Name: Chiara Brust, Krithika Karunakaran
Date: 8/6/19

Goals:

1. Heat kill on restriction digest of Dino III mini preps from 7/17/19 samples #1-#10
2. Gel extraction on linearized Dino III with GFP
3. Transform Dino III with CO RFP into E. Coli OneShot chemically competent cells

Name: Chiara
Date: 8/6/19

Goal:

1. Heat kill on restriction digest of Dino III mini preps from 7/17/19 samples #1-#10

Protocol:

Heat Kill

1. Placed digested DNA in water bath at 65° C for 20 minutes

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Date: 8/6/19

Goal:

1. Gel extraction of linearized DinoIII with GFP minipreps from 7/17/19 samples # 1-10

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 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
6. Gel solidified
7. Changed the orientation of casting tray so the rubber sides were not in contact with the sides of the system.
8. Poured in 1X TBE into the gel electrophoresis system to the fill line, making sure to submerge the gel.

Loading

1. Loaded 5 µL of the GeneRuler 1kb Plus ladder in the first well .
2. Loaded 25 µL of each sample including 5 µL of loading dye, and skipped every other lane

Running

1. Ran for 1 hour and 15 minutes at 94 volts.

Gel Extraction

QIAQuick Gel Extraction Kit Lot # 42141174

1. Ran a restriction digest on the targeted DNA part using restriction enzymes yesterday and ran an agarose gel for 1 hour before starting
2. Cut the targeted DNA sequence out using a razor blade, making 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.
6. The DinoIII fragments weighed _____ mg.
7. Added 3 times of the weight of the gel of Buffer QG (ex if gel weighs 100 mg, add 300 µL of Buffer QG).
    May have to transfer to a 15 mL falcon tube if the volume exceeds 1.5 mL
8. Incubated the tubes at 50° C for 10 minutes and vortexed every 2 minutes to help dissolve the gel
    a. Checked to make sure the color of the mixture is yellow
9. Once dissolved, added 1X of isopropanol to the Dino III fragment. (if the gel weighs 100 mg add 100 µL).
10. Placed a QIAquick spin column in a provided 2 mL collection tube.
11. Added 700 µL of the solution to the spin column at a time and centrifuged at 13,000 rpm for 1 minute and discarded the flow through. Repeated until all of the solution had ran through.
12. Added 500 µL of Buffer QG to the spin columns to remove traces of agarose and centrifuged for 1 minute.
13. Added 750 µL of Buffer PE to the column to wash and centrifuged for 1 minute.
14. Discarded the flow through and centrifuged for an additional 1 minute at 13,000 rpm to remove residual buffer.
15. Placed the spin column in a clean 1.5 mL Eppendorf tube
16. Added 40 µL of warmed Buffer EB to the center of the spin column, allowed to sit for 2 minutes, and centrifuged for 1 minute.
17. Measured and recorded the concentrations.

Results:

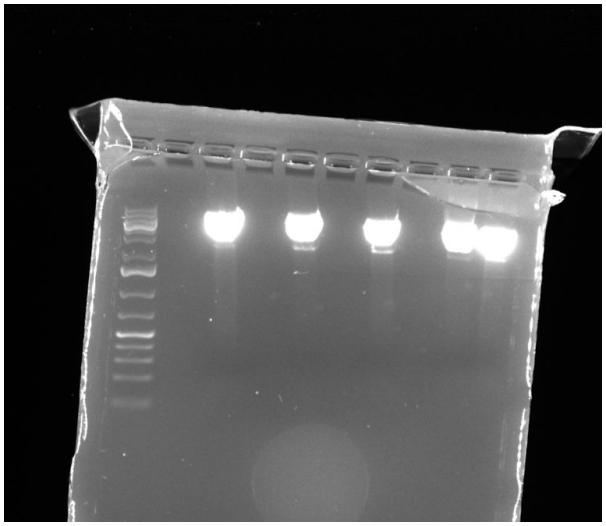
Gel 1 Key

Lane #	**Sample**
1	1 Kb Plus DNA MW ladder
2	Blank
3	1
4	Blank
5	2
6	Blank
7	3
8	Blank
9	4
10	5

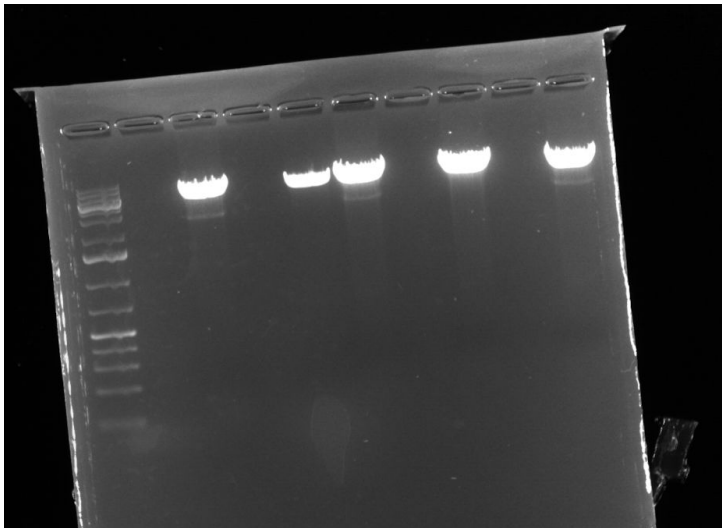
Gel 2 Key

Lane #	**Sample**
1	1 Kb Plus DNa MW ladder
2	Blank
3	6
4	Blank
5	7
6	8
7	Blank
8	9
9	Blank
10	10

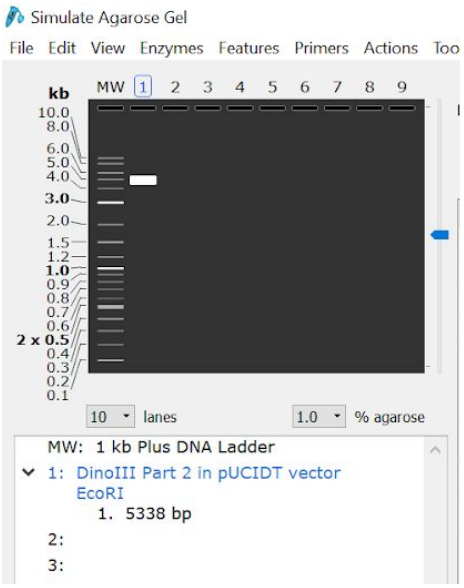
Gel 1



Gel 2



Expected Results:



DNA Concentration

Sample	[DNA] (ng/µL)	260/280
1	1.1	2.444
2	.55	3.667
3	.45	4.500
4	-.05	.2500
5	.55	3.667
6	.40	4.000
7	-.15	1.000
8	.45	4.500
9	.55	2.750
10	.15	---

Conclusion:

The gel extractions were not successful.

Name: Krithika Karunakaran
Date: 8/6/19

Goals:

1. Transform Dino III with CO RFP from 8/2/19 ligation into E. Coli OneShot chemically competent cells

Protocol:

Heat Shock Transformation

1. OneShot chemically competent cells were thawed on ice
2. 2 µL of Dino III with CO RFP was added into competent cells
3. The cells were incubated on ice for 35 minutes.
4. After the ice incubation, the samples were placed into a 42° C water bath for 30 seconds.

Transformation Plating

5. Immediately after the heat shock, 250µL of SOC medium was added to the sample
6. The samples were placed in a 37° C shaking water incubator for 1 hour at 200 rpm.
7. After shaking for 1 hour, 150 µL of the solution was streaked onto an ampicillin plate
8. The plates were then placed in the incubator at 37°C for at least 24 hours.