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Date: 6/20/19

Goal:

1. Miniprep overnight cultures
    a. Ligation 1 (K592009 + J23102)
    b. Ligation 2 (K592009 + J23102)
    c. Pcb302 (in E. Coli) from papers A & B
2. Glycerol stocks for overnight cultures.
3. Prepare ASP-8A media

Name: Kennex, Saleh, Chiara
Date: 6/20/19

Goal:

1. Minipreps
    a. Ligation 1((K592009 + J23102)
    b. Ligation 2 (K592009 + J23102)
    c. Pcb302 (in E. Coli)

Protocol:

QIAprep Spin Miniprep Kit Protocol

a. Centrifuged 3 mL of bacterial overnight culture in two separate Eppendorf tubes (1.5 mL in each) at 8,000 rpm for 3 minutes at room temperature.
b. Discarded the supernatant and resuspended pelleted bacterial cells in one tube with 250 µL Buffer P1 and transfered to the other and resuspended until one eppendorf tube contains the pelleted cells resuspended in 250 µL Buffer P1.
c. Added 250 µL of Buffer P2 and inverted 5 times.
d. Added 350 µL of Buffer N3 and immediately mixed by inverting 5 times.
e. Centrifuged for 10 minutes at 13,000 rpm.
f. Micropipetted 800 µL of the clear supernatant into a spin column and centrifuged for 60 seconds and discarded the excess liquid.
g. Added 500 µL of PB and centrifuged the spin columns for 60 seconds. Discarded the flow through.
h. Added 750 µL of PE to the spin columns, centrifuged for 60 seconds, and discarded the flow through.
i. Centrifuged the spin columns again for 60 seconds to remove residual wash buffer and discarded the flow through.
j. Transfered the spin columns to a clean eppendorf tube and added 50 µL of EB to the center of the spin column to elute the DNA.
k. Allowed the spin column to stand for one minute and then centrifuged for one minute.
l. Recorded the concentrations for each sample.
    i. Blanked with EB buffer

*Did not do minipreps on ligation 1 (colonies #8 100uL, #10 100uL, #12 100uL , and #12 150uL) and ligation 2 (#12 150uL). Due to those being pink colonies.
(Glycerol stock of 6/19/2019 25 samples are done)

Results

Ligation 1 100 µL

Colony #	**Concentration (ng/µL)**	**260/280**
7	0.4	2.000
9?	0.6	1.714
11?	0.5	2.500

*?- indicates unknown colony #. The labels on the overnight culture tubes rubbed off

Ligation 1 150 µL

Colony #	**Concentration (ng/µL)**	**260/280**
7	0.45	3.000
8	0.20	-2.000
9	0.15	3.000
10	0.40	2.667
11	0.45	2.250

Ligation 2 100 µL

Colony #	Concentration (ng/µL)	260/280
7	.65	2.167
8	.40	4.000
9	.50	2.500
10	.45	4.500
11	.55	2.750
12	.55	3.667

Ligation 2 150 µL

Colony #	Concentration (ng/µL)	260/280
7	1.35	2.250
8	.45	4.500
9	.65	2.600
10	.60	2.400
11	.35	7.000

Pcb302

Colony #	Concentration (ng/µL)	260/280
7	20	2.667
8	12.5	5.00
9	15	2.00
10	5	----
11	10	----
12	12.5	5.0

Conclusion

Every transformation resulted in very low DNA concentrations. We should re-order the pcb302 plasmid and attempt the ligations again.

***Note:**

All of the above readings excluding pcb302 were recorded using a contaminated blank and are, therefore, invalid.

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Goal:

1. Prepare ASP-8A media

Protocol:

PII TRACE METAL (10x 1L)

1. Diluted 480mg CoSO4.7H2O in 100ml H2O to get 1000x CoSO4.7H20 solution.
2. Used the table below to make 10x 1L PII TRACE METAL stock.

COMPONENTS	**MW**	**10X STOCK (1L)**
CoSO4.7H2O	281.12	10mL 1000x CoSO4.7H2O
EDTA.2Na	372.2	11.07g
FeCl3.6H2O	270.3	0.49g
H3BO3	61.8	11.4g
MnSO4.4H2O	223	1.64g
ZnSO4.7H2O	287.5	0.22g

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Goal:

1. Glycerol stocks
    a. Ligation 1 (K592009 + J23102)
    b. Ligation 2 (K592009 + J23102)
    c. Pcb302 in E. Coli

Protocol:

Glycerol Stocks

1. Took 1 mL of 50% glycerol and 1 mL of the overnight culture (after incubation) and added to a glycerol stock tube.
2. Labeled with name, date, and the contents and stored in the -80° C freezer in CLSO 442