Name: Rehmat

Date: 10/16/19

Goal: Linearize the two Dino III plasmids using EcoRI

Protocol:

Linearizing Dino RFP and Dino GFP

1. Combined 1,090 µL of diH2O, 273 µL of EcoRI, 273 of Fast Digest Buffer, and 1,090 µL of Dino III GFP mini prep DNA at 260 ng/µL in one eppendorf tube.
2. Combined 830 µL of diH2O, 208 µL of EcoRI, 208 of Fast Digest Buffer, and 830 µL of Dino III RFP mini prep DNA at 170 ng/µL in another eppendorf tube.
3. Incubated at 37°C for 2-3 hours.

Name: Chiara
Date: 10/16/19

Goal:

1. Run gel of linearized Dino GFP and DinoRFP

Protocol:

Preparing, Loading, and Running a 1% Agarose Gel

Preparing

1. Added 1 g of Agarose in 100 mL of 1X TBE in an Erlenmeyer flask
2. Heated in the microwave until fully dissolved (usually about 45 seconds to 1 minute)
3. Allowed the solution to cool until comfortable to touch
4. Added 10 µL GelRed Nucleic Acid Gel Stain and mix
5. Inserted casting tray, make sure the rubber on the sides is not overlapping
6. Carefully poured the agarose into the tray and place the comb to create the wells
7. Allowed the gel to solidify
8. Once solidified, changd the orientation of casting tray where the rubber sides are not in contact with the sides of the system.
9. Poured in 1X TBE into the gel electrophoresis system to the fill line, being sure to submerge the gel, and removed the comb

Loading

1. Prepared samples to load by adding in 1 µL of 6X Loading dye for every 5 µL of DNA and loaded
    a. Added 260 µL of 6X loading dye to 1,560 µL of the linearized Dino GFP plasmid, loaded about 75 µL per well

    b. Added 130 µL of 6X loading dye to 780 µL of the linearized Dino RFP plasmid, loaded about 75 µL per well

Running

1. Once the gel had been loaded, slid on the cover making sure the negative electrode is closest to the DNA and the positive electrode is at the bottom of the gel
2. Ran at 34 volts overnight

Name: Krithika, Asma

Date: 10/16/19

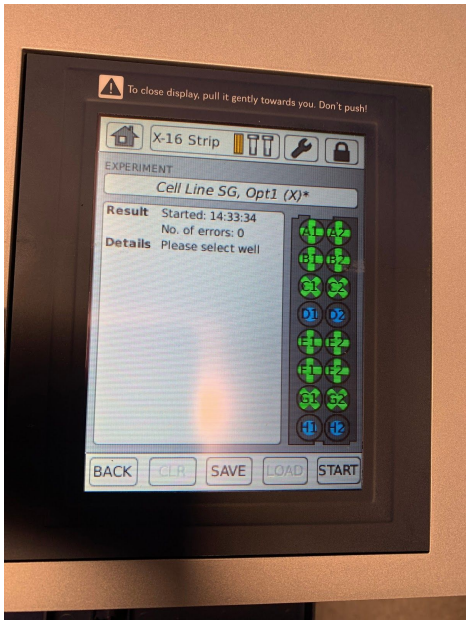
Goal: Transform S. microadriaticum and O. marina using LONZA

Protocol:

1. 1.5 mL of the symbiodinium (cell count: 1,092,000 cells/mL) was added to an eppendorf tube and spun down at 900g for 3 minutes. 3.5 mL of the o. Marina (cell count: 420,000 cells/mL) was added to a 50mL falcon tube and spun down at 1000g for 3 minutes.
2. The supernatant was removed from both tubes and 98 µL of SG and 22 µL of the supplement were added to EACH of the tubes and were swirled to resuspend the pellet.
3. 40 µL aliquots were made of each cell type (6 tubes total)
4. O. marina tubes
    a. 20 µL of Dino III RFP (72.5 ng/µL)
    b. 20 µL of Dino III GFP (92 ng/µL)
    c. 20 µL diH2O (for the blank)
5. S. microadriaticum tubes
    a. 40 µL of Dino III RFP (42.5 ng/µL)
    b. 20 µL of Dino III GFP (92 ng/µL)
    c. 20 µL diH2O (for the blank)
6. The Lonza machine was set for the correct pulse codes (DS-137 and DS-130) and 25 µL of each sample was added to the corresponding cuvette well
7. After shocking, 80 µL of media (ASP8A for s. microadriaticum and F/2 for o. marina) was added to the cuvette well and pipetted up and down three times. The contents of the cuvette were then transferred to the corresponding well in a 24-well plate.
8. The 24-well plates were placed in artificial light (hub) for incubation.

Notes:

- On the 24-well plate, A stands for pulse code DS-137 and B stands for pulse code DS-130
- The 24-well plate on top contains transformed O. marina and the one on the bottom contains transformed S. microadriaticum



This image shows there were no errors during or immediately after electroporation