Group 3 Notebook: June

WEDNESDAY, 20/06/2018

Preparation of LB medium

1. Add 5 g of LB broth to 200 mL of deionized water in a 500 ml bottle.
2. Swirl to mix and autoclave.

Preparation of chloramphenicol LB agar plates

1. Add 8 g of LB broth with agar to 200 ml of deionized water in a flask.
2. Swirl to mix and autoclave.
3. Add 200 ul of 34 ug/ml chloramphenicol.
4. Pour ~20 mL of agar onto each petri dish and leave them to harden.

Preparation of 100 mM $CaCl_2$ solution

1. Add 2.2 g of anhydrous $CaCl_2$ (MW = 111 g/mol) to 200 ml of deionized water in a 500 ml bottle
2. Swirl to mix and filter sterilize.
3. Store at 4°C

Preparation of 50% glycerol solution

1. Add 50 ml of 100% glycerol to 50 ml of deionized water in a 500 ml bottle
2. Swirl to mix and filter sterilize.
3. Store at 4°C

Inoculation of DH5α and BL21 (DE3) for preparation of competent cells

1. Inoculate *E. coli* DH5α and BL21 (DE3) in 10 ml LB broth in 50 ml centrifuge tubes.
2. Incubate at 37°C, 220 rpm overnight.

THURSDAY, 21/06/2018

Preparation of DH5α and BL21 glycerol stock

1. Add 500 ul of overnight culture to 500 ul of 50% glycerol solution in 1.5 ml Eppendorf tubes.
2. Store at -80°C.

Preparation of DH5α and BL21 competente cells

1. Transfer 1% overnight culture to 10 ml of LB broth.
2. Incubate at 37°C, 220 rpm for ~3 hrs.
3. Measure OD600 by performing 10X dilution (100 ul of culture and 900 ul of deionized water) and using a spectrophotometer.
4. Once OD600 reaches 0.5-0.7, keep culture in ice.
5. Centrifuge at 4000 rpm, 4°C for 20 min and discard supernatant.
6. Resuspend in 25 ml of 100 mM of $CaCl_2$.
7. Incubate in ice for 1-3 hrs.
8. Centrifuge at 4000 rpm, 4°C for 20 min and discard supernatant.
9. Resuspend in 400 ul of 25% glycerol 50 mM $CaCl_2$ mixture.
10. Dispense 50 ul into Eppendorf tubes and store immediately in -80°C.

MONDAY, 25/06/2018

PCR Amplification of mRFP-Spinach

Table1

	A	B	C	D	E	F	G	H
1		Volume added (ul)						
2	mRFP-Spinach gblock	2.0			Initial denaturation	98°C	30s	
3	10 uM FP	2.5		34X	Denaturation	98°C	10s	
4	10 uM RP	2.5			Annealing	**71°C**	30s	
5	10 mM dNTP	1.0			Elongation	72°C	**60s**	~1kb
6	Q5 reaction buffer	5.0			Final elongation	72°C	120s	
7	Q5 polymerase	0.5				12°C	∞	
8	Nuclease-free water	36.5						
9	Total	50.0						

Gel Electrophoresis

TUESDAY, 26/06/2018

Transformation of empty pSB1C3 into DH5α

1. Add 2 ul of pSB1C3 to competent DH5α. Incubate in ice for 30 min.
2. Heat shock at 42°C for 1 min. Incubate in ice for ~5 min.
3. Add 1 ml of LB broth. Incubate at 37°C, 220 rpm for 1 hr.
4. Add 200 ul of culture to CmR agar plate and spread. Incubate at 37°C overnight.

WEDNESDAY, 27/06/2018

1. Check colonies (2 red colonies, possibly due to contamination of plasmid or culture).
2. Inoculate a white colony in 10 ml of LB broth with 10 ul of chloramphenicol.
3. Inoculate competent DH5α and BL21 cells in 10 ml of LB broth.
4. Incubate at 37°C, 220 rpm overnight.

THURSDAY, 28/06/2018

Preparation of DH5α-pSB1C3 glycerol stock

Plasmid Extraction of pSB1C3

Concentration of pSB1C3: 266.2 ng/ul