Name: Asma, Krithika
Date: 9/25/19

Goal:

1. Transform pcb302 into A. Tumefaciens
    a. Use DNA extracted from E. Coli transformations Plate B Colony 4
2. Test (blank) yeast transformation protocol on S. microadriaticum

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Goal:

3. Transform pcb302 into A. Tumefaciens
    a. Use DNA extracted from E. Coli transformations Plate B Colony 4

Agrobacterium tumefaciens LBA4404

Protocol:

Electroporation of Agrobacterium tumefaciens

1. Thawed Agrobacterium tumefaciens cells on wet ice
2. Combined 1 µL of pCB302-gfp-MBD plasmid DNA and 20 µL of cells in an Eppendorf Tube
3. Pipetted the cells into a cuvette and electroporated at 2 kV
4. Added 1 mL of YM media and transferred to a 15 mL falcon tube
5. The tubes were incubateXDSQ1`1`1Q1``d at 30°C at 200 rpm for 3 hours
6. 400 µL of each culture was streaked onto a LB kanamycin plate.
7. 300 µL of cultures 1 & 3 was streaked onto a YM kanamycin plate.
8. 300 µL of culture 2 was streaked onto a LB kanamycin plate.
9. 200 µL of each culture was also streaked onto a LB kanamycin plate.
10. The plates were incubated at 30°C for 48 hours

*Incubating 9/25 @6:30pm

Name: Krithika
Date: 9/25/19

Goal:

4. Blank transformation on S. microadriaticum using yeast protocol

Protocol:

1. 90 µL of symbiodinium at an OD of 0.678 was added to an ice cold electroporation cuvette
2. The symbiodinium was incubated on ice for 5 minutes
3. The cells were pulsed at 1.5V and given 1mL 10% glycerol recovery
4. The cells were transferred to a 15 mL tube and placed in a non-shaking water bath at 30 °C for 1 hour
5. The cells were then put in 2 mL of ASP8A media and placed in artificial light for rapid growth