Name: Rehmat, Kennex, Chiara, Shakera
Date: 8/15/19

Goal:

1. Linearize the Dino III plasmids
2. Run gel electrophoresis on mCherry PCR
3. Run a gel overnight for each plasmid
4. Start overnight cultures for characterization of Test Device 1, Test Device 5, Positive Control, Negative Control colony 1 and 2 for each in LB, TB, YM, and YPD media
5. Miniprep on overnight mcherry cultures
6. Glycerol Stock mCherry Overnights
7. Glycerol stock of pcb302 from 8/12/19
    a. Papers A & B
8. Gel extraction from kit and using Whattman paper for kit plate parts (promoter, RBS, coding, terminator)

Name: Kennex Lam
Date: 8/15/19

Goal:

1. Make more overnights from the mCherry overnights that grew.

Protocol:

1. 250 uL of chloramphenicol was added into a flask with 250 mL of LB media.
2. 20 mL of the Chloramphenicol + LB media was added into 9 Falcon tubes.
3. 1 mL of individual overnight was added into the larger, new overnights.
4. Shook overnight at 37°C.

Results:
All the overnights grew.

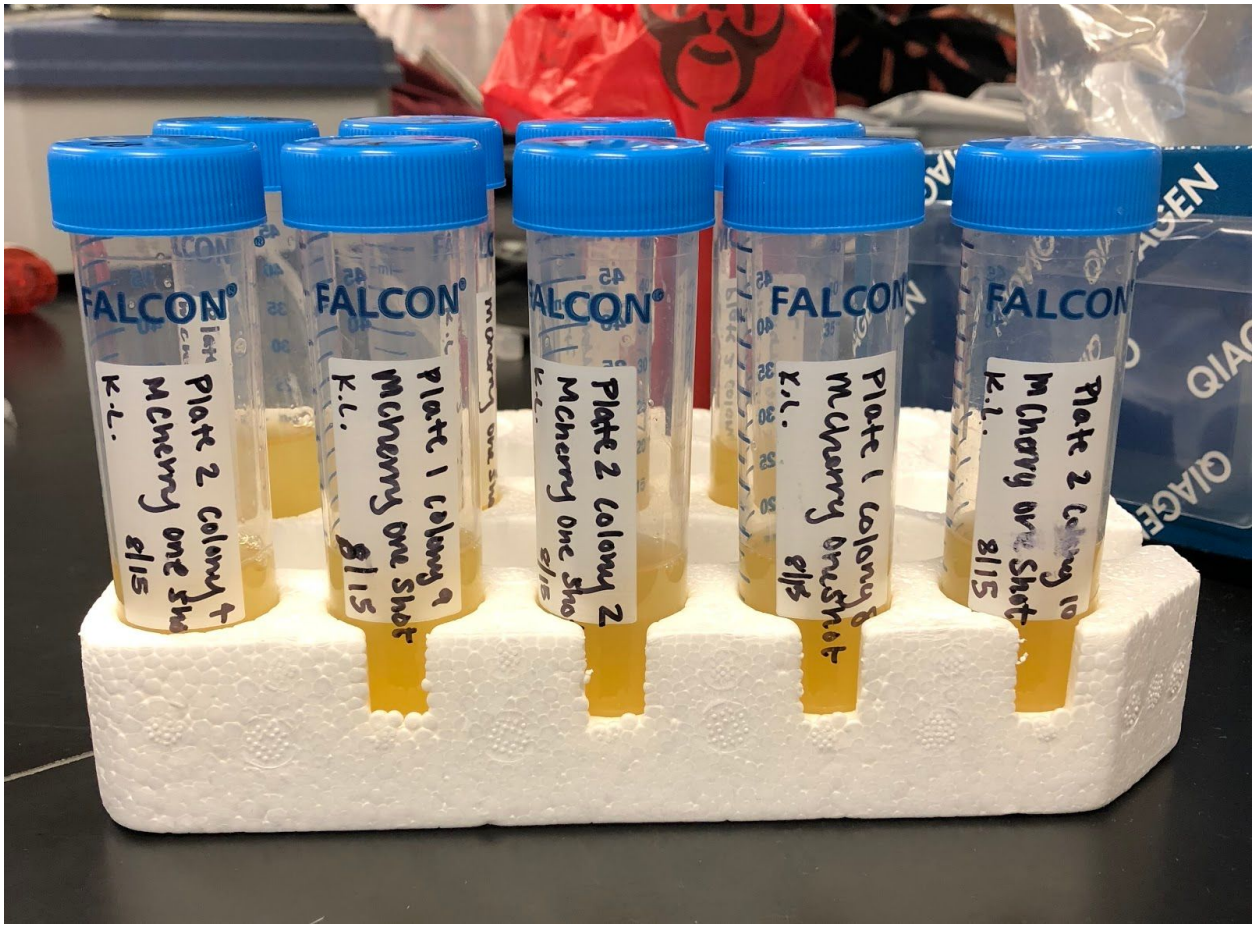

FIGURE 1. Overnights for mCherry Transformed with One Shot Plate 1 Colonies 3, 8, 9 and Plate 2 Colonies 2, 4, 5, 6, 9, 10.

Conclusion:

We will do a miniprep on these overnights tomorrow. After shaking these overnights for about 2 hours (let cells grow a bit), we added IPTG to aid in the fluorescence.

Name: Kennex, Shakera
Date: 8/15/19

Goal:

1. Miniprep on overnight mcherry cultures

Protocol:

QIAprep Spin Miniprep Kit Protocol

a. Centrifuged 3 mL of bacterial overnight culture in three separate Eppendorf tubes (1.5 mL, 1.5, 1.0 in each) at 8,000 rpm for 3 minutes at room temperature.
b. Discarded the supernatant and resuspend pelleted bacterial cells in one tube with 250 µL Buffer P1 and transfer to the other and resuspend until one eppendorf tube contains the pelleted cells resuspended in 250 µL Buffer P1.
c. Add 250 µL of Buffer P2 and invert 5 times.
d. Add 350 µL of Buffer N3 and immediately mix by inverting 5 times.
e. Centrifuge for 10 minutes at 13,000 rpm.
f. Micropipette 800 µL of the clear supernatant into a spin column and centrifuge for 60 seconds and discard the excess liquid.
g. Add 500 µL of PB and centrifuge the spin columns for 60 seconds. Discard the flow through.
h. Add 750 µL of PE to the spin columns, centrifuge for 60 seconds, and discard the flow through.
i. Centrifuge the spin columns again for 60 seconds to remove residual wash buffer and discard the flow through.
j. Transfer the spin columns to a clean eppendorf tube and add 50 µL of EB to the center of the spin column to elute the DNA.
k. Allow the spin column to stand for one minute and then centrifuge for one minute.
l. Record the concentrations for each sample.

Results:

Samples	DNA Concentrations
Plate 1 Colony 3	6 ng/uL
Plate 2 Colony 6	2.5 ng/uL
Plate 2 Colony 5	0.5 ng/uL
Plate 1 Colony 9	2.5 ng/uL

Plate 2 Colony 4	5 ng/uL
Plate 2 Colony 10	1.5 ng/uL
Plate 2 Colony 2	3 ng/uL
Plate 1 Colony 8	3 ng/uL
Plate 2 Colony 9	2.5 ng/uL

Conclusion:
Because the concentrations are too low, we are unable to send mCherry out for sequencing.

Name: Kennex
Date: 8/15/19

Goal:

1. Glycerol Stocks on mCherry Minipreps

Protocol:

1. 1 mL of overnight culture + 1 mL of 50% glycerol was placed into 9 cryotubes.
2. Tubes were placed in -80°C freezer.

Results:
Only Plate 1 Colony 3, 8, 9 and Plate 2 Colony 2, 4, 5, 6, 8, 9, 10 were preserved.

Conclusion:
Because the miniprep concentrations were so low, do we really need the glycerol stocks?

Name: Chiara

Date: 8/15/19

Goal:

1. Glycerol stock of pcb302 from 8/12/19
    a. Papers A & B

Protocol:

Glycerol Stocks

1. Took 1 mL of 50% glycerol and 1 mL of the overnight culture (after incubation) and added to a glycerol stock tube.
2. Labelled as pcb302 in E. Coli glycerol 8/15/19, Paper-identifying letter & colony #, C.B. and stored in the -80° C freezer in CLSO 442

Name: Rehmat

Date: 8/15/19

Goal: Linearize the two Dino III plasmids using EcoRI

Protocol:

1. Combined 100 µL of diH2O, 25 µL of EcoRI, 25 of Fast Digest Buffer, and 100 µL of Dino III GFP mini prep DNA at 260 ng/µL in one eppendorf tube.
2. Combined 100 µL of diH2O, 25 µL of EcoRI, 25 of Fast Digest Buffer, and 100 µL of Dino III RFP mini prep DNA at 180 ng/µL in another eppendorf tube.
3. Incubated at 37°C for 2-3 hours.

Name: Rehmat

Date: 8/15/19

Goal: Run a gel for gel extractions on the linearized Dino III plasmids

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 15 µL of the GeneRuler 1kb Plus ladder in the first well .
2. Prepared samples to load by adding in 50 µL of 6X Loading dye to the 250 µL digest reactions for each plasmid.
3. Loaded 50 µL of the sample into the wells.

Running

1. Ran for overnight at 34 volts.

Name: Rehmat and Chiara

Date: 8/15/19

Goal: Gel extraction for the kit plate parts (Promoter, RBS, coding, terminator)

Protocol:

Did two methods of gel extractions for each part, the Qiagen Quick Gel Extraction Kit and a method using the Whattman paper to bind the DNA from the agarose gel.

1. **Qiagen Quick Gel Extraction Kit**
    a. Excised the bands from the gel and weighed the gel fragments
        i. RBS: 130 mg
        ii. Coding 110 mg
        iii. Terminator 160 mg
    b. Added 390 µL Buffer QG to the RBS gel fragment, 330 µL Buffer QG to the coding gel fragment, 480 µL to the terminator gel fragment
    c. Allowed tubes to incubate at 52°C and vortexed periodically until dissolved
    d. Added 130 µL isopropanol to the RBS, 110 µL isopropanol to the coding, and 160 µL isopropanol to the terminator
    e. Added solution to a spin column and centrifuged for 1 minute and discarded the flow through
    f. Added 500 µL of Buffer QG to each spin column, centrifuged for 1 minute, and discarded flow through
    g. Added 750 Buffer PE to each column, centrifuged for 1 minute and discarded flow through
    h. Centrifuged spin columns again for 1 minute to remove residual buffer.
    i. Added 50 µL of warmed EB to each column and allowed to sit for 1 minute and centrifuged for 1 minute, collected flow through in a clean eppendorf tube.
2. **Whattman Paper Gel Extraction**
    a. Cut slits in gel in front of (towards positive cathode) the desired DNA band for extraction
    b. Ran the gel for about 10 to 20 more minutes to allow the DNA to bind to the paper
    c. Removed the paper and placed it in a syringe all the way into the bottom
    d. Added 50 µL of warmed water and centrifuged for 1 minute, collected flow through in a clean 15 mL falcon tube.

Results

Qiagen Quick Gel Extraction

RBS	7.5 ng/μL
Coding	7.5 ng/μL-
Terminator	7.5 ng/μL

Whattman Paper

Promoter
RBS
Coding
Terminator

Name: Kennex
Date: 8/15/19

Goal:

1. Gel Electrophoresis on mCherry PCR

Protocol:

Preparing, Loading, and Running a 1% Agarose Gel for PCR on mCherry

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 GelRed 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

Gel 1:

1. Loaded 10 µL of the GeneRuler 1kb Plus ladder in the first well .
2. Loaded 8 uL of mCherry Plate 1 Colonies 1-10 in lanes 2-11.
3. Loaded 8 uL of mCherry Plate 2 Colonies 1-2 in lanes 13-14.

Gel 2:

1. Loaded 10 µL of the GeneRuler 1kb Plus ladder in the first well .
2. Loaded 8 uL of mCherry Plate 2 Colonies 3-10 in lanes 2-9.

Running

1. Ran for 2 hours at 90 volts.

Results:

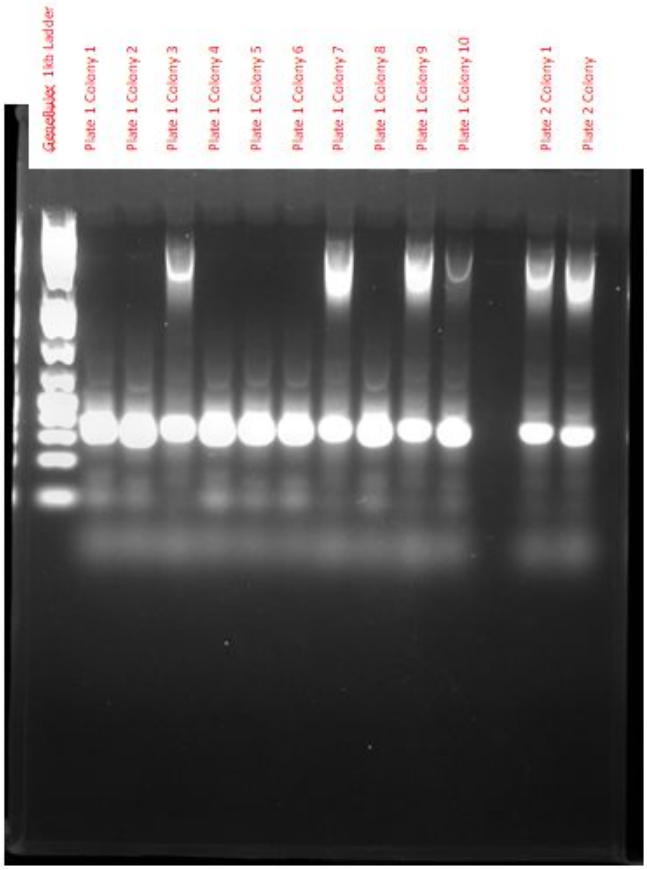

FIGURE 1. Gel 1 of mCherry PCR.