Name: Laura, Asma, Rehmat, Chiara

Date: 09/03/19

Goal:

1. Original mCherry overnights from Glycerol stocks created on 8/31/19
2. Check pCB302 in A. Tumefaciens OD
3. Digest Dino III RFP for gel extraction
4. Run overnight gel for DinoIII RFP gel extraction
5. Transform S. Microadriaticum
    a. DinoGFP
    b. DinoRFP

Name: Laura Das Neves
Date: 9/3/19

Goal:

1. Overnight Cultures of Original Mcherry parts

Protocol:

Overnight culture:

1. Prepared 150 mL of Lb with 150 uL of chloramphenicol
2. Labeled falcon tubes with the corresponding colony and plate number from glycerol stocks of mCherry part
3. 7.5 mL of the Lb w/chloramphenicol was distributed to each labeled tube
4. A sample of each glycerol stock was derived by dipping a pipette tip at the surface and then ejected into the falcon tube.
5. The samples were placed into the shaker at 37°C at 1pm.

Name: Asma Khimani
Date: 09/03/19

Goal:

1. Check pCB302 in A. Tumefaciens OD

Protocol:

1. 3 samples were checked (using 800µL of the sample)
    a. OD = .476
    b. OD = .368
    c. OD = .345

Conclusion:

Samples are not at the desired OD of 1.5. Many samples seem to have clumping at the bottom of the tube.

Name: Rehmat
Date: 9/3/19

Goal: Digest Dino III RFP to linearize it for gel extraction

Protocol:

1. Combined 200 µL of diH2O, 50 µL of EcoRI, 50 µL of Fast Digest Buffer, and 200 µL of Dino III RFP mini prep DNA at 180 ng/µL in one eppendorf tube.
2. Incubated at 37°C for 2-3 hours.

Name: Chiara Brust
Date: 9/3/19

Goal:

1. Run overnight gel for DinoIII RFP gel extraction

Protocol:

Preparing, Loading, and Running a 1% Agarose Gel

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 (usually about 45 seconds to 1 minute)
3. Allowed the solution to cool until comfortable to touch
4. Added 10 µL GelRed Nucleic Acid Gel Stain and mixed
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 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, being sure to submerge the gel, and removed the comb

Loading

1. Loaded 50 µL of linearized DinoIII RFP into each well

Running

1. Once the gel had been loaded, 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 overnight at 35 V starting at 6:30 pm on 9/3/19

Name: Chiara
Date: 9/3/19

Goal:

1. Transform DinoIII-GFP and Dino-RFP into S. Microadriaticum

Protocol:

1. Cultured Symbiodinium Microadriaticum cells in ASP-8A with filtered seawater medium under natural sunlight for about 2 months and O. Marina cells in f/2 media for about 2 months with no antibiotics

- Culture:
  - S. Microadriaticum: ASP 8a-SW media 75 mL labeled 8/21/19; [cell]= 4.6 x10^5 cells/mL

2. Harvested the cells by centrifugation at 800 g for 5 min at 4°C.

- S. Microadriaticum: Pelleted 5 mL for each transformation= 2.3 x10^6 cells

3. Used 500 µL of 0.1M EDTA to resuspend the cell pellet

4. Centrifuged at 800 g for 2 min at 4°C.

5. Washed cells with 10% Glycerol 3 times, centrifuged at 800 g for 2 min in 4°C.

6. Resuspended the pellet in 1 mL of 10% Glycerol1

7. Incubated ~100µl of cells with (~1µg) of DNA on ice for 5 minutes.

- 6 µl of DinoGFP EtOH precipitate 170 ng/µl 8/20/19 sample
- 20 µl DinoRFP EtOH precipitate 50 ng/µl 8/19/19 sample for SHS & SC2
- 25 µl DinoRFP EtOH precipitate 42.5 ng/µl 8/19/19 sample for DIC

8. Put cells into a 0.2 cm cuvette, and electroporated using SHS (2.0 kV, 1 pulse), SC2 (1.5 kV, 1 pulse), or DIC (1.0 kV, 2 pulses, 1.0 msec) program with Bio-Rad MicroPulser 165-2100.

9. Added 1mL of ASP-8A SW medium to the 0.2 cm cuvette, mixed well, and transferred to a 15 mL tube

10. Left the cultures to grow under the programmed lights

11. Observed the cells under a normal microscope in 1-3 days and according to the need.

Results:

The cells were present at the end of the transformation protocol (Their concentration was not calculated). The following day, they were gone.

Conclusion:

We should redo the transformation but be more gentle during the resuspension step by simply swirling the tube rather than pipetting up and down.