Name: Kennex, Asma, Chiara, Justin, Sijia, Jiazi
Date: 07/25/2019

Goals:

1. Overnight cultures (100 mL)
    a. DinoIII-GFP P2 from glycerol stocks
2. MiDi prep
    a. Pcb302 in E. Coli from papers 1 & 2 from glycerol stocks made on 7/3/19
3. Transform improved mCherry into E. Coli
4. Send parts for sequencing
    a. K1357009
    b. J23102

Name: Justin
Date: 07/25/2019

Goals:

1. Overnight cultures (100 mL)
    a. DinoIII-GFP P2 from glycerol stocks

Protocol:

1. One liter of Lb+Amp was created
2. Four overnight cultures were then created from the stock LB+Amp. DinoIII P2(4)- 100mL and 150mL and DinoIII P2(4)- 100mL and 150mL.
3. The DinoIII was allowed to culture for 18 hours at 37°C and 220rpm.
4. The excess LB+Amp was stored for later use.

Name: Asma Khimani, Kennex Lam
Date: 07/25/2019

Goals:

1. Transformation of improved mCherry

Protocol:

Plasmid Resuspension

1. Centrifuged the tube prior to opening to ensure DNA is at the bottom of the tube.
2. Resuspended DNA in 60 uL IDTE buffer so the final concentration is 66 ng/ul.
3. Incubated the tube at room temperature for 30 mins.
4. Centrifuged for 1 min..

Electroporation of mCherry with NEB® 5-alpha Electrocompetent E. coli

1. Prepared 17 mm x 100 mm round-bottom culture tubes (e.g. VWR #60818-667) at room temperature. Placed SOC recovery medium in a 37°C water bath. Pre-warmed ampicillin plates at 37°C for 1 hour.
2. Placed electroporation cuvettes (1 mm) and microcentrifuge tubes on ice.
3. As a positive control for transformation, diluted the control pUC19 by 1:5 to a final concentration of 10 pg/µl using sterile water. **Deviations**: Made a solution of 12ul (10 ul of diH2O and 2 ul of pUC19) **1:6 dilution**.
4. Thawed NEB 5-alpha Electrocompetent cells on ice (about 10 min) and mixed cells by flicking gently. Transferred 25 µl of the cells to a chilled microcentrifuge tube. Added 1 µl of the DNA solution.
5. Carefully transferred the cell/DNA mix into a chilled cuvette without introducing bubbles and made sure that the cells deposited across the bottom of the cuvette. Electroporated using the following conditions: bacteria Eco1.
6. Immediately added 975 µl of 37°C SOC to the cuvette, gently mixed up and down twice, then transferred to the 17 mm x 100 mm round-bottom culture tube. **Deviations**: mCherry #1: SOC medium was added 1 min later. mCherry 2: SOC added immediately.
7. Shaken vigorously (250 rpm) at 37°C for 1 hour. Deviation: Shook at 300 rpm.
8. Diluted the cells as appropriate then spread 100-200 µl cells onto a pre-warmed selective plate.
9. Incubated plates overnight at 37°C.

Results:

Observed on 7/26/19:

All 7 plates had colony growth. mCherry 2 Transformed had the most growth while the puc19 plate had the least growth.

mCherry 1 Transformed

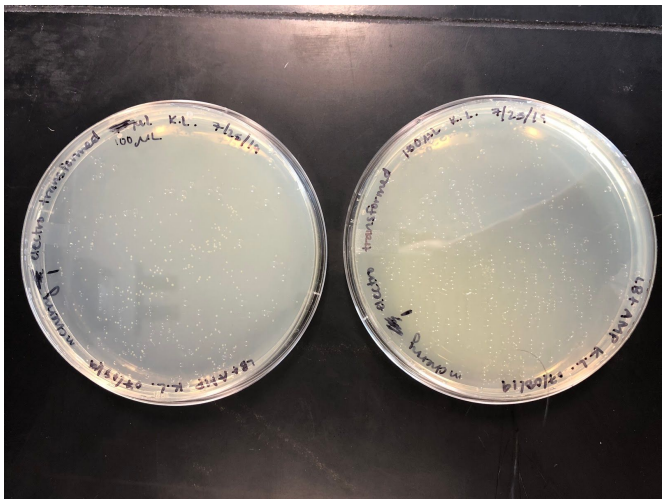

FIGURE 1. There were 2 mCherry 1 plates: 100 uL and 150 uL.

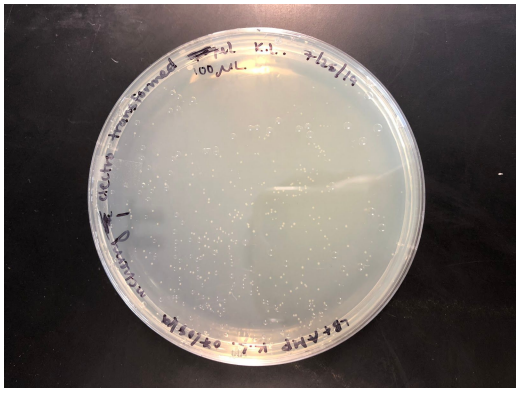

FIGURE 2. A closer picture of mCherry 1 Transformed 100 uL.

mCherry 2 Transformed

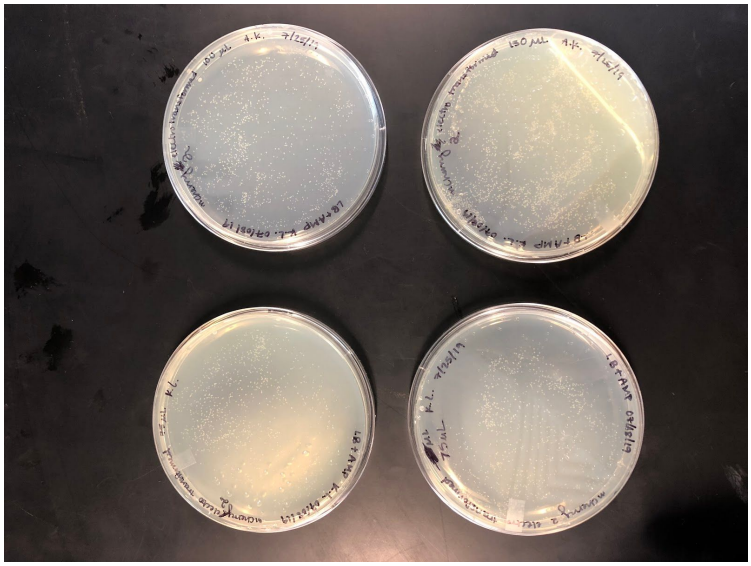

FIGURE 3. There were 4 mCherry 2 plates: 75 uL, 75 uL, 100 uL, and 150 uL.

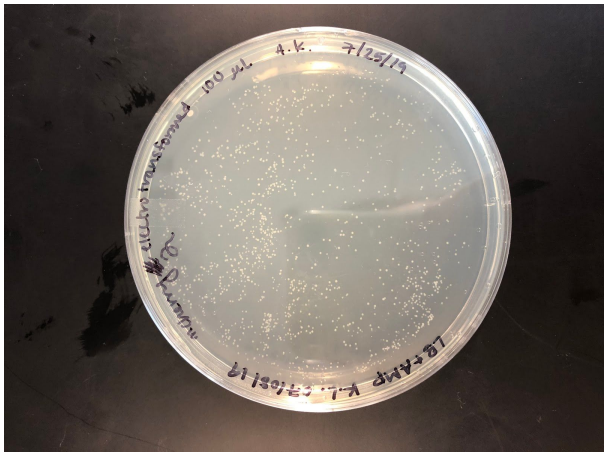

FIGURE 4. A closer image of mCherry 2 Transformed 100 uL.

Puc19 Control 100 uL

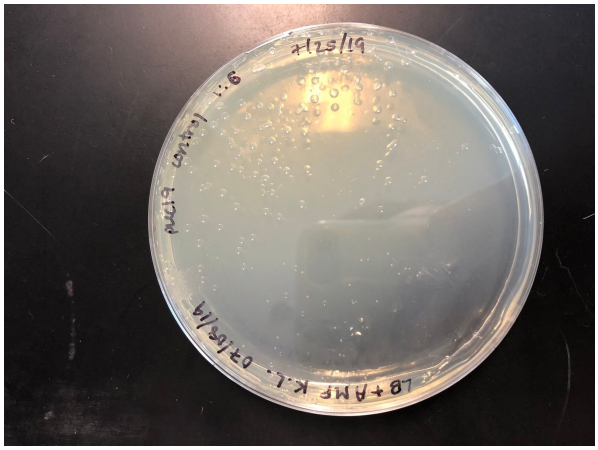

FIGURE 5. puc19 control 1:6 dilution 100 uL.

Conclusion:

We did 3 transformations, two mCherrys and one puc19, using electroporation competent cells. mCherry 1 Transformation was a bit flawed as the SOC media was not immediately added, so we did mCherry 2 Transformation. puc19 diluted 1:6 was used as a control. After plating, we had to wait for the results tomorrow.

Observed on 7/26/19:

As expected, mCherry 2 grew more colonies because the SOC media was not immediately added to mCherry 1. mCherry 150 uL had the most colonies while puc19 had the least most likely due to the puc19 being too diluted, but there was still growth.

Name: Chiara, Sijia Qin, Jiazi Tian
Date:7/25/19

Goals:

1. Mi**D**i prep pcb302 in E. Coli

Protocol:

QIAGEN Plasmid Midi Kit

1. Separated 100 mL of bacterial overnight culture into 5 separate 50 mL falcon tubes and centrifuged at **5,000 rpm** for 15 minutes at 4°C.
2. Poured out supernatant.
3. Added 4 mL of Buffer P1 to one tube, pipet mixed, and transferred to another tube. Mixed and transferred contents to the next tube with pelleted cells. Repeated until all tubes are combined.
4. Added 4 mL of Buffer P2 to the tube containing 4 mL of Buffer P1 and the combined resuspended pelleted cells. Inverted 6 times.
5. Incubated at room temperature for 5 minutes.
6. Added 4 mL of Buffer P3 and vigorously inverted 6 times.
7. Incubated on ice for 15 minutes.
8. Centrifuged at 20,000 x g at 4°C for 30 minutes.
9. After centrifuging, clear supernatant was transferred to another centrifuge tube while avoiding all of the flakes on the sides and in the solution. Used a syringe filter for this.
10. Centrifuged the tube again at 20,000 x g at 4°C for 15 minutes
11. While that ran, the QIAGEN-tip was equilibrated by adding 4 mL of QBT to the QIAGEN-tip.
12. Added the clear solution (from step 10) to the QIAGEN-tip and allowed it to enter the resin by gravity flow.
13. Next, 10 mL of Buffer QC was added to the QIAGEN-tip and allowed to gravity drip.
14. Once that passed through, 10 mL more of Buffer QC was added and allowed to flow through.
15. Then, 5 mL of Buffer QF was added and flowed through into a new tube.
16. Added 3.5 mL of room temperature isopropanol to elute the DNA and mixed. Then centrifuged at 15,000 x g for 30 minutes at 4°C.
17. Carefully removed the supernatant making sure not to disrupt the clear pellet.
18. Added 2 mL of room-temperature 70% ethanol and centrifuged for 10 minutes at 15,000 x g at 4°C. Discarded the supernatant leaving as little liquid behind as possible, being careful not to disrupt the clear pellet.

19. Air-dried the pellet overnight.

Conclusion:

Since the samples need to air-dry for several hours, we put them in the hood overnight to measure the concentration tomorrow.

Name: Chiara

Date: 7/25/19

Goal:

1. Send parts for sequencing
    a. K1357009
    b. J23102

Protocol:

1. J23102
    a. Diluted sample 6 from 6/13/19 minipreps by mixing 4 µL of sample with 8 µL of DI water to reach a concentration of about 100 ng/µL
2. K1357009
    a. Diluted sample 150 µL from 7/24/19 midipreps by mixing 5 µL of sample with 5 µL of DI water to reach a concentration of about 85 ng/µL