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Date: 6/28/19

Goal:

1. PCR on pcb302 plasmid straight from paper solutions A & B
2. Transfer O. Marina, S. Microadiatic, D. Tertiolecta into other cultures and observe the algae

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1. PCR on pcb302 plasmid straight from paper solution

Protocol:

PCR (pcb302) 20 µL Reaction

1. Prepared a PCR concentration cocktail with the following proportions: 7 µL of diH2O, 10 µL PCR Mastermix, 1 µL of the forward primer, and 1 µL of the reverse primer.
    - Primers 1 & 2
    - Primers 3 & 4
    - Primers 1 & 4
    - Samples A (½, ¾, & ¼) & B (½) used PCR mastermix
    - Samples B (¾, & ¼) used PCR supermix high fidelity
2. Added 19 µL of the concentration cocktail into a PCR tube along with 1 µL of the DNA.
    - Pcb302 from paper A
    - Pcb302 from paper B
2. Placed PCR tube in the thermocycler at the following generic settings:
    1. 95° C for 3:00 minutes
    2. 95° C for 1:00 minute
    3. **52° C for 1:00 minute**
    4. 72° C for 1:00 minute
    5. 30X (Go to Step 2)
    6. 72° C for 5:00 minutes

    Lid Temperature: 105° C

Conclusion:

On Monday, we should run a gel on the PCR products. Independent of the results, the pcb302 plasmid should be sent off for sequencing. Finally, a restriction digest, gel extraction, ligation, and transformation should be performed on K592015 and J23102.

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1. Transfer O. Marina, S. Microadiatic, D. Tertiolecta into other cultures and observe the algae

Protocol:

Transfer the alga into different cultures

Part 1.

A. 9 mL of filtered autoclaved seawater with 1 mL / Liter F/2 medium into individually labeled falcon tubes and 1 mL of each alga was added
B. 75 mL of filtered autoclaved seawater with F/2 media and 1mL into each 125 mL flask
C. 25 mL of filtered autoclaved seawater with F/2 into each individual 50 mL falcon tube with 1 mL of each alga

Part 2.

A. 9 mL of ASP8A medium and 1 mL of each alga
B. 75 mL of ASP8A medium and 1mL into each 125 mL flask
C. 25 mL of ASP8A medium into each individual 50 mL falcon tube with 1 mL of each alga

Each culture was covered with aluminum foil to enable gas exchange.

The O. marina was given 1½ mL of D.tertiolecta into each of the individual cultures from part 1 and 7 mL of the D.tertiolecta was placed into the original culture to allow sufficient food supply for the weekend.

Results:

N/A

Conclusion:

We should set up the image capturing system on the microscope and record our daily observations of the algae. It would also be of value to determine the concentration of each culture solution.