Name: Chiara Brust, Sijia, Jaizi
Date: 7/16/19

Goals:

1. PCR new primers (pcb302 GFP Fwd & Rev)
    a. Pcb302 straight from papers 1& 2
    b. Pcb302 in A. Tume from papers 1 & 2 from minipreps done on 7/15/19
2. Overnight cultures
    a. Pcb302 in A. Tumefaciens from papers 1 & 2 glycerol stocks made on 7/15/19
    b. pGEX-HCG from glycerol stocks made on
    c. DinoIIIP2
3. Gel Electrophoresis
    a. Pcb302 in A. Tume from papers 1 & 2 PCR from miniprep done on 7/15/19

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Goal:

1. PCR new primers
    a. Pcb302 straight from papers
    b. Pcb302 from minipreps done on 7/15/19

Protocol:

PCR 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 the forward primer, and 1 µL of the reverse primer.
    - Pcb302 GFP Fwd
    - Pcb302 GFP Rev
2. Added 19 µL of the concentration cocktail into a PCR tube along with 1 µL of the DNA.
2. Placed PCR tube in the thermocycler at the following settings:
    1. 95° C for 3:00 minutes
    2. 95° C for 1:00 minute
    3. 43° C for 1:00 minute
    4. 72° C for 1:00 minute
    5. 30X (Go to Step 2)
    6. 72° C for 5:00 minutes

    Lid Temperature: 105° C

Results:

N/A

Conclusion:

N/A

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Goal:

1. Overnight cultures of A. Tumefacien (pcb302)

Protocol:

Overnight Cultures

1. Added about 15 mL of YM to a 50 mL Falcon tube along with 15 µL of antibiotics
    a. 1000x Kanamycin
2. Scraped some of the ice of a glycerol stock with the p20 tip and dropped into the tube
        i. NOTE: did NOT let the glycerol stock defrost!
3. Incubated at 28° C at 200 rpm for 48-56 hours

Results:

N/A
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Conclusion:

N/A

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Goal:

1. Overnight cultures
    a. DinoIIIP2 from transformations done on 7/15/19
    b. PGEX-HCG from glycerol stocks made on

Protocol:

Overnight Cultures

1. Added about 5 mL of LB to a 15 mL Falcon tube along with 5 µL of antibiotics
    a. DinoIII: 1000x Ampicillin
    b. PGEX-HCG: 1000x Ampicillin
2. Dip a p10 tip into your selected colony and drop into the tube
    a. Forn PGEX-HCG glycerol stock, simply scraped some of the ice with the p10 tip and dropped into the tube
3. Incubated at 37° C at 200 rpm for 16-18 hours

Results:

N/A

Conclusion:

N/A

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Goal:

1. Run gel electrophoresis on pcb302 PCR
    a. Pcb302 straight from papers
    b. Pcb302 from minipreps done on 7/15/19

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
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

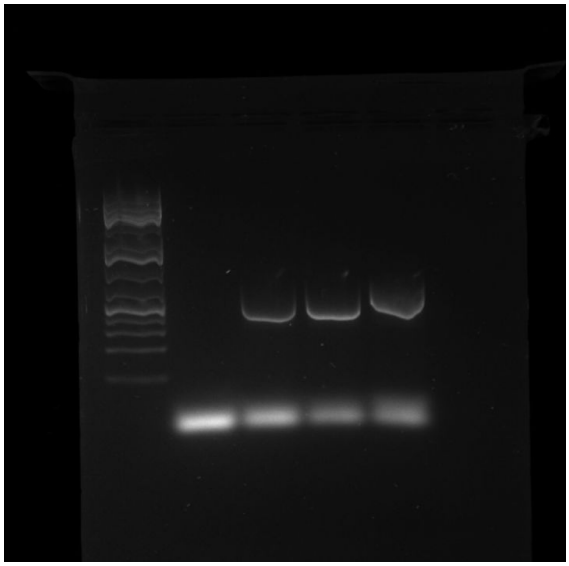
Loading

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

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 for about 45 minutes to an hour at 117 V

Results:



Gel Key

Lane #	**Sample**
1	MW 1 Kb Plus DNA Ladder
2	Pcb302 paper # 1
3	Pcb302 paper # 2
4	Pcb302 miniprep # 25
5	Pcb302 miniprep # 51

Expected Results: