Name: Kennex, Jiayi Lan, Xuecheng Ye, Amirah, Rehmat
Date: 8/1/19

Goal:

1. Gel extraction of DinoIII
2. Transformation on mCherry
3. Colony pCR for pCB302 using gfp primers and primers 3 and 4
4. PCR of source DNA for pCB302 using gfp primers and primers 3 and 4
5. Run E-gel on gel extraction product
6. Restriction digest on RFP for ligation

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 10 µL of the GeneRuler 1kb Plus ladder in the first well .
2. Loaded 30 µL of the digested DinoIII into third well.
3. Loaded 30 uL of digested RFP optimized codon into fifth well.

Running

1. Ran for 1 hour at 100 volts.

Results:

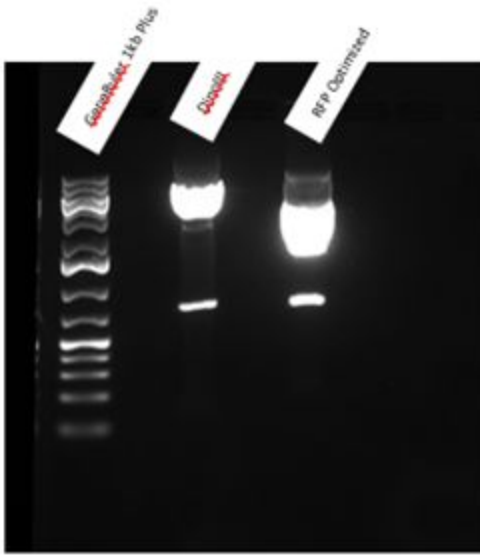
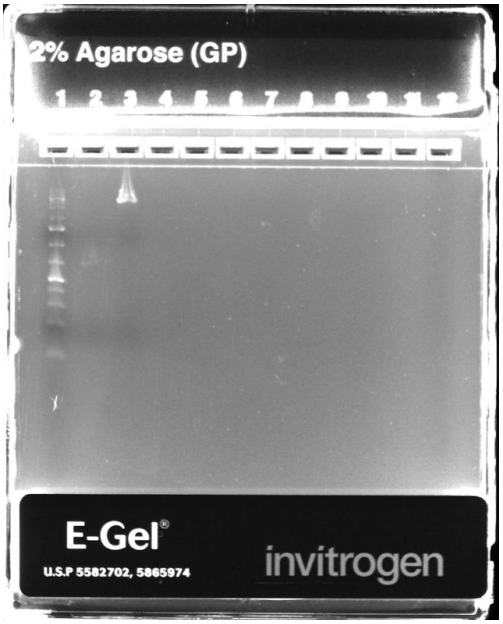


FIGURE 1. Gel image of digested RFP and DinoIII.
Lane 1: 1 KB Plus Ladder

Lane 2: Empty
Lane 3: Dino III with GFP digested with XbaI and BglII
Lane 4: Empty
Lane 5: Codon Optimized RFP in pUC vector digested with XbaI and BglII



Lane 1: 1 KB Plus Ladder
Lane 2: Codon optimized RFP gel extraction product
Lane 3: Dino III gel extraction product

FIGURE 3. E-Gel Image of Gel Extracted RFP and DinoIII.

Conclusion:
The concentrations were relatively low but when we ran the gel extraction products on an E-gel, the band for the DinoIII was relatively bright. We will redo the gel extraction for the RFP and perhaps combine multiple samples and do a precipitate reaction to increase the yield and then ligate using that.

Name: Kennex, Rehmat
Date: 8/1/19

Goal:

1. Gel extraction of DinoIII Miniprep #4 from 7/17 and RFP Codon Opt. Midiprep #3 from 7/24

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 fragment weighed 560 mg and the RFP fragment weighed 380 mg.
7. Added 1680 µL of Buffer QG to the Dino III fragment and 1140 µL to the RFP fragment.
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 560 µL of isopropanol to the Dino III fragment and 380 µL to the RFP fragment 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.

Dino III	40 ng/µL

RFP	5 ng/μL

Name: Jiayi Lan and Xuecheng Ye
Date: 8/1/19

Goal:

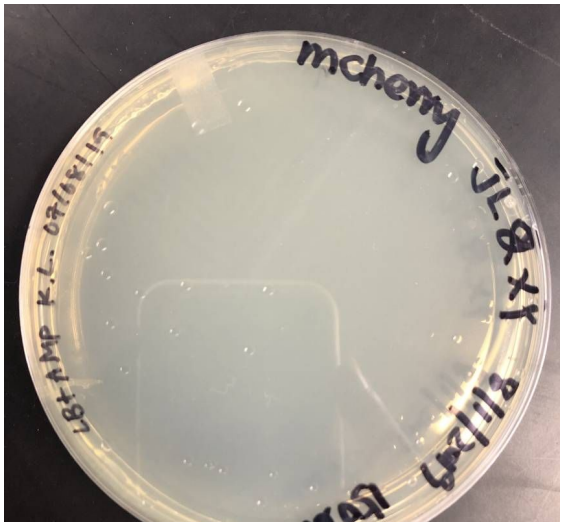
1. Transformation of mCherry with One Shot™ BL21 Star™ (DE3) Chemically Competent E. coli

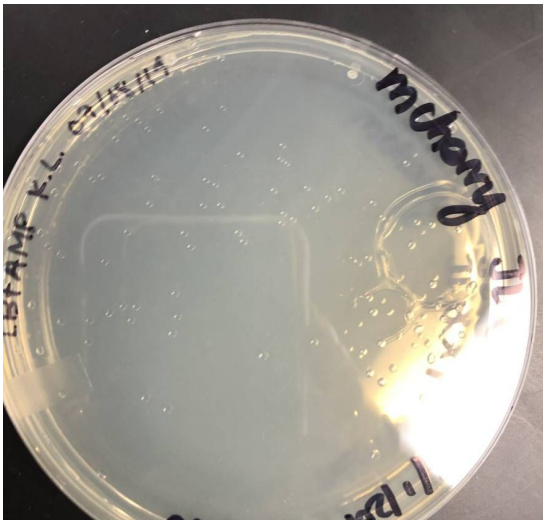
Protocol:

Transformation using One Shot BL21 Star (DE3) Cells

1. One vial of One Shot® cells was thawed on ice for one transformation.
2. Added 1 uL of 66ng/uL of DNA, in a volume of 5 µL to the cells and mixed by tapping gently. Did not mix cells by pipetting.
3. Incubated the vial on ice for 30 minutes.
4. Heat shocked the cells by incubating the vial(s) for exactly 30 seconds in the 42°C water bath. Did not mix or shake.
5. Removed the vial(s) from the 42°C bath and quickly placed on ice.
6. Added 250 µL of pre-warmed SOC medium to the vial(s). (SOC is a rich medium; used proper sterile technique to avoid contamination.)
7. Placed in a shaking incubator, and shook the vial(s) at 37°C for 1 hour at 225 rpm. **Deviation**: 300 rpm.
8. Plated two different volumes of the transformation reaction onto LB plates containing the appropriate antibiotic for plasmid selection. Included 34 µg/mL chloramphenicol if using BL21(DE3)pLysS or BL21(DE3)pLysE cells. Selected two volumes ranging from 20–200 µL to ensure well-spaced colonies on at least one plate. We selected two volume, 100µL and 150µL. The remaining transformation reaction was stored at 4°C and plated out the next day, if needed.
9. Inverted the plates and incubated at 37°C overnight.

Results:





Conclusion:

The cells didn't grow.

Name: Amirah
Date: 8/1/19

Goal:

1. Gel extraction of DinoIII Miniprep #4 from 7/17 and RFP Codon Opt. Midiprep #3 from 7/24

Protocol:

PCR Protocol

20 µL Reaction

1. Prepared a 20X PCR concentration cocktail with the following proportions: 7 µL of diH2O, 10 µL PCR Mastermix, 1 µL of primer 3, and 1 µL of primer 4.
2. Add 19 µL of the concentration cocktail into a PCR tube along with 1 µL of the source pCB302 DNA.
2. Place PCR tube in the thermocycler at the following settings:
    1. 95° C for 3:00 minutes
    2. 95° C for 1:00 minute
    3. 49° C for 1:00 minute
    4. 72° C for 1:00 minute
    5. 30X (Go to Step 2)
    6. 72° C for 5:00 minutes

    Lid Temperature: 105° C

Colony PCR Protocol

20 µL Reaction

1. Prepared a 20X PCR concentration cocktail with the following proportions: 7 µL of diH2O, 10 µL PCR Mastermix, 1 µL of the gfp rev primer, and 1 µL of the gfp fwd primer.
2. Added 19 µL of the concentration cocktail into a PCR tube.
3. Using a 10 µL micropipette, touched the tip onto the glycerol stock and put a piece of ice the PCR tube.
4. Placed PCR tube in the thermocycler at the following settings:
    1. 95° C for 3:00 minutes
    2. 95° C for 1:00 minute
    3. 47° C for 1:00 minute *Annealing temperature varies depending on primer
    4. 72° C for 1:00 minute
    5. 30X (Go to Step 2)
    6. 72° C for 5:00 minutes

    Lid Temperature: 105° C

- Primers used
    - Pcb302 gfp
        - Forward
        - Reverse
    - Primer 4
    - Primer 3

Pcr tube number	Primer used	Dna used
1	Pcb302 gfp	A1 150ul LB
2	Pcb302 gfp	A1 col5 250ul
3	Pcb302 gfp	2B col5 Na ul ym
4	Pcb302 gfp	2b col3 200ul ym
5	Pcb302 gfp	1b col1 400ul ym
6	Pcb302 gfp	1A colb 150 lb
7	Pcb302 gfp	2a col3 300ul ym
8	Pcb302 gfp	2A col1 200ul ym
9	Pcb302 gfp	1B col1 300ul ym kana
10	Pcb302 gfp	1B col1 400ym
11	Pcb302 gfp	Source pcb302 plasmid 1
12	Pcb302 gfp	Source pcb302 plasmid 2
13	Primers 3&4	A1 150ul LB
14	Primers 3&4	A1 col5 250ul
15	Primers 3&4	2B col5 Na ul ym
16	Primers 3&4	2b col3 200ul ym
17	Primers 3&4	1b col1 400ul ym
18	Primers 3&4	1A colb 150 lb
19	Primers 3&4	2a col3 300ul ym
20	Primers 3&4	2A col1 200ul ym

21	Primers 3&4	1B col1 300ul ym kana
22	Primers 3&4	1B col 400ul ym
23	Primers 3&4	Source pcb302 plasmid 1
24	Primers 3&4	Source pcb302 plasmid 2

Name: Kennex Lam, Rehmat Babar
Date: 8/1/19

Goal:

1. Restriction Digest on
    a. RFP Codon Opt. Midiprep #3 from 7/24
    b. RFP Codon Opt. Miniprep #10 from 7/24
    c. RFP Codon Opt. Midiprep #7 from 7/24

Protocol:

Restriction Digest Protocol

30 µL Fast Digest Restriction Digest

1. Prepare a Fast Digest concentration cocktail with the following proportions: 1 µL XbaI, 1 µL BglII, 8 µL of 10X Fast Digest Buffer, and 5 µL of diH2O.
2. Add 15 µL of this cocktail to a clean 1.5 Eppendorf tube and then add 15 µL of DNA.
3. Incubate at 37° C for 30 minutes.

Results:

Will run a gel tomorrow before the gel extraction.