

Name: Rehmat, Krithika, Laura

Date: 9/26/19

Goal:

1. Transform pcb302 into *A. Tumefaciens*
  - a. Use DNA extracted from *E. Coli* transformations Plate B Colony 4
2. Cell count/observe *S. microadriaticum* after yesterday's transformation

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Materials:

Agrobacterium tumefaciens LBA4404

Protocol:

### **Electroporation of Agrobacterium tumefaciens**

1. Thawed Agrobacterium tumefaciens cells on wet ice
2. Combined 1  $\mu$ L of pCB302-gfp-MBD plasmid DNA and 20  $\mu$ L 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 incubate at 30°C at 200 rpm for 3 hours
6. 400  $\mu$ L of each culture was streaked onto a LB kanamycin plate.
7. 300  $\mu$ L of cultures 1 & 3 was streaked onto a YM kanamycin plate.
8. 300  $\mu$ L of culture 2 was streaked onto a LB kanamycin plate.
9. 200  $\mu$ L of each culture was also streaked onto a LB kanamycin plate.
10. The plates were incubated at 30°C for 48 hours

Results:

Plates were removed from the incubator on 9/30/2019 and seem to have overgrown. However, colonies were still picked to start over nights.

Name: Krithika

Date: 9/26/19

Goal:

1. Count symbiodinium cells from blank transformation (yeast protocol) using hemocytometer

Results:

10  $\mu$ L volume: 7 cells/5 squares = 1.4 cells/square

$1.4 \times 10^4 = \mathbf{14,000 \text{ cells/mL}}$