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Liquid culture of BL21De3 pET43.1a-E1-1 and pET43.1a-E3:

Aim: Transform competent bacteria with plasmids

Equipment:

- Bacteria transform BL21.De3 pET43.1a-E1-1 or -E3
- 1.5 ml Eppendorf tubes
- Erlenmeyers
- P1000, P200, P10 pipettes + paired cones
- Incubator 37°C with or without a stirrer/agitator
- Sterile rake/scrapper/comb
- Timer

Bacteria transformed:

- BL21De3 pET43.1a-E1-1 colony 1 & 2
- BL21De3 pET43.1a-E3 colony 1 & 2

Protocol:

- 1) Retrieve the 10 ml liquid cultures (seeded the 2017/08/08) from the incubator after 16 hours
- 2) Centrifuge the falcon tubes of BL21De3 pET43.1a-E1-1 colony 1 and BL21De3 pET43.1a-E3 colony 1 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 910µl 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 E1-1 (col1)	Time	OD _{600nm} : BL21De3 pET43.1a E1-1 (col1) + IPTG
T = 0	0	T = 0	0
T = 1 hour	0.09	T = 2h30	0.73 +IPTG
T = 2 hour	0.44	T = 1h	1.3 = 0.65 x2
T = 2h20	0.6		
T = 2h30	0.73		

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Time	OD _{600nm} : BL21De3 pET43.1a E3 Colony 1	Time	OD _{600nm} : BL21De3 pET43.1a E3 Colony 1 + IPTG
T = 0	0	T = 0	0
T = 1 hour	0.098	T = 2h30	0.72+IPTG
T = 2 hour	0.47		
T = 2h20	0.6	T = 1h	1.34 = 0.67 x 2
T = 2h30	0.72		

Add 8.05 ml of IPTG 50 mM per 500 ml of LB/CARB