Name: Rehmat Babar and Chiara Brust

Date: 7/1/19

Goal:

1. Transform Pcb302 (new samples; 1& 2) into E. coli
2. Transform blue chromoprotein composite (K592015)
3. Make YM media plates with Kanamycin and YM broth
4. Verify algal life. Take pictures (Algae Log)
5. Run gel on PCR of pcb302 in E. Coli from papers A & B from 6/28/19
6. Check concentrations on Pcb302 from new paper samples (1 & 2)

Name: Rehmat Babar

Date:7/1/19

Goal:

1. Transform
    a. Blue chromoprotein (K592015)
    b. pcb302-GFP plasmid into E. Coli

Materials

Invitrogen Chemically competent DH5alpha cells

Protocol:

Heat Shock for Blue Chromoprotein

1. Thawed One Shot TOP10 chemically competent cells on ice.
2. Added 3 µL of DNA sample into competent cells
3. Incubated the cells on ice for 35 minutes.
4. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
5. **Quickly** took them out and **immediately** added 250 µL of SOC medium
6. Placed the samples into a 37° C shaking water incubator for 1 hour at 200 rpm.
7. After shaking for 1 hour, streaked 150 µL of the solution onto an agar plate with chloramphenicol and another with 75 µL.
8. Incubated plates at 37°C for at least 24 hours.

Heat Shock for pCB302-gfp-MBD plasmid 1

9. Thawed One Shot TOP10 chemically competent cells on ice.
10. Added 6 µL of DNA sample into competent cells
11. Incubated the cells on ice for 35 minutes.
12. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
13. **Quickly** took them out and **immediately** added 250µL of SOC medium
14. Placed the samples into a 37° C shaking water incubator for 1.5 hours at 200 rpm.
15. After shaking for 1.5 hours, streaked 150 µL of the solution onto an agar plate with kanamycin and another with 75 µL.
16. Incubated plates at 37°C for at least 24 hours.

Heat Shock for pCB302-gfp-MBD plasmid 1

17. Thawed One Shot TOP10 chemically competent cells on ice.
18. Added 6 µL of DNA sample into competent cells
19. Incubated the cells on ice for 35 minutes.
20. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
21. **Quickly** took them out and **immediately** added 250µL of SOC medium
22. Placed the samples into a 37° C shaking water incubator for 1.5 hours at 200 rpm.

23. After shaking for 1.5 hours, streaked 150 µL of the solution onto an agar plate with kanamycin and another with 75 µL.
24. Incubated plates at 37°C for at least 24 hours.

YM Media Recipe
Dissolve in 900 mL of diH2O
0.4 g Yeast extract
10 g Mannitol
0.1 g NaCl
0.2 g MgSO4ï 7H2O
0.38 g K2HPO4

Autoclaved for 45 minutes and brought to a pH of 7

Results:

The two sources of the pCB302-gfp-MBD plasmid both had a concentration of 0.65 ng/µL.

Observed on 7/2/19:

There was no growth on the plates after 15 hours for the blue chromoprotein however the two plates with pCB302 plasmid transformed cells had lots of growth with small circular colonies.

Conclusion:

We will start overnights and do colony PCR for the pCB302 plates with colonies.

Name: Chiara Brust

Date: 7/1/19

Goal:

1. Gel electrophoresis of pCB302-gfp-MBD plasmid PCR

Protocol:

Preparing

1. Added 1 g of Agarose in 100 mL of 1X TBE in an Erlenmeyer flask
2. Heated in the microwave until fully dissolved (1.5 min.)
3. Allowed the solution to cool until comfortable to touch
4. Added 10 µL GelRed Nucleic Acid Gel Stain and mix
5. Inserted casting tray, made sure the rubber on the sides was not overlapping
6. Carefully poured the agarose into the tray and placed the comb to create the wells
7. Allowed the gel to solidify
8. Once solidified, changed the orientation of the casting tray where the rubber sides are not in contact with the sides of the system.
9. Poured in 1X TBE into the gel electrophoresis system to the fill line, made to submerge the gel, and removed the comb

Loading

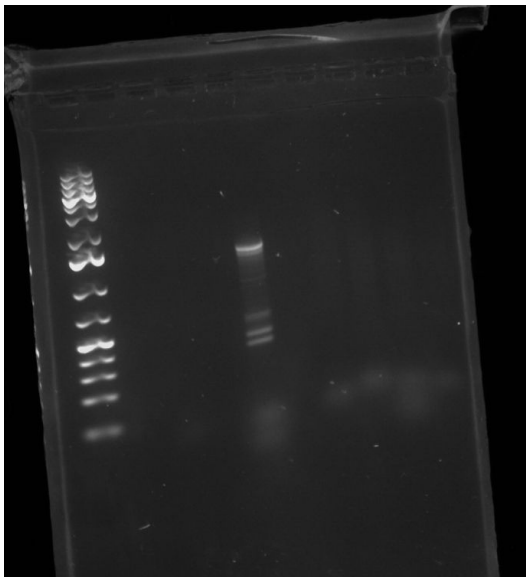
1. Loaded ~5 µL of the ladder in the first well
2. Prepared your samples to load by adding in 1 µL of 6X Loading dye + 4 µL PCR product

Running

1. Once the gel had been loaded, we slid on the cover making sure the negative electrode is closest to the DNA and the positive electrode is at the bottom of the gel
2. Ran for about 1.5 hours at 95 V

Results

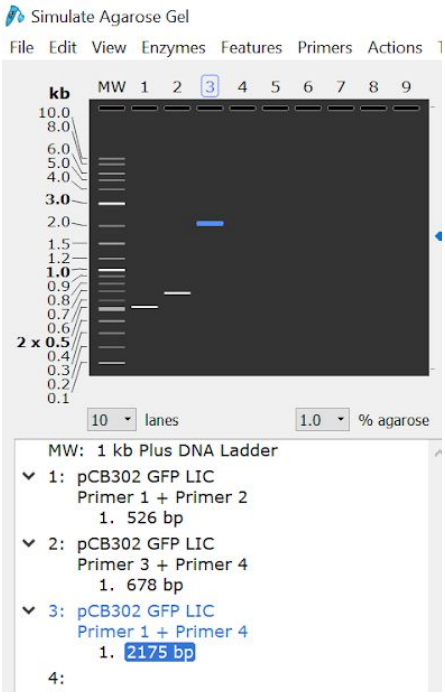
Pcb302 PCR gel



Pcb302 PCR Gel Key

Lane #	1	2	3	4	5	6	7	8	9	10	11
Sample	1 Kb Plus DNA ladder	Blank	A/ P1, P2	Blank	A/ P3, P4	Blank	A/ P1, P4	Blank	B/ P1, P2	B/ P3, P4	B/P1, P4

Expected Results



Conclusion

The results are not as expected. Because we were given new samples of the pcb302 plasmid, we will stop using the old samples and start fresh with the new ones.