Date: 20170810
Operators: Gaétan, Gabriel

Liquid culture of BL21De3 pET43.1a-E1-2 and pET43.1a-E2:

Aim: Transform competent bacteria with plasmids

Equipment:

- Bacteria transform BL21.De3 pET43.1a–E1-2 or -E2
- 1.5 ml Eppendorf tubes
- Erlenmeyers
- P1000, P200, P10 pipettes + paired cones
- Incubator 37°C with or without a stirrer/agitator
- Sterile rake/scraper/comb
- Timer

Bacteria transformed:

- BL21De3 pET43.1a-E1-2 colony 1 & 2
- BL21De3 pET43.1a-E2 colony 1

Protocol:

1) Retrieve the 10 ml liquid cultures (seeded the 2017/08/08) from the incubator after 16 hours
2) Centrifuge the falcon tubes at 3 000 x g for 5 min at Room Temperature (R/T)
3) Remove supernatant, add 5 ml LB broth and resuspend pellet
4) Centrifuge again at 3 000 x g for 5 min at RT
5) Remove supernatant completely (use a p1000 for the last drops) and resuspend the pellet in 1 ml of LB broth
6) Mix 250 µl of your 1 ml liquid cultures to 100 ml of LB/CARB broth
7) Incubate at 37°C at 150 rpm, and follow the OD_{600nm} growth kinetics
8) Measure the OD_{600nm} with a 1 ml sample, every hour until $OD_{600nm} = 0.3$
9) When $OD_{600nm} = 0.3$ measure the OD_{600nm} every 20 minutes, until $OD_{600nm} = 0.7$
10) When $OD_{600nm} = 0.7$, sample 1 ml of culture in an 1.5 ml Eppendorf tube twice and then add IPTG

Kinetic analysis, OD_{600nm} measurements

1) Sample 1ml of solution and measure the OD_{600nm} using the UV5 spectrophotometer, every hour
2) When $OD_{600nm} = 0.3$ measure the OD_{600nm} every 20 minutes
3) When $OD_{600nm} = 0.7$ Sample 1ml of solution twice.
4) Then add - 920 µl to BL21De3 pET43.1a.E1_2 colony 1
   - and 910 µl to BL21De3 pET43.1a.E2 and BL21De3 pET43.1a.E1_2 colony 2
   of IPTG (50 mM) to obtain a solution of IPTG at 0.5 mM
5) Measure the OD_{600nm} every 40 minutes for 3 hours
6) After 3 hours do an SDS migration gel and reveal using

Time	OD_{600nm}: BL21De3 pET43.1a E2 (col1)	Time	OD_{600nm}: BL21De3 pET43.1a E2 (col1)

			+ IPTG
T = 0	0	T = 0	0
T = 1 hour	0.03	T = 3h52	0.67 +IPTG
T = 2 hour	0.1	T = 5h	2.20
T = 3h	0.4	T = 6h	1.3
T = 3h20	0.45	T = 6h15 min	1.2
T = 3h40	0.59	T = 7h	1.5
T = 3h52	0.67		

Time	OD_{600nm}: BL21De3 pET43.1a E1_2 Colony 1	Time	OD_{600nm}: BL21De3 pET43.1a E1_2 Colony 1 + IPTG
T = 0	0	T = 0	0
T = 1 hour	0.06	T = 3h10	0.69 +IPTG
T = 2 hour	0.27		
T = 2h20	0.49	T = 4h14 min	1.05
T = 2h50	0.6	T = 5h14 min	2.4
T = 3h0	0.65	T = 6h15 min	1.4
T = 3h10	0.69		

Time	OD_{600nm}: BL21De3 pET43.1a E1_2 Colony 2	Time	OD_{600nm}: BL21De3 pET43.1a E1_2 Colony 2 + IPTG
T = 0	0	T = 0	0
T = 1 hour	0.06	T = 3h10 min	0.75+IPTG
T = 2 hour	0.29	T = 4h14 min	1.1
T = 2h10	0.43	T = 5h14 min	2.4
T = 2h45 min	0.56	T = 6h15 min	1.5
T = 3h	0.63		
T = 3h10	0.75		

Date: 20170810
Operators: Gabriel, Karima

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
- Microcentriguge 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 pET43.1aE1-2 colony 1 & 2 and BL21De3 pET43.1aE2 colony 1 (stored at -20°C)

Transformed Bacteria:

- BL21De3 pET43.1aE1-2 colony 1
- BL21De3 pET43.1aE1-2 colony 2
- BL21De3 pET43.1aE2 colony 1

Protein:

- E1-2
- E2

Protocol:

Sample of cell pellet name	Time	OD_{600nm}	Lysis solution B-PER = 100 x OD_{600nm} (µl)
BL21De3 pET43.1aE1-2 colony 1	T = 0	0.71	71 µl
BL21De3 pET43.1aE1-2 colony 1	T = 2		…… µl
BL21De3 pET43.1aE1-2 colony 1	T =3		µl
BL21De3 pET43.1aE1-2 colony 2	T =0	0.7	70 µl
BL21De3 pET43.1aE1-2 colony 2	T =2		…… µl

BL21De3 pET43.1aE1-2 colony 2	T =3		µl
BL21De3 pET43.1aE2 colony 1	T =0	0.69	690 µl
BL21De3 pET43.1aE2 colony 1	T =2		µl
BL21De3 pET43.1aE2 colony 1	T =3		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 pET43.1aE1-2 colony 1	T =0		
BL21De3 pET43.1aE1-2 colony 1	T =2		
BL21De3 pET43.1aE1-2 colony 1	T =3		
BL21De3 pET43.1aE1-2 colony 2	T =0		
BL21De3 pET43.1aE1-2 colony 2	T =2		
BL21De3 pET43.1aE1-2 colony 2	T =3		
BL21De3 pET43.1aE2 colony 1	T =0		
BL21De3 pET43.1aE2 colony 1	T =2		
BL21De3 pET43.1aE2 colony 1	T =3		

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
Volume(µl)	40	40	40	40	40	40	40	40	40	40
Sample name	E2 Sup T0	E2 Pellet T0	E2 Sup T3	E2 Pellet T3	E12 Sup T0	E12 Pellet T0	E12 Sup T3	E12 Pellet T3	E2 Sup T2	Ladder
SDS Gel 2	1	2	3	4	5	6	7	8	9	10
Volume(µl)	40	40	40	40	40	40	40	40	40	40
Sample name	E12 Sup T0	E12 Pellet T0	E12 Sup T3	E12 Pellet T3	E2 Pellet T2	E12 Sup T2	E12 Pellet T2	E12 Sup T2	E2 Pellet T2	Ladder

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

*Lysis Solution : Tris-Cl 50 mM, NaCl 100 mM, Glycerol 5% v/v