Name: Kennex, Rehmat, Asma, Yujie Huang, Jiayi Lan, Xuecheng Ye
Date: 8/2/19

Goal:

1. Run a gel on the PCR products from yesterday
2. Run a gel and do gel extraction on the digested codon optimized RFP
3. Ligation of RFP and DinoIII plasmid
4. Ligation for composite part

Name: Yujie Huang, Jiayi Lan, Xuecheng Ye
Date: 8/2/19

Goal:

1. Run a gel on the PCR products from yesterday

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 5 µL of the GeneRuler 1kb Plus 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. Ran for 1 hour at 100 volts.

Results:

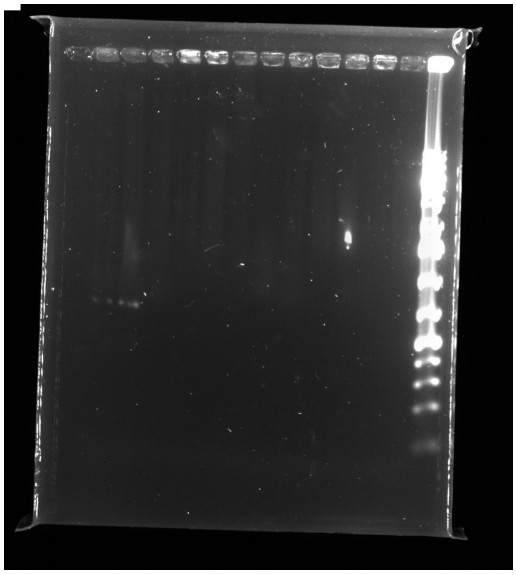


Figure 1: from right to left is ladder, 1-12

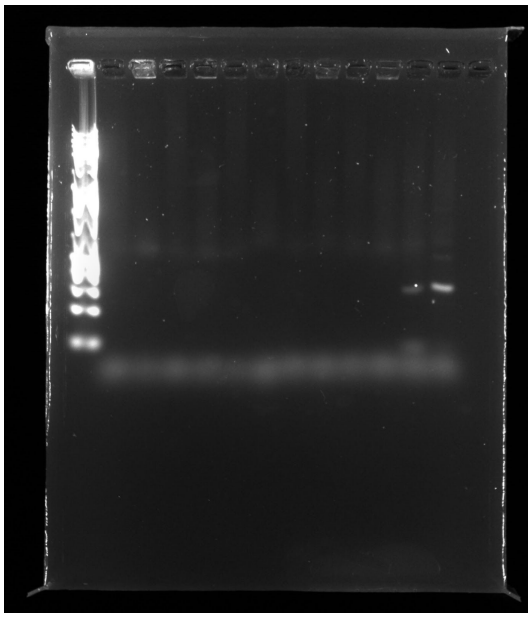


Figure 2: from left to right is ladder, 13-24

Name: Kennex, Asma
Date: 8/2/19

Goal:

1. Gel Extraction of RFP Midiprep #3 and #7 from 7/24 and RFP Miniprep #10 from 7/11

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. 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.

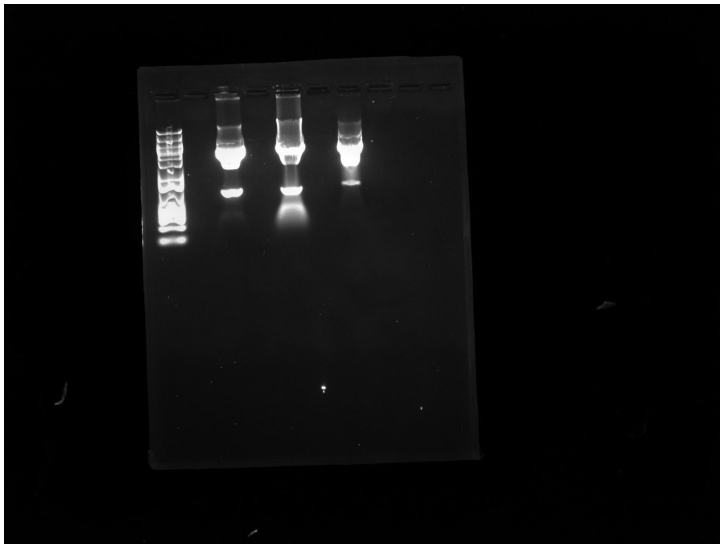
Loading

1. Loaded 10 µL of the GeneRuler 1kb Plus ladder in the first well .
2. Loaded 30 µL of digested RFP #3 + 6 uL of Purple Loading Dye into third well.
3. Loaded 30 uL of digested RFP #7 + 6 uL of Purple Loading Dye into fifth well.
4. Loaded 30 uL of digested RFP #10+ 6 uL of Purple Loading Dye into seventh well.

Running

1. Ran for 1 hour at 100 volts.

Results:



Gel Extraction

QIAQuick Gel Extraction Kit Lot # 42141174

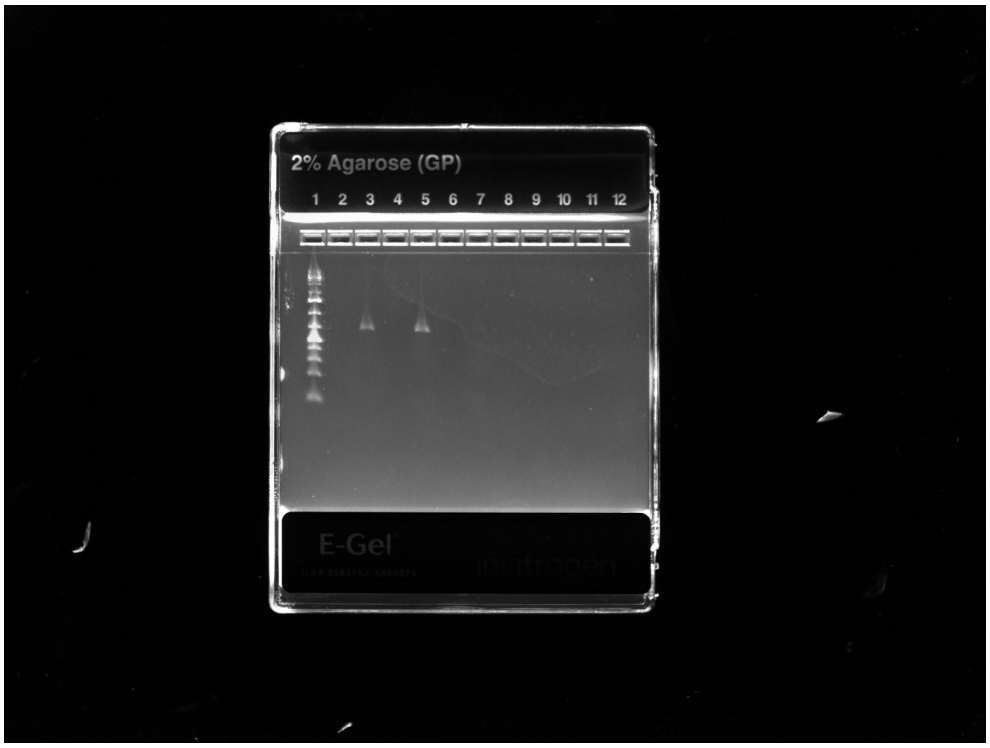
1. Ran a restriction digest on the targeted DNA part using restriction enzymes yesterday and ran an agarose gel for 1 hour before starting
2. Cut the targeted DNA sequence out using a razor blade, making sure to get as much DNA while limiting the amount of agarose extracted
3. Pre-weighed empty Eppendorf tubes before adding the gel exicisions.
4. Added the gel extracts to Eppendorf tubes and weighed again.
5. Calculated the mass of the gel using the difference of the two measurements.
6. The RFP fragment 3 weighed 350 mg, RFP fragment 7 weighed 330 mg, and the RFP fragment 10 weighed 400 mg.
7. Added 1050 µL of Buffer QG to the RFP fragment 3, 990 µL to the RFP fragment 7, and 1200 µL to the RFP fragment 10.
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 350 µL of isopropanol to the RFP fragment 3, 330 µL to the RFP fragment 7, and 400 µL to the RFP fragment 10, and mixed.
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 1 minute, and centrifuged for 1 minute.
17. Measured and recorded the concentrations.

Results

RFP 3	12.5 ng/µL
RFP 7	10 ng/µL
RFP	5 ng/µL

Lane 1: 1 KB Plus Ladder
Lane 2: Empty
Lane 3: RFP 3
Lane 4: Empty
Lane 5: RFP 7
Lane 6: Empty
Lane 7: RFP 10



Name: Rehmat, Kennex, Asma
Date: 8/2/19

Goal:

1. Ligate the DinoIII plasmid and the codon optimized RFP

Protocol:
Ligation

1. Added 6 µL of diH2O to a clean 1.5 mL Eppendorf tube, 1µL of T4 DNA Ligase Buffer, 1µL of the Dino III plasmid and 1µL of the codon optimized RFP part and mixed.
2. Added 1µL of T4 DNA Ligase.
3. Pipet mixed the tube and incubated at room temperature for 10 minutes.

Conclusion:
We will transform this ligation into DH5alpha E. Coli cells to see if our ligation worked.