Name: Chiara, Asma, Rehmat, Yilin Lu, Jia Deng, Zeshi Wang, Xuecheng Ye, Jiayi Lan, Yujie Huang, Xinyi Liu, Krithika, Kennex Lam
Date: 8/12/19

Goal:

1. Transform pcb302 into E. Coli
    a. From papers A & B
2. Make New Algae Cultures for O. Marina and S. Microdraticum
3. Transform mCherry using Rosetta-Gami competent cells
4. 500 mL cultures for Dino III with RFP and GFP
5. Overnight cultures of Test Device 1 and + and - controls in LB, TB, YM, and YPD

Name: Chiara, Krithika
Date: 8/12/19

Goal:

1. Transform pcb302 into E. Coli
    a. From papers A & B

Protocol:

Electroporation

1. Combined 40 µL of electrically competent DH5a cells and 1 µL of ligated DNA to an Eppendorf tube.
2. Transferred the contents of the Eppendorf tube to a cuvette and lightly tapped the cuvette on the table to evenly distribute the contents and to get rid of air bubbles.
3. Placed the cuvette into the Bio-Rad MicroPulser and delivered an electric shock.
4. **Immediately after**, added 900 µL SOC medium to the cuvette and micropipette mixed the solution.
5. Transferred the solution from the cuvette to a shaker tube and placed in a shaker at 37°C at 200 rpm for 1 hour.
6. After shaking for 1 hour, streaked 150 µL of the solution onto an LB Kanamycin agar plate.
7. Incubated plates at 37°C for at least 24 hours.

Name: Asma
Date: 08/12/2019

Goal: Transform mCherry using Rosetta-Gami competent cells

Protocol:

Heat Shock

1. Thawed Rosetta-gami 2(DE3) pLysS chemically competent cells on ice.
2. For Standard Kits: added 20 ul of competent cells in pre-chilled 1.5 eppendorf tubes
3. Added 1 ul of DNA in each tube (Test Plasmid, Transformation 1, Transformation 2)
4. Placed tubes on ice for 5 mins
5. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
6. Placed on ice for 2 mins and added 80µL of SOC medium to each tube
7. Placed the samples into a 37° C shaking water incubator for 1 hour at 250 rpm.
8. After shaking for 1 hour, plated onto ampicillin resistant plates
9. Incubated plates at 37°C for at least 24 hours.

Results:

No growth

Conclusion:

Will attempt transformation again

Name: Rehmat, Yilin Lu, Jia Deng, Zeshi Wang, Xuecheng Ye, Jiayi Lan, Yujie Huang, Xinyi Liu

Date: 8/12/19

Goal:

1. Make new LB Medium TB Medium YB Medium YPD Medium for overculturing
2. Start overculture of DinoIII-GFP in these medium

Protocol:

1. **LB medium**

How to make a 1 L LB media solution

LB media recipe

The recipe below is used to prepare a 1 L LB media solution.

Reagent	Weight / Volume
Tryptone	10 grams
NaCl	10 grams
Yeast extract	5 grams
Distilled water	Up to 1 L

1. Weighed out 10 g tryptone, 10 g sodium chloride (NaCl) and 5 g yeast extract and added to a 1 L Duran bottle.
2. Measured out approximately 900 mL of distilled water and added to the Duran bottle.
3. Shook the bottle to dissolve the reagents.
4. Once the reagents had fully dissolved, adjusted the pH to 7.0 by using sodium hydroxide (NaOH) solution (1 N).
5. Once the pH was adjusted, topped up the solution to 1 L by using distilled water.
6. To sterilize, autoclaved the solution on a liquid cycle (20 min at 15 psi).

2. **YM medium**

How to make a 1 L YM media solution

To make medium from scratch, followed the formulation below:

- Yeast Extract……3.0 g
- Malt Extract……3.0 g
- Dextrose……10.0g
- Peptone……5.0 g
- Agar (if required)……20.0 g
- DI Water……1000 ml

pH *6.2 +/- 0.2* Autoclave at 121ºC.

The actual pH is 6.37.

3. **YPD medium**

How to make a 1 L YPD media solution

Materials

- Graduated Cylinder
- Nanopure Water
- Flask/Bottle
- A weigh boat and 3 spatulas
- 10g Yeast Extract
- 20g Peptone
- 20g Glucose
- Aluminum Foil
- Stir Bar

Method

1.Measured ~700ml nanopure water using the graduated cylinder and add to flask. Added stir-bar and began stirring the water at ~medium speed.

Note: The graduated cylinder is used instead of the flask because it is more accurate than the cylinder, so ignore the apparent discrepancy you will see when the water is added.

2.Placed empty weight boat on the balance and tared it.

3.Weighed yeast extract, and added to flask.

4.Repeated with peptone and glucose. Weighed each component separately, but reused the same weigh boat.

5.Added agar when other ingredients had dissolved (no powder can be seen).

6.Poured solution (NOT including the stir-bar) back into the graduated cylinder.

7.Brought the volume up to 1000ml using nanopure water. This ensured that the final volume is 1000ml, including the ingredients added.

9.Tore off a small piece of aluminum foil and covered the top of the flask.

10.Placed a small piece of autoclave tape on the aluminum foil and autoclaved.

12.Cooled to 50 °C and carefully poured into sterile, labeled petri dishes aiming for speed, accuracy and the least amount of bubbles.

13.Disposed of excess media in the gel bucket before washing glassware. Did NOT dispose in the sink as this could clog the drain.

4. **Terrific Broth (TB) medium**

How to make a 1 L TB media solution

Reagent	Quantity	Final concentration
Yeast extract	24 g	24 g/L
Tryptone	20 g	20 g/L
Glycerol	4 mL	4 mL/L
Phosphate buffer (0.17 M KH_2PO_4, 0.72 M K_2HPO_4)	100 mL	0.017 M KH_2PO_4, 0.072 M K_2HPO_4

1. Added 900 mL of deionized water to 24 g of yeast extract, 20 g of tryptone, and 4 mL of glycerol. Shook or stirred until the solutes have dissolved and sterilized by autoclaving for 20 min at 15 psi (1.05 kg/cm2).

2. Allowed the solution to cool to ~60°C and added 100 mL of sterile phosphate buffer.

3. Stored TB at room temperature; it will keep for at least 1 yr.

Result:

See the medium tomorrow

Name: Rehmat
Date: 8/12/2019

Goal: Start overnight cultures for the characterization and the Dino III with GFP and Dino III with RFP

Protocol:

Overnight Cultures

1. Added 7 mL of LB to a 50 mL Falcon tube along with 7 µL of Chloramphenicol
2. Scraped some of the ice with the p10 tip and drop into the tube for:
    a. Test Device 1 colony 1 and 2, Test Device 5 colony 1 and 2, Positive Control colony 1 and 2, Negative Control colony 1 and 2
3. Incubated at 37° C at 220 rpm for 16 hours

4. Added 500 mL of LB to a 50 mL Falcon tube along with 500 µL of Ampicillin
5. Scraped some of the ice with the p10 tip and drop into the tube for:
    a. Dino III with GFP and Dino III with RFP
6. Incubated at 37° C at 220 rpm for 18 hours

Name: Kennex Lam
Date: 8/12/19

Goal:

1. Make new algae cultures for O. Marina and S. Microadriaticum

Protocol:

1. 4 flasks (two for O. marina and 2 for S. microadriaticum) were gathered as one set, and 2 sets were made.
2. 3 mL of S. Microadriaticum from the stock solution and 1 mL from our ASP-8A cultured symbiodinium (6/28) were added into 75 mL of new F/2 medium. The same was done to a 75 mL new ASP-8A medium.
3. 3 mL of stock O. Marina was added into one flask containing 75 mL of autoclaved, filtered seawater and 75 mL of F/2 medium.
4. One set as placed under a hood with a regulated 14 hour light/ 10 hour dark interval. The other 4 flasks were paced by the window.

Results:

FIGURE 1. S. Microdriaticum in ASP-8A media and F/2 media along with O. Marina in filtered seawater and F/2 media by the window.

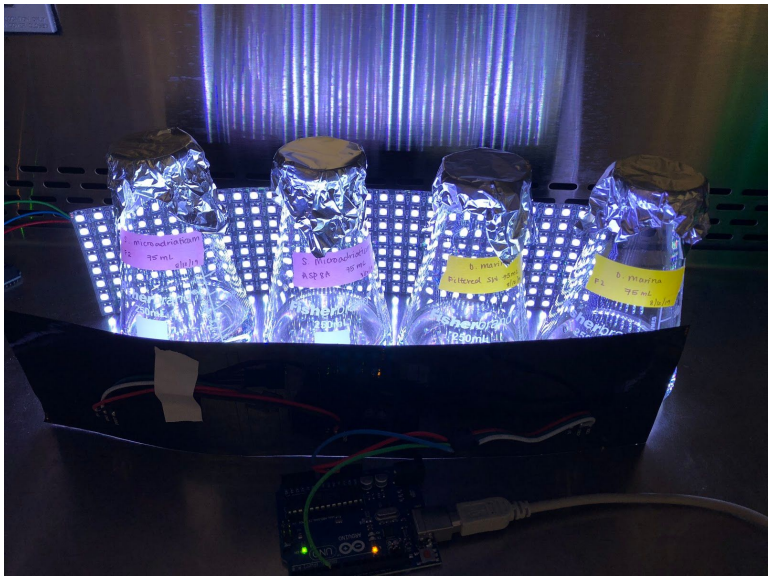

FIGURE 2. S. Microdriaticum in ASP-8A media and F/2 media along with O. Marina in filtered seawater and F/2 media under hood with regulated lighting.

Conclusion:

New cultures were made for O. Marina and S. Microadriaticum to observe their growth rate quantitatively (temperature and light intensity). We will compare how the different environmental factors will affect the algae's growth. The ASP-8A medium used was made last Friday while the F/2 medium was made today, so the media are new. I added 1 mL of our cultured Symbiodinium into these new cultures because many of the stock cells were non-moving. Our cultured cells appeared healthiest and more active compared to the stock cells.