Name: Chiara, Asma, Rehmat, Kennex, Shakera
Date: 8/14/19

Goal:

1. Mass miniprep of pcb302 250 mL overnight cultures
    a. From papers A & B
2. Diagnostic restriction digest on Dino III RFP and Dino III GFP
3. Run a gel alongside undigested mini prep DinoIII GFP/RFP DNA with diagnostic restriction digest and pCB302 mini preps
4. Make overnight cultures on mcherry transformations
5. Perform colony pcr on mcherry transformations

Name: Chiara
Date: 8/14/19

Goal:

1. Mass miniprep of pcb302 250 mL overnight cultures
    a. From papers A & B

Protocol:

Mini Prep from 500 mL cultures

1. Pelleted the cultures in 50 mL falcon tubes at 5000 rpm for 5 minutes
2. The pelleted cells were resuspended in 2.5 mL of P1 total and mixed by vortexing
3. 400 µL of this solution was placed in an Eppendorf tube
4. 400 µL of P2 was added to the tubes and the tubes were inverted about 5 times
5. 600 µL of N3 was added and the tubes were immediately inverted about 5 times
6. Centrifuged at 13,000 rpm for 10 minutes
7. The clear supernatant was carefully removed and added to a spin column until full, centrifuged for 1 minute at 13,000 rpm, and removed the flow through.
8. This was repeated for each spin column 2-3 times until all of the clear supernatant had been collected
9. Added 500 µL of PB and centrifuged the spin columns for 60 seconds. Discarded the flow through
10. Added 750 µL of PE to the spin columns, centrifuged for 60 seconds, and discarded the flow through
11. Centrifuged the spin columns again for 60 seconds to remove residual wash buffer and discarded the flow through
12. 50 µL of EB buffer was added to each of the spin columns and eluted into an Eppendorf tube.
13. The samples were combined so there were 100-300 µL in each tube.
14. The concentrations were recorded
    a. Blanked with the same EB buffer that was used to elute

Results:

Sample	**[DNA]**	**260/280**
Plate B Colony 2	90.0	1.895
B3	67.5	1.929
B4	108.0	1.955
A2	52.5	2.333
A3	72.5	1.933

Conclusion:

The concentrations were really good. We should now sequence this DNA and transform it into A. Tumefaciens.

Name: Rehmat

Date: 8/14/19

Goal: Diagnostic restriction digest on the mini preps from yesterday

Protocol:

Restriction Digest Protocol

30 µL Fast Digest Restriction Digest

1. Prepared a Fast Digest concentration cocktail with the following proportions: 2 µL HindIII, 3 µL of 10X Fast Digest Buffer, and 15 µL of diH2O.
2. Added 20 µL of this cocktail to a clean 1.5 Eppendorf tube and then added 10 µL of Dino III GFP.
3. Performed the same reaction for Dino III RFP.
4. Incubated at 37° C for 30 minutes.

Name: Rehmat

Date: 8/14/19

Goal: Run a gel on the diagnostic RD of Dino III GFP and Dino III RFP alongside undigested mini prep DNA of those samples, and the pCB302 mini prep samples

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 until fully dissolved.
3. Added 10 µL SYBR Gold Nucleic Acid Gel Stain when it cooled enough to touch.
4. Inserted casting tray.
5. Poured the agarose into the tray and placed the comb to create the wells. Gel solidified
6. Changed the orientation of casting tray so the rubber sides were not in contact with the sides of the system.
7. Poured in 1X TBE into the gel electrophoresis system to the fill line, making sure to submerge the gel.

Loading

1. Loaded 5 µL of the GeneRuler 1kb Plus ladder in the first well .
2. Prepared samples to load by adding in 1 µL of 6X Loading dye for every 5 µL of DNA and loaded

Running

1. Ran for 1 hour at 100 volts.

Results:

Key

Lane 1: GeneRuler 1 kb Plus
Lane 2: RFP diagnostic RD
Lane 3: GFP diagnostic RD
Lane 4: Dino III RFP mini prep 170 ng/µL
Lane 5: Dino III RFP mini prep 180 ng/µL
Lane 6: Dino III GFP mini prep 248 ng/µL
Lane 7: Dino III GFP mini prep 260 ng/µL
Lane 8: B4 pCB302 mini prep
Lane 9: B2 pCB302 mini prep
Lane 10: A3 pCB302 mini prep

Conclusion:

There was an issue with the gel and the bands didn't run properly, the ladder did not appear. We will run this gel again.

Name: Shakera Thomas
Date: 8/14/2019

Goal:

1. Make overnight cultures on mcherry transformations from 8/13/19

Protocol:

Overnight Cultures

1. Made a cocktail mix containing 150 mL of LB, 150 µL of ampicillin, and 150 µL of 1M IPTG.
2. 10 colonies were picked from both plate 1 and plate 2
3. The picked colonies DNA was diluted.
    a. A p10 tip of the colonies was swirled in 10 µL of DI H2O
4. Each falcon tube was labeled based on the corresponding colony. (ex: Plate 1 Colony 1)
5. Distributed 6 mL of the cocktail into each 15 mL Falcon tube.
6. Added 3 µL of the diluted DNA into each 15 mL Falcon tube.
7. The samples were shaken at 220 rpm overnight.

Name: Shakera Thomas
Date: 8/14/2019

Goal:

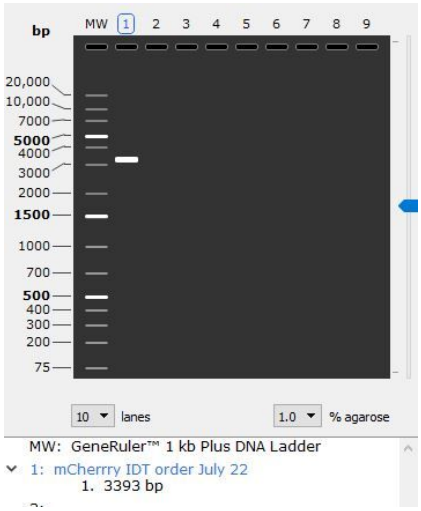
1. Colony PCR on mcherry transformations from 8/13/19

Protocol:

20 µL Reaction

1. Prepared a PCR concentration cocktail with the following proportions: 7 µL of diH2O, 10 µL PCR Mastermix, 1 µL of diluted forward primer, and 1 µL of diluted reverse primer.
2. PCR tubes were labeled by corresponding colony. (ex: P1,1 means plate 1 colony)
3. Added 19 µL of the concentration cocktail into a PCR tube.
4. Added 1 µL of of the diluted DNA into the PCR tube
5. Place PCR tube in the thermocycler at the following generic settings:
    a. 95° C for 3:00 minutes
    b. 95° C for 0:30 minute
    c. 58° C for 0:30 minute *Annealing temperature varies depending on primer
    d. 72° C for 1:00 minute
    e. 30X (Go to Step 2)
    f. 72° C for 5:00 minutes
    g. 4° C for ∞
    h. Lid Temperature: 105° C

Expected Results



Name: Chiara
Date: 8/14/19

Goal:

1. Re do overnight cultures of pcb302 from transformations done on 8/12/19 because we forgot to do glycerol stocks before the minipreps
    a. Papers A & B

Protocol:

Overnight Cultures

1. Added about 5-7 mL of LB to a 25 mL Falcon tube along with 5-7 µL of antibiotics
    a. Kanamycin
2. Dipped a p10 tip into the selected colony and dropped into the tube
3. Incubated at 37° C at 220 rpm for 16-18 hours