Name: Sijia, Jaizi, Rehamt, Chiara, Shakera, Kennex, Amirah
Date: 7/15/19

Goals:

1. Make glycerol stock for overnight cultures for the transformations done on 7/9/19
    a. Pcb302 in A. Tumefaciens from papers 1 & 2
2. Miniprep for pcb302 in A. Tume from papers 1 & 2 overnight cultures for the transformations done on 7/9/19
3. Transform
    a. K1357009
    b. DinoIII part 2
4. Run gel of K1357009 colony PCR
5. Overnight cultures
    a. PGEX-HCG from glycerol stocks

Name: Rehmat Babar, Shakera Thomas
Date: 7/15/19

Goal:

1. Miniprep Extract pCB302-gfp-MBD plasmid from agrobacterium overnights

Protocol:

Mini Preps for Agrobacterium tumefaciens

1. Centrifuged 10 mL of overnight for 15 minutes at 3500 rpm and resuspended in 250 µl buffer P1 containing 0.1 mg/ml RNase A.
2. Added 250 µl lysis buffer P2 to the tube and inverted gently 6 times to mix.
3. Added 350 µl neutralization buffer N3 to the tube and inverted immediately but gently 6 times.
4. Centrifuged the lysate for 10 min at 13,000 rpm
5. Placed a QIAprep Spin Column in a 2 mL collection tube.
6. Transferred the cleared lysates from step 4 to the QIAprep Spin Column by decanting or pipetting.
7. Centrifuged for 60 seconds at 13,000 rpm and discarded flow-through.
8. Washed the QIAprep Spin Column by adding 500 µL of Buffer PB and centrifuged for 60 seconds at 13,000 rpm and discarded flow-through.
9. Washed the QIAprep Spin Column by adding 750 µL of Buffer PE and centrifuging at 60 seconds at 13,000 rpm and discarded the flow through.
10. Centrifuged for an additional 1 min to remove residual wash buffer at 13,000 rpm.
11. Placed the QIAprep Spin Column in a clean 1.5 mL microcentrifuge tube.
12. Added 50 µL of Buffer EB to the center of each QIAprep Spin Column, let stand for 1 min, and centrifuged for 1 min.

Results:

Samples	DNA Concentrations (ng/µL)
1	0.35
2	0.15
3	0.40
4	0.25
5	0.20
6	0.10

7	0.20
8	0.40
9	0.10
10	0.25
11	0.15
12	0.15
13	0.25
14	0.40
15	0.40
16	0.40
17	0.10
18	0.10
19	0.15
20	0.15
21	0.15
22	0.20
23	0.15
24	0.25
25	2.00
26	0.10
27	0.10
28	0.15
29	0.15
30	0.10
31	0.25
32	0.10

33	0.15
34	0.55
35	0.55
36	0.15
37A*	0.25
37B*	0.55
38	0.60
40	0.50
41	0.35
42	N/A
43	0.15
44	0.10
45	0.15
46	0.20
47	0.15
48	0.20
49	0.20
50	0.15
51	0.90
52	0.35
53	0.45

Conclusion:

The DNA concentrations were too low

*These samples were mislabeled and consequently unidentifiable.

Name: Sijia Qin, Jiazi Tian
Date:7/15/19

Goals:

1. Make glycerol stock for overnight cultures for the transformations done on 7/9/19
    a. Pcb302

Protocol:

1. 500 µl of 53 samples and 500 µl of 50% glycerol was added.
2. The box was labeled as glycerol stock of o/n cultures on 7/9/19 in the top of freezer.

Conclusion:
N/A

Name: Chiara Brust
Date: 7/15/19

Goal:

1. Transform K1357009

Protocol:

Heat Shock Transformation

1. Thawed One Shot TOP10 chemically competent cells on ice.
2. Added 2 µL of DNA sample into competent cells
    a. K1357009
    b. RFP positive control
3. Incubated the cells on ice for 35 minutes.
4. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
5. **Quickly** took them out and **immediately** added 250µL of SOC medium
6. Placed the samples into a 37° C shaking water incubator for 1 hour at 200 rpm.
7. After shaking for 1 hour, smeared 150 µL of the solution onto an agar plate with the respective antibiotics.
    a. Chloramphenicol
8. Incubated plates at 37°C for at least 24 hours.

Results:

N/A

Conclusion:

N/A

Name: Chiara Brust
Date: 7/15/19

Goal:

1. Resuspend DinoIII Part 2 plasmid
2. Transform DinoIII part 2 plasmid into E. Coli

Protocol:

Resuspension

1. Centrifuged the tube prior to opening at 13,000 RPM for 5 minutes
2. Resuspended DNA in IDTE buffer to the desired concentration
3. Vortexed for 20 seconds
4. Incubated the tube at room temperature for 30 minutes
5. Centrifuged for 1 minute

Transformation

Heat Shock

1. Thawed One Shot TOP10 chemically competent cells on ice.
2. Added 1 µL of DNA sample into competent cells
    a. DinoIII part 2
3. Incubated the cells on ice for 35 minutes.
4. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
5. **Quickly** took them out and **immediately** added 250µL of SOC medium
6. Placed the samples into a 37° C shaking water incubator for 1 hour at 200 rpm.
7. After shaking for 1 hour, smeared 150 µL of the solution onto an agar plate with the respective antibiotics.
    a. Ampicillin
8. Incubated plates at 37°C for at least 24 hours.

Results:

N/A

Conclusion:

N/A

Name: Chiara Brust
Date: 7/15/19

Goal:

1. Overnight cultures of PGEX-HCG from glycerol stocks

Protocol:

Overnight Cultures

1. Added about 6 mL of LB to a 15 mL Falcon tube along with 6 µL of antibiotics
    a. 1000X Chloramphenicol
2. Scraped some of the glycerol stock ice with the p10 tip and dropped into the tube
3. Incubated at 37° C at 220 rpm for 16-18 hours

Results:

N/A

Conlcusion:

N/A

Name: Amirah Hurst
Date:7/15/19

Goals:

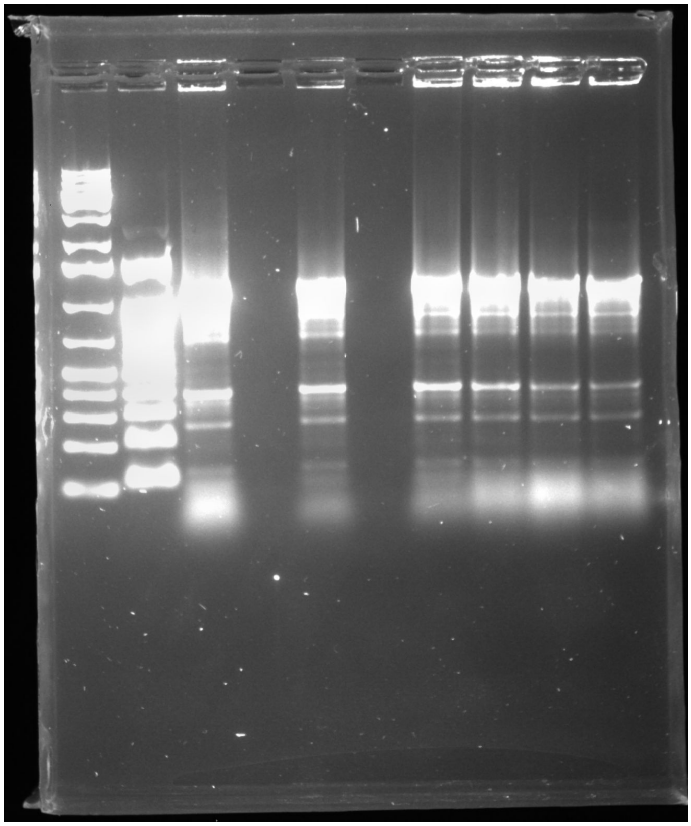
1. To run gel electrophoresis on the PCR product from the K1357009 plasmid done on 7/12/19
    a. Samples:
        i. A1
        ii. A2
        iii. A3
        iv. B1
        v. B2
        vi. B3
        vii. C1
        viii. C2
        ix. C3
        x. D1
        xi. D2
        xii. D3

Protocol:

1. Made two 1% Agarose gel
    a. 1 g Agarose
    b. 100 ml 1x TBE
        i. Mixed in Erlenmeyer flask
        ii. Heat in microwave until fully dissolved, swirling flask intermittently
    c. Solution cooled
    d. Added 10 ul of gel red
    e. Made gel with cast
2. Loaded 5 ul of samples and ladder onto gel
3. Ran gel at 130v at high voltage for one hour

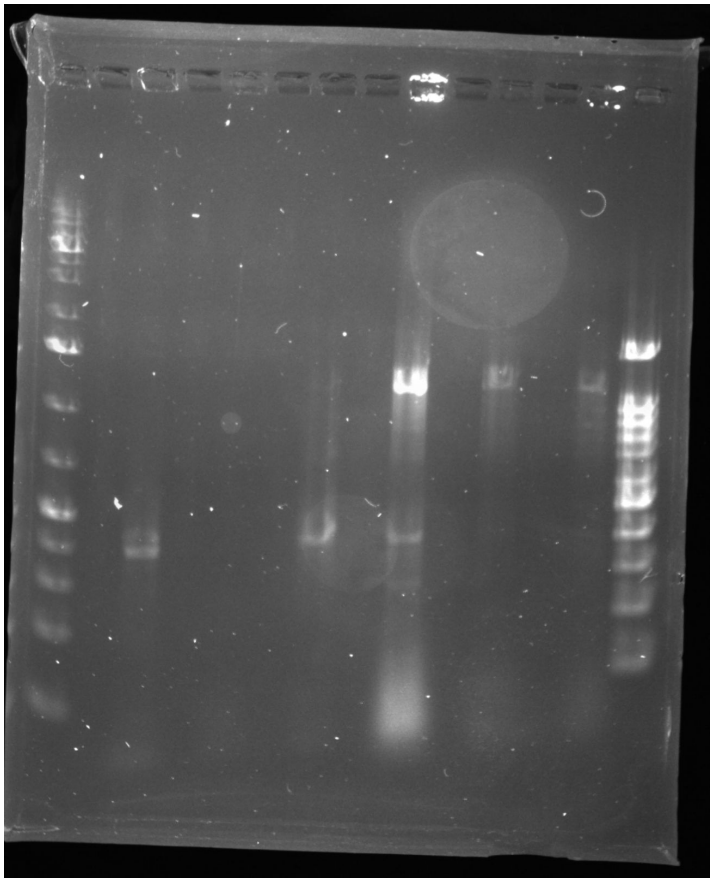
Results:

Gel 1



- Gel 1 key
    1. 1 kb ladder
    2. 100 bp ladder
    3. A1
    4.
    5. A2
    6.
    7. A3
    8. B1
    9. B2
    10. B3

Gel 2



- Gel 2 key
    1. 1 kb ladder
    2.
    3. C1
    4.
    5. C2
    6.
    7. C3
    8.
    9. D1
    10.
    11. D2
    12.
    13. D3
    14. 100 bp ladder