Date: 20170818
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Bacteria lysis and SDS PAGE gel migration

Aim: Verify the synthesis and production of proteins from liquid culture of bacteria

Equipment:

- Lysis solution B-PER
- Lysis solution Tris-Cl*
- Pipet p100, p200, and p10 and corresponding cones
- Microcentrifuge tubes (1.5 and 0.5 ml)
- Heater block
- SDS Page gel
- SDS 20X solution
- Blue SDS 2X solution
- Coomassie Blue staining solution
- Pellets of induced liquid culture of transformed bacteria: BL21De3 pET43.1aE1-1 colony 1 and BL21De3 pET43.1aE3 colony 3 (stored at -20°C)

Transformed Bacteria:

- BL21De3 pET43.1aE1-1 colony 1
- BL21De3 pET43.1aE3 colony 1

Protein:

- E1-1
- E3
- E1-2 purified on the 17th of August

Protocol:

Sample of cell pellet name	Time	OD_{600nm}	Lysis solution B-PER = 100 x OD_{600nm} (µl)
BL21De3 pET43.1aE1-1 colony 1	T = 0	0.73	71 µl
BL21De3 pET43.1aE1-1 colony 1	T =3	1.64	164 µl
BL21De3 pET43.1aE3 colony 1	T =0	0.72	70 µl
BL21De3 pET43.1aE3 colony 1	T =3	1.6	160 µl

1) Re-suspend cell pellet in 71 µl = 100 x OD_{600nm}, of lysis solution B-PER
2) Centrifuge 5 min at 3 000 x g
3) Separate supernatant from pellet, and place supernatant in new tube

Sample name	Time	Supernatant volume (µl)	Lysis solution Tris-Cl* = supernatant volume (µl)
BL21De3 pET43.1aE1-1 colony 1	T =0	110	110
BL21De3	T =3	245	245

pET43.1aE1-1 colony 1			
BL21De3 pET43.1aE3 colony 1	T =0	110	110
BL21De3 pET43.1aE3 colony 1	T =3	245	245

4) Re-suspend pellet in …….. µl = separated supernatant volume of lysis solution Tris-Cl*
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 dry-heater set at 90°C fro 5 minutes
7) Prepare 400 ml of SDS Page Buffer 1X solution:

Mix

SDS 20X	Deionized water
20 µl	380 µl

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

SDS Gel 1	1	2	3	4	5	6	7	8	9	10	11	12
Volume (µl)	40	40	40	40	40	40	40	40	40	40	40	40
Sample name	E11 Pellet T0	E11 Sup T0	E11 Pellet T3	E11 Sup T3	E3 Pellet T0	E3 Sup T0	E3 Pellet T3	E3 Sup T3	E12 Pellet not purified	E12 Sup not purified	E12 raw not pur not cent	Ladder MW
SDS Gel 2	1	2	3	4	5	6	7	8	9	10	11	12
Volume (µl)	40	40	40	40	40	40	40	40	40	40	0	40
Sample name	E12 5	E12 6	E12 7	E12 8	E12 9	E12 10	E12 11	E12 12	E2 FT	Wash	0	Ladder mw

11) Set voltage at 75 V and let migrate for 1 hour
12) Wash gels 3 times for 5 minutes in clean distilled water
13) Stain gels by incubating them in 20 ml of Coomassie blue staining solution for 20 minutes
14) Wash gels 3 times for 5 minutes in clean distilled water
15) Wash gels by incubating them in clean distilled water over-night
16) Reveal image by white imaging

*Tris-Cl : Tris-Cl 50 mM pH 7.4, NaCl 100 mM, Glycerol 5% v/v

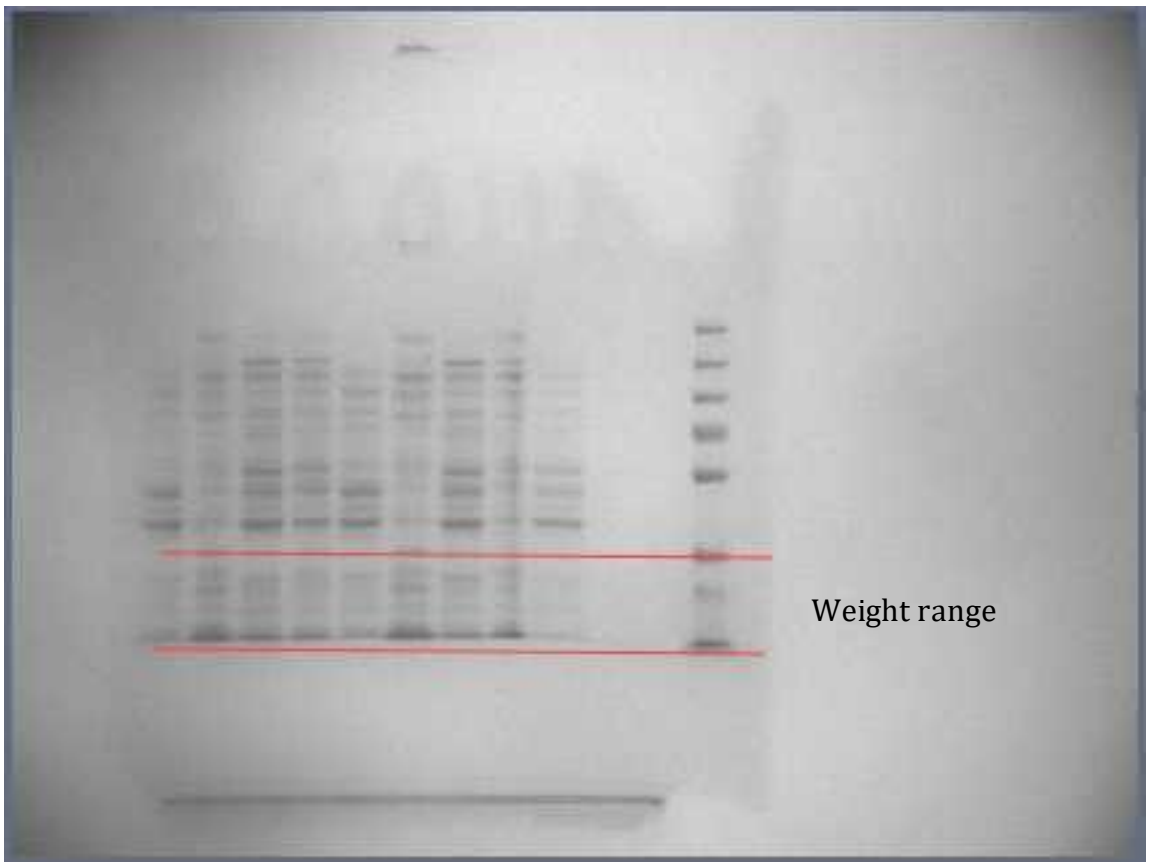

Figure 1 : SDS Gel 1

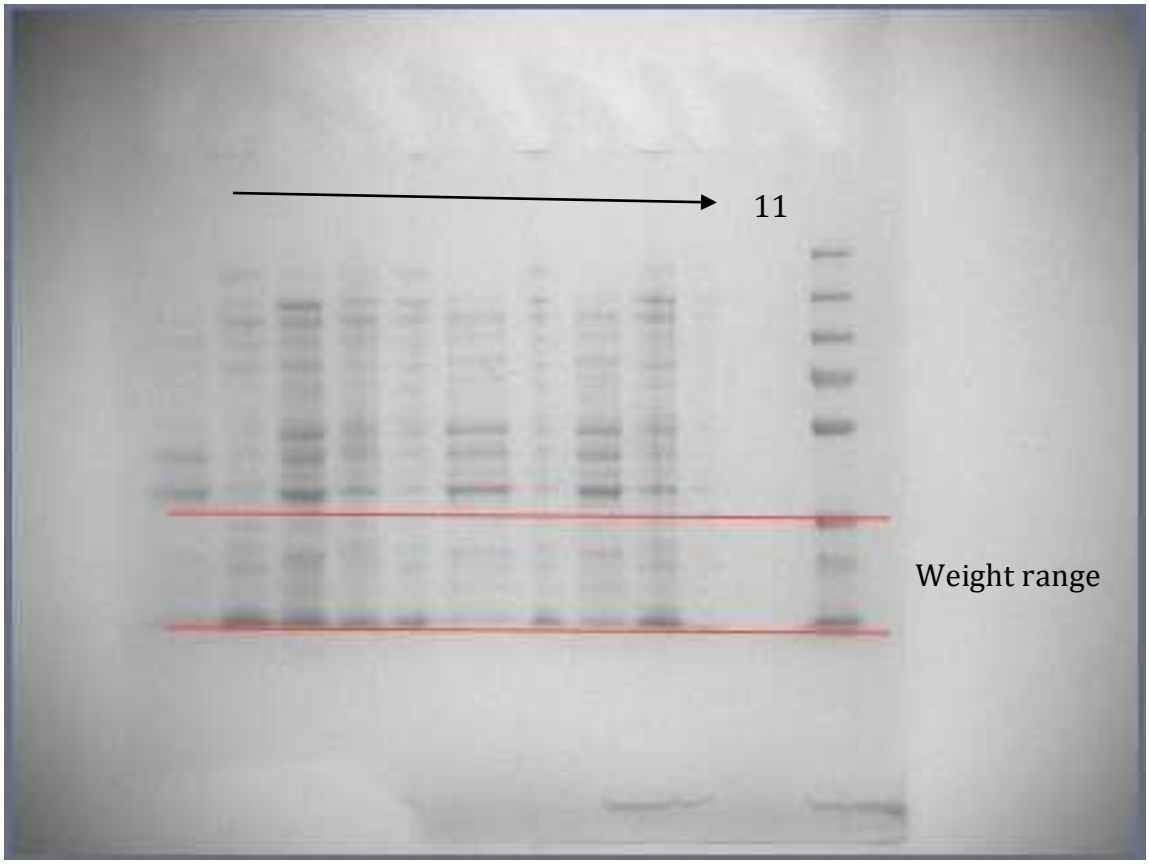

Figure 2 : SDS Gel 2