Name: Chiara, Krithika
Date: 8/22/19

Goal:

1. Digest DinoIII-RFP
2. Run overnight gel of DinoIII-RFP
3. Overnights on pcb302 in A. Tumefaciens from 8/16/19 transformation (Trial #2)

Name: Krithika

Date: 8/22/19

Goal:

1. Overnights on pcb302 in A. Tumefaciens from 8/16/19 transformation (Trial #2)
    a. Papers A & B

Protocol:

1. 7 mL of YM broth was added to a 15 mL Falcon tube along with 7 µL of kanamycin
    a. *Note: Tubes 1-18 contain 8/19 YM and 2016 Kan; tubes 19-23 contain 7/19 YM and 2019 Kan*
2. A p10 tip was dipped into the selected colony and was dropped into its respective tube
3. Tubes were placed in the back shaking incubator at 220rpm at 30 °C at 6:00 pm and should be removed on Monday. **OD reading of 1.5 needed**

Name: Chiara
Date: 8/22/19

Goal:

1. Digest DinoIII-RFP to linearize it
    a. Enzyme: EcoRI

Protocol:

1. Combined 200 µL of diH2O, 50 µL of EcoRI, 50 µL of Fast Digest Buffer, and 200 µL of Dino III RFP mini prep DNA at 180 ng/µL in one eppendorf tube.
2. Incubated at 37°C for 2-3 hours.

Name: Chiara
Date: 8/22/19

Goal:

1. Run an overnight gel of linearized DinoIII-RFP

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 mixed
5. Inserted casting tray, made sure the rubber on the sides was not overlapping
6. Carefully poured the agarose into the tray and placed the comb to create the wells
7. Allowed the gel to solidify
8. Once solidified, changed 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. Loaded ~5 µL of the ladder in the first well
2. Loaded 50 µL of DNA

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 overnight at 34 V