Name: Chiara Brust, Laura Das Neves, Sijia Qin, Vicky Liu
Date: 10/11/19

Goal:

1. Transform S. Microadriaticum using Agrobacterium Tumefaciens Bead Beating
2. PCR on transformed algae
    a. DinoIII Fwd/Rev primers

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1. Transform S. Microadriaticum using Agrobacterium Tumefaciens Bead Beating
    a. Mini bead beater
    b. Lambert's bead beater

Protocol:

Agrobacterium Tumefaciens Bead Beating Protocol
Pcb302 into S. Microadriaticum

1. Placed Symbiodinium cells in 1 mL culture medium (ASP-8A) in a 2mL cryotube containing a dry volume of 200 µL (about 500 mg) acid-washed, sterile glass beads.
    a. Mini-bead beater [S. Micro]= 3.0 x10^5 total cells from flask: S. Micro ASP-8A 8/2/19 100 mL; 2
    b. Lambert Bead Beater [S. Micro]= 9.6 x 10^5 total cells from flask: S.M. 75 mL + 1mL ASP-8A 8/21/19

2. Then, added 350 µL of 20% polyethylene glycol (PEG) to the suspension.

3. Shaked the tube in a bead beater at 4200 rpm for 90 seconds.
    a. Mini-bead beater [S. Micro]= 1.6 x10^4 cells/mL
    b. Lambert Bead Beater [S. Micro]= 6.0 x10^4 cells/mL

4. After shaking, transferred the cells to a new sterile 2 mL cryotube and washed to remove the PEG by pelleting at 3,000 g for 3 minutes then resuspending in 1 mL ASP-8A.
    a. Mini-bead beater [S. Micro]= 0 visible cells
    b. Lambert Bead Beater [S. Micro]= 4.4 x10^4 cells/mL (some seen swimming)

5. Added 150 µL of Agrobacterium culture (OD600= 1.5) harboring the pcb302 plasmid.

6. Incubated in fresh ASP-8A medium without antibiotics in the dark for 2 days before selection.

7. To select, added 50 µg/mL Kanamycin and 50 µg/mL Ampicillin. In addition, 50 µg/mL Kanamycin was always present to prevent any bacterial growth during the selection process.
    a. 50 µL of 1000x kanamycin
    b. 25 µL of 50 µg/mL Ampicillin

8. The cultures were then maintained in the same solution (Final volume 25 mL) in 50 mL sterile tubes under the standard photoperiod conditions and monitored for the appearance of bright-green fluorescence detected by microscopy (under phase contrast and epifluorescence 40x and 63x).

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Goal: PCR on transformed algae

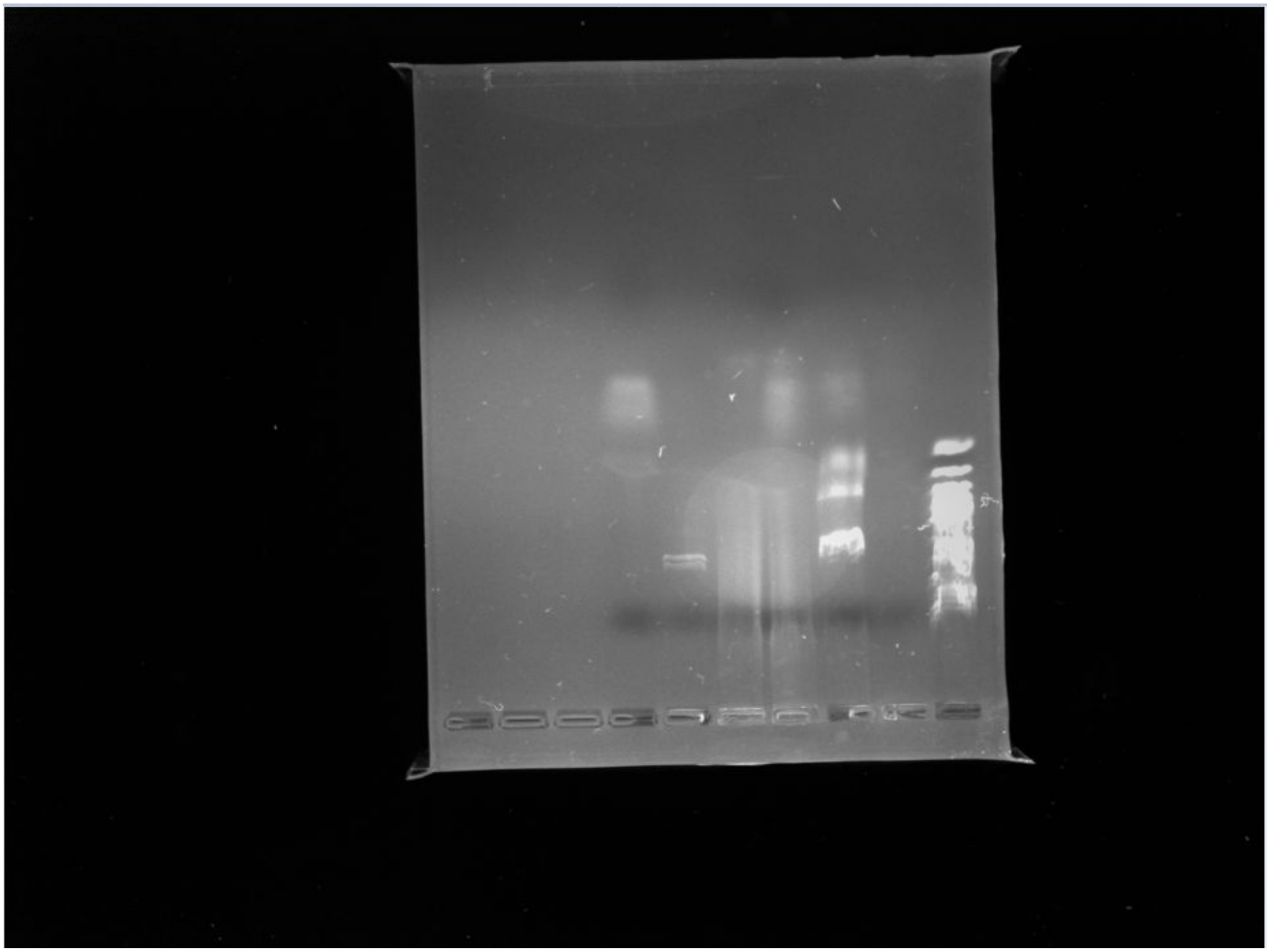
a. DinoIII Fwd/Rev primers

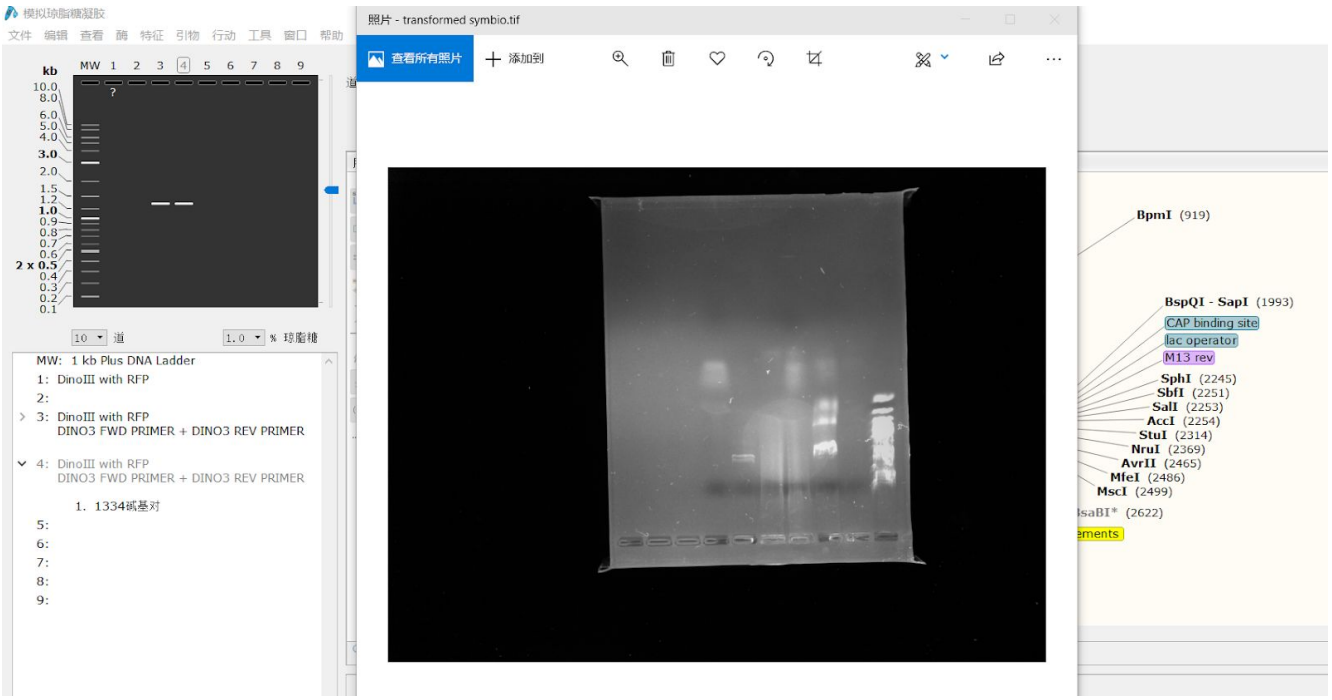
Protocol:

1. Took 200ul from algae solution and spun at 1,400 rpm for 2min, resuspended in 20ul ddH2O. Took 10 ul into PCR tube.
2. Prepared a PCR concentration cocktail with the following proportions:,10 µL PCR Mastermix, 1 µL of the Dino forward primer, and 1 µL of the Dino reverse primer.
3. Labeled PCR tubes as following:
   1: S.micro ASP8A blank transt
   2: 'blank' Symbio #1 transt
   3: Dino RFP S.micro 3
   4: Dino RFP S.micro 1
   5: 'blank' Symbio #2 transt
   6: Symbiodium original

4. Placed PCR tube in the thermocycler at the following generic settings:
   95° C for 3:00 minutes
   95° C for 1:00 minute
   50° C for 1:00 minute
   72° C for 1:00 minute
   30X (Go to Step 2)
   72° C for 5:00 minutes
   Lid Temperature: 105° C

Results:




横拟琼脂糖凝胶
文件 编辑 查看 酶 特征 引物 行动 工具 窗口 帮助
kb
MW 1 2 3 4 5 6 7 8 9
10.0
8.0
6.0
5.0
4.0
3.0
2.0
1.5
1.2
1.0
0.9
0.8
0.7
0.6
2 x 0.5
0.4
0.3
0.2
0.1
10 道
1.0 % 琼脂糖
MW: 1 kb Plus DNA Ladder
1: DinoIII with RFP
2:
3: DinoIII with RFP
DINO3 FWD PRIMER + DINO3 REV PRIMER
4: DinoIII with RFP
DINO3 FWD PRIMER + DINO3 REV PRIMER
1. 1334碱基对
5:
6:
7:
8:
9:
照片 - transformed symbio.tif
查看所有照片
添加到
BpmI (919)
BspQI - SapI (1993)
CAP binding site
lac operator
M13 rev
SphI (2245)
SbfI (2251)
SalI (2253)
AccI (2254)
StuI (2314)
NruI (2369)
AvrII (2465)
MfeI (2486)
MscI (2499)
saBI* (2622)
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