Name: Laura Das Neves, Jiazi Tian, Sijia Qin, Chiara Brust
Date: 7/23/19

Goals:

1. M..... Sike, we're doing a miniprep
    a. K1357009 from glycerol stocks of unknown date
    b. Codon-optimized-RFP from glycerol stocks of unknown date
2. Run gel electrophoresis of restriction digest
    a. DinoIIIP2
    b. Codon-optimized-RFP
3. Gel extraction
    a. Remove GFP from DinoIIIP2
    b. Isolate RFP from codon-optimized-RFP
4. Overnights in 100 mL cultures
    a. K1357009 from glycerol stocks of unknown date
    b. Codon optimized RFP from glycerol stocks of unknown date
    c. Pcb302 in E. Coli from glycerol stocks made on 7/3/19

Name: Chiara, Laura
Date: 7/23/19

Goal:

1. Miniprep
    a. K1357009
    b. Codon-Optimzed-RFP

Protocol:

QIAprep Spin Miniprep Kit Protocol

a. Centrifuged 5 mL of bacterial overnight culture in falcon tubes at 8,000 rpm for 3 minutes at 4 degrees Celcius
    i. Was supposed to be at room temperature
b. Discarded the supernatant and resuspended pelleted bacterial cells in one tube with 250 µL Buffer P1.
c. Added 250 µL of Buffer P2 and inverted 5 times.
d. Added 350 µL of Buffer N3 and immediately mixed by inverting 5 times.
e. Centrifuged for 10 minutes at 13,000 rpm.
f. Micropipetted 800 µL of the clear supernatant into a spin column and centrifuged for 60 seconds and discarded the excess liquid.
g. Added 500 µL of PB and centrifuged the spin columns for 60 seconds. Discarded the flow through.
h. Added 750 µL of PE to the spin columns, centrifuged for 60 seconds, and discarded the flow through.
i. Centrifuged the spin columns again for 60 seconds to remove residual wash buffer and discarded the flow through.
j. Transferred the spin columns to a clean eppendorf tube and added 50 µL of EB to the center of the spin column to elute the DNA.
k. Allowed the spin column to stand for one minute and then centrifuged for one minute.
l. Recorded the concentrations for each sample.

Results:

Sample	**[DNA] (ng/μL)**	**260/280**
K1	22.5	1.8
K2	25	2
K3	20	2
K4	20	1.6
K5	17.5	3.5
K6	17.5	2.33
K7	22.5	3
K8	17.5	3.5
K9	40	1.778
RFP 2	50	1.818
RFP 4	47.5	2.111
RFP 5	55	2.000
RFP 6	42.5	2.125
RFP 7	62.5	1.923
RFP 8	57.5	2.091
RFP 9	55	1.750
RFP 10	47.5	1.9

Conclusion:

None of the samples had a high enough concentration to perform a successful gel extraction. We will do midipreps tomorrow.

Name: Sijia Qin, Jiazi Tian
Date:7/23/19

Goal:

1. Make 100ml overnight cultures for codon optimized RFP, K1357009 and pcb302

Protocol:

Overnight cultures for Codon optimized RFP from 7/11/19, sample 3&7&10

1. Added 100ml LB medium and 100ul ampicillin into a flask.
2. Used 10ul pipette tip to dip the glycerol stock of these samples.
3. Incubated at 37 degrees C for 24 hours .

Overnight cultures for K1357009 from 7/18/19 100ul 2&150ul 2

1.Added 100ml LB medium and 100ul chloramphenicol into a flask.
2.Used 10ul pipette tip to dip the glycerol stock of these samples.
3.Incubated at 37 degrees C for 24 hours.

Overnight cultures for PCB302 from 7/3/19 75ul 7&150ul 1&150ul 3

1.Added 100ml YM medium and 100ul kanamycin into a flask.
2.Used 10ul pipette tip to dip the glycerol stock of these samples.
3.Incubated at 28 degrees C for 48 hours.

Results:
N/A

Conclusion:
N/A

Name: Chiara
Date: 7/23/19

Goal:

1. Gel electrophoresis of restriction digests on:
    a. DinoIII-GFP P2
    b. Codon-optimized RFP

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

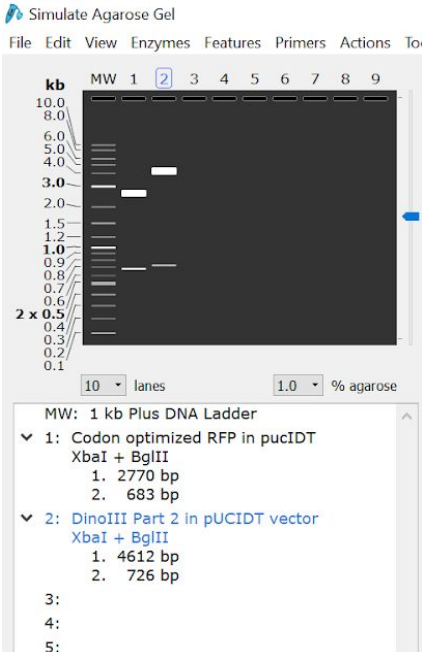
Loading

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

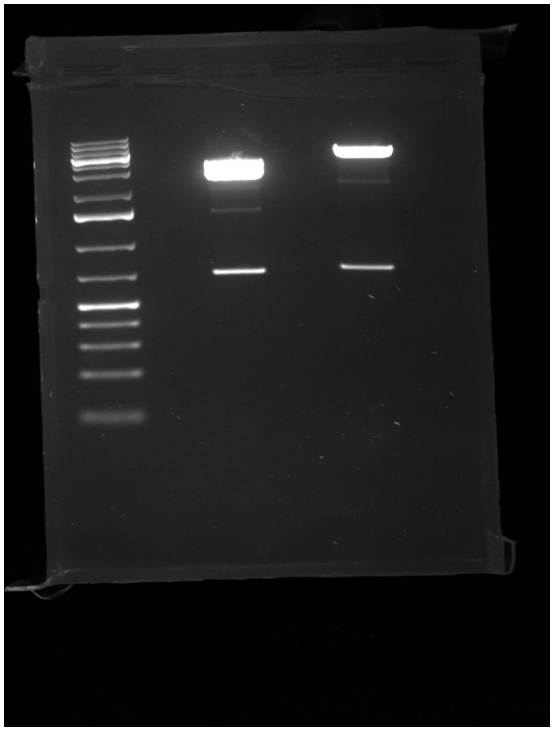
Running

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 1 hour and 35 minutes at 94 V

Expected Results:



Results:



Gel Key:

Lane #	**Sample**
1	MW 1 Kb Plus DNA Ladder
2	Blank
3	Codon-Optimized-RFP
4	Blank
5	DinoIIIP2

Conclusion:

The digest was successful. We will now do a gel extraction.

Name: Chiara
Date: 7/23/19

Goal:

1. Gel extraction of DinoIII-GFP P2 and codon optimized RFP

Protocol:

Gel Extraction

Qiagen QIAEX II Gel Extraction Kit

1. Ran a restriction digest on the targeted DNA part using restriction enzymes and ran an agarose gel
2. Cut the targeted DNA sequence out using a razor blade and UV imager, made sure to get as much DNA while limiting the amount of agarose extracted
3. Pre-weighed empty Eppendorf tubes before adding the gel exicisions.
4. Added the gel extracts to the Eppendorf tubes and weighed again.
5. Calculated the mass of the gel using the difference of the two measurements.
    - DinoIIIP2= 141.1 mg
    - RFP= 122.4 mg
6. Multiply the mass by a factor of 3 to get the volume of Buffer QX1 needed.
    - DinoIIIP2= 423.3 µL
    - RFP= 367.2 µL
7. Added the respective amounts of Buffer QX1 to each of the tubes
8. Added 30 µL of QIAEX II to the samples.
    a. Vortexed QIAEX II before using
9. Incubated the tubes at 50° C for 14 minutes and vortex every 2 minutes to help dissolve the gel
    a. Checked to make sure the color of the mixture is yellow
10. Once dissolved, centrifuged for 30 seconds and carefully removed the supernatant.
11. Added 500 µL of Buffer QX1 to the tubes and resuspended by vortexing
12. Resuspended the pellet in 500 µL of Buffer PE and centrifuged for 30 seconds. Removed supernatant and repeated this step.
13. Air dried pellet for 30 minutes
14. Eluted the DNA by adding 20 µL deionized water, vortexed it, and incubated at room temperature for 11 minutes.
15. Centrifuged for 30 seconds and pipetted the supernatant into a clean tube.
16. Measured and recorded the concentrations.

Results:

Sample	**[DNA]**	**260/280**
DinoIII GFP P2	12.5	2.5
Codon optimzed RFP	----	----

Conclusion:

Both gel extractions resulted in too low of concentrations. We should do midipreps on both DInoIII and codon optinized RFP and try the extraction again.