Name:Sijia Qin, Jiazi Tian, Justin Benton, Shakera Thomas, Chiara Brust, Kennex Lam
Date: 7/8/19

Goals:

1. Minipreps for pcb302 in Agrobacterium tumefaciens from papers 1 & 2
2. Glycerol stock for overnight pcb302 Agrobacterium cultures papers 1 & 2
3. Determine algae concentration

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Goal:

1. Miniprep pcb302 in A. Tume

Protocol:

Mini Preps for Agrobacterium tumefaciens

1. Centrifuged 10 mL of overnight for 15 minutes at 3500 rpm and resuspended in 250 µl buffer P1 containing 0.1 mg/ml RNase A.
2. Added 250 µl lysis buffer P2 to the tube and inverted gently 6 times to mix.
3. Added 350 µl neutralization buffer N3 to the tube and inverted immediately but gently 6 times.
4. Centrifuged the lysate for 10 min at maximum speed in a tabletop microcentrifuge 13,000 rpm
5. Placed a QIAprep Spin Column in a 2 ml collection tube.
6. Transferred the cleared lysates from step 4 to the QIAprep Spin Column by decanting or pipetting.
7. Centrifuged 30–60 s (13,000 rpm or ≥10,000 x g). Discard flow-through.
8. Washed the QIAprep Spin Column by adding 0.5 ml of Buffer PB and centrifuging 30–60 s (13,000 rpm or ≥10,000 x g). Discarded flow-through.
9. Washed the QIAprep Spin Column by adding 0.75 ml of Buffer PE and centrifuging 30–60 s (13,000 rpm or ≥10,000 x g).
10. Discarded flow-through and centrifuged for an additional 1 min to remove residual wash buffer (13,000 rpm or ≥10,000 x g). IMPORTANT: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.
11. Placed the QIAprep Spin Column in a clean 1.5 ml microcentrifuge tube.
12. To elute DNA, added 50 µl of Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep Spin Column, let stand for 1 min, and centrifuged for 1 min.

Results:

Sample	**[DNA]**	**260/280**
7	2.5	0.5
2	2.5	---
3	---	---

Conclusion:

Pcb302 plasmid may not have been transformed into A. Tumefaciens. We should re-do the transformations. It would be best to sequence pcb302 because we may just be starting with too low of concentrations. However, new primers need to be designed in order to do that.

Name: Jiazi Tian
Date: 8 July 19

Goal:

1. Create a glycerol stock of Agrobacter+pCB302

Protocol:

Glycerol Stock
500 ul of overnight cultures of pcb302 in agrobacterium and 500 ul of glycerol were stored in the freezer and labeled as pcb302 agrobacterium, 7/8/19.

Name: Chiara Brust, Kennex Lam
Date: 8 July 19

Goal:

1. Determine the concentration of algae

Protocol:

1. 50 µL of algae solution was dropped onto a microscope slide
2. The algae were individually counted on a 10x magnification microscope
3. This number was then divided by .00129 mL to get the total number of algal cells per mL of solution
    a. .00129 mL is the volume of liquid seen on a 10x microscope view

Results:

Algae	**Density**
O. Marina in original solution	2325.58 cells/mL
D. Tertiolecta in original solution	2.8667 x 10^6 cells/mL