffer Tris-Cl 50 mM pH 7.4, NaCl 100 mM, Glycerol 5% v/v, PMSF 15 µM*
- Pipet p100, p200, and p10 and corresponding cones
- Microcentriguge tubes (1.5 and 0.5 ml)
- Heater block at 95°C
- SDS Page gel
- SDS* 20X solution
- SDS 2X gel loading solution
- Protein Molecular weight ladder (Page ruler)
- Gel code blue Coomassie Blue
- Pellets of induced liquid culture of transformed bacteria:……. (stored at -20°C or -80°C)

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Induced Bacteria: Small scale 200 ml culture and induction

1. Grow one colony of BL21De3 (pLysS) cells containing the pET32/43a plasmid with the insert of interest overnight in 20 ml of LB supplemented with carbenicillin at 50 µg/ml in a 37°C shaking incubator.
2. The next day, after 16 hrs, spin the cells at 3000 x g and resuspend the pellet in 20 ml LB medium.
3. Spin the cells one more time.
4. Use 1 ml of the resuspended cells to innoculate 100 ml of LB supplemented with carbenicillin at 50 µg/ml.
5. Grow the culture at 37°C shaking.
6. Take the absorbance at 600 nm (UV5 Mettler Toledo Spectrophotometer) of a 1 ml sample after two hours, and subsequently every 20 minutes to follow the growth profile.
7. After reaching an OD_{600} of approximately 0.7, induce the protein expression with IPTG* at 0.5 mM for three hrs.
8. Keep an aliquot of uninduced cells, spin at 3000 g and store the pellet at -20°C.
9. After the three hours of induction take a 1 ml aliquot measure the OD600 , spin the cells down at 3000 x g and store the pellet at -20°C.

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Proteins

1. Thaw the pellets and resuspend them in B-PER (Pierce / GE HealthCare) solution in a volume ratio such as to obtain 10 OD/µl.
2. Add PMSF to 15 µM.
3. Vortex the cells for 5 minutes at RT and centrifuge for 10 mins at 16 000 g in a microfuge.
4. Transfer the supernatant in a clean labeled tube.
5. Resuspend the lysed pellet in an equivalent volume as for the initial intact cell pellet.
6. Proceed to adding SDS PAGE 2X loading buffer to 10-20 µl of lysed cell extract, mix, heat at 95°C for 5 mins, place on ice.
7. Load up to 40 µl on a 14-16% SDS PAGE gel (Novex).
8. Run at 150 V in 1X SDS PAGE Tris-Glycine buffer until the blue dye has reached the bottom of the gel.
9. Open the gel cast plate and transfer the gel to a clean container.
10. Wash with de-ionized water for 5 minutes. Repeat two to three more times.
11. Add 15 ml of Gel Code Blue coomassie stain. Stain for 20 minutes.
12. Wash three times with deionized water.
13. Place the gel on a transilluminator for observation and in a Gel Doc for imaging.

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Protocol

Sample of cell pellet name	Time	OD	Lysis solution B-PER = 10.0 x OD (µl)
			…… µl
			…… µl
			…… µl
			…… µl

1) Re-suspend cell pellet in …… µl = 10.0 x OD, of lysis solution B-PER.
2) Centrifuge 10 min at 16 000 g.
3) Separate supernatant from pellet, and place supernatant in new tubes.

Sample name	Time	Supernatant volume (µl)	Lysis solution Tris-Cl* = supernatant volume (µl)
			…… µl
			…… µl
			…… µl
			…… µl

4) Re-suspend pellet in …….. µl = lysis solution B-PER.
5) Mix 20 µl of supernatant or re-suspended pellets with 20 µl of blue SDS 2X solution in microcentriguge 0.5 ml tubes.
6) Place the tubes in heater block set at 95°C fro 5 min.
7) Prepare 400 ml of SDS PAGE Buffer 1X solution:

SDS 20X	De-ionized water
20 ml	380 ml

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8) Place the SDS PAGE gel in the electrophoresis tube.
9) Pour the SDS PAGE Buffer 1X in the tub containing the gel.
10) Add 40 µl of each sample in the wells of the SDS gel.

Lane	1	2	3	4	5	6	7	8	9	10	11	12
Volume (µl)												
Name												

11) Set voltage at 150 V and let migrate for 1 h.
12) Wash gels 3 times for 5 min in de-ionized water.
13) Stain gels by incubating them in 15 ml of Coomassie blue staining Gel Code blue solution for 20 min.
14) Wash gels 3 times for 5 min in clean de-ionized water.
15) Wash gels by incubating them in clean distilled water overnight.
16) Reveal image by white imaging on a transilluminator.

*PMSF: Phenyl methyl sulfonyl fluoride: protease inhibitor, SDS: Sodium dodecyl sulfate.