Name: Laura das Neves, Chiara Brust, Rehmat Babar
Date: 7/9/19

Goal:

1. Run a gel on the PCR products of pCB302-gfp-MBD plasmid in E. Coli that came off of the filter papers 1 & 2
2. Transform codon optimized RFP into E. Coli
3. Transform Blue chromoprotein K592015 into E. Coli
4. PCR on pcb302 in A. Tume from papers 1 & 2 minipreps performed on 7/8/19
5. Run gel of pcb302 PCR in A. Tume from papers 1 & 2 from minipreps
6. Transform pcb302 into A. Tumefaciens from papers 1 & 2
7. Overnight cultures of pcb302 in A. Tume from papers 1 & 2 from glycerol stocks of minipreps done on 7/8/19

Name: Sijia Qin, Jiazi Tian
Date: 7/9/19

Goal:

Colony PCR on pcb302 in A. Tume overnight cultures from miniprep 7/8/19

Materials:

Dream Taq PCR mastermix (2x)
pcb302 from minipreps performed on 7/8/19

Protocol:

Colony PCR Protocol

20 µL Reaction

1. Prepared a PCR concentration cocktail with the following proportions: 7 µL of diH2O, 10 µL Dream Taq PCR Mastermix (2x), 1 µL of the forward primer, and 1 µL of the reverse primer.
   - 3 reactions with primers 1&2
     200ul, colony 2, **labeled as 1**
     200ul, colony 7, **labeled as 2**
     100ul, colony 3, **labeled as 3**
   - 3 reactions with primers 3&4
     200ul, colony 2, **labeled as 4**
     200ul, colony 7, **labeled as 5**
     100ul, colony 3, **labeled as 6**
   - 3 reactions with primers 1&4
     200ul, colony 2, **labeled as 7**
     200ul, colony 7, **labeled as 8**
     100ul, colony 3, **labeled as 9**
2. Added 19 µL of the concentration cocktail into a PCR tube.
3. Added 1 ul of pcb302 overnight cultures from miniprep 7/8/19.
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. **50° 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

Name: Laura Das Neves
Date: 7/9/19

Goal:

1. Run gel of PCR pcb302 in E. Coli from original papers

Materials:

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 were 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 your samples to load by adding in 1 µL of 6X Loading dye for every 5 µL of DNA and loaded

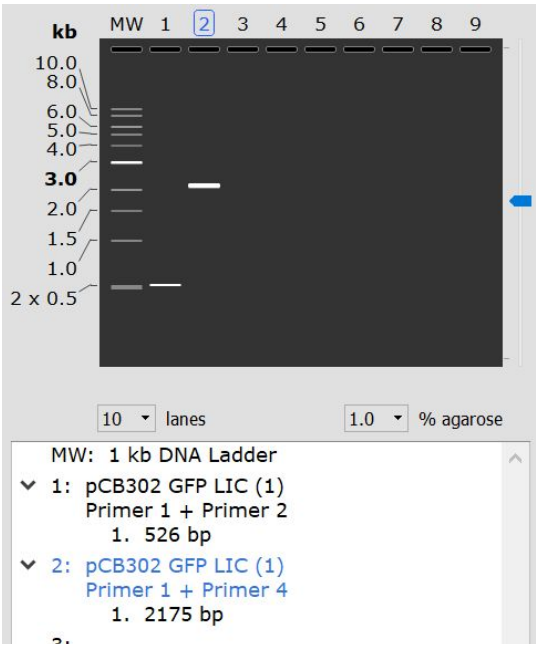
Running

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 an hour at 120 V

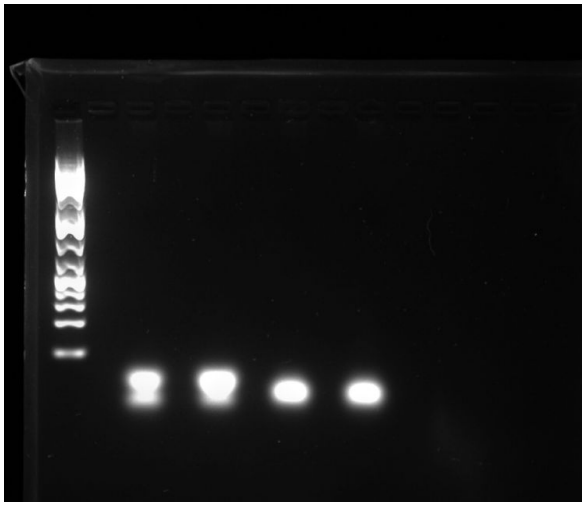
Gel Key

Lane 1: Gene ruler ladder 1 Kb plus
Lane 2: empty
Lane 3: 10 ul sample 1 of 1&2
Lane 4: empty
Lane 5: 10 ul Sample 2 of 1&2
Lane 6: empty
Lane 7: 10 ul Sample 1 of 1&4
Lane 8: empty
Lane 9: 10 ul Sample 2 of 1&4
Lane 10: Empty
Lane 11: Empty
Lane 12:Empty
Lane 13:Empty
Lane 14: Empty

Expected Results:



Results:



Conclusion:

The primers formed dimers. Another PCR reaction will be performed using the higher annealing temperature of 50 degrees Celcius. New primers should also be designed.

Name: Chiara Brust
Date: 7/9/19

Goal:

1. Transformation
    a. Blue chromoprotein (K592015)
    b. Codon-optimized-RFP

Protocol:

Transformations

Heat Shock

1. Thawed One Shot TOP10 chemically competent cells on ice.
2. Added 2 µL of DNA sample into competent cells
3. Incubated the cells on ice for 35 minutes.
4. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
5. **Quickly** took them out and **immediately** add 250µL of SOC medium
6. Placed the samples into a 37° C shaking water incubator for 1 hour at 200 rpm.
7. After shaking for 1 hour, smeared 100 µL of the solution onto an agar plate with the respective antibiotics.
    a. K592015: Chloramphenicol resistance
    b. Codon-optimized-RFP: Ampicillin resistance
8. Incubated plates at 37°C for at least 24 hours.

Name: Rehmat

Date: 7/9/19

Goal: 1. Transform the pCB302 plasmid into Agrobacterium tumefaciens

Materials:
Agrobacterium tumefaciens LBA4404
Protocol:
Electroporation of Agrobacterium tumefaciens

1. Thawed Agrobacterium tumefaciens cells on wet ice
2. Combined 1 µL of pCB302-gfp-MBD plasmid DNA and 20 µL of cells in an Eppendorf tube
3. Combined 1 µL of RFP construct positive control plasmid DNA and 20 µL of cells in an Eppendorf tube
4. Pipetted the cells into a cuvette and electroporated at 2 kV
5. Added 1 mL of YM media and transferred to a 15 mL falcon tube
6. The tubes were incubated at 30°C at 200 rpm for 3 hours
7. 400 µL of each culture was streaked onto a YM kanamycin plate.
8. 300 µL of each culture was streaked onto a YM kanamycin plate.
9. 200 µL of each culture was also streaked onto a YM kanamycin plate.
10. The plates were incubated at 30°C for 48 hours

11. Thawed Agrobacterium tumefaciens cells on wet ice
12. Combined 1 µL of pCB302-gfp-MBD plasmid DNA and 20 µL of cells in an Eppendorf tube
13. Combined 1 µL of RFP construct positive control plasmid DNA and 20 µL of cells in an Eppendorf tube
14. Pipetted the cells into a cuvette and electroporated at 2 kV
15. Added 400 µL of SOC media and transferred to an Eppendorf tube
16. The tubes were incubated at 30°C at 200 rpm for 3 hours
17. 200 µL of each culture was streaked onto a LB kanamycin plate.
18. 150 µL of each culture was also streaked onto a LB kanamycin plate.
19. The plates were incubated at 30°C for 60 hours

Take out Friday morning!!!

The positive controls were plated on chloramphenicol plates

Name: Chiara Brust
Date: 7/9/19

Goals:

1. Overnight cultures of A. Tumefacien (pcb302)

Protocol:

Overnight Cultures

1. Added about 15 mL of YM to a 50 mL Falcon tube along with 15 µL of antibiotics
    a. 1000x Kanamycin
2. Scraped some of the ice of a glycerol stock with the p20 tip and dropped into the tube
        i. NOTE: did NOT let the glycerol stock defrost!
3. Incubated at 28° C at 220 rpm for 48-56 hours

Name: Chiara Brust
Date: 7/9/19

Goal:

1. Run gel of pcr pcb302 from minipreps done on 7/8/19

Protocol:

Preparing, Loading, and Running a 1% Agarose Gel

Preparing

10. Added 1 g of Agarose in 100 mL of 1X TBE in an Erlenmeyer flask
11. Heated in the microwave until fully dissolved
12. Allowed the solution to cool until comfortable to touch
13. Added 10 µL GelRed Nucleic Acid Gel Stain and mixed
14. Inserted casting tray, made sure the rubber on the sides was not overlapping
15. Carefully poured the agarose into the tray and placed the comb to create the wells
16. Allowed the gel to solidify
17. Once solidified, changed the orientation of casting tray where the rubber sides were not in contact with the sides of the system.
18. Poured in 1X TBE into the gel electrophoresis system to the fill line, being sure to submerge the gel, and removed the comb

Loading

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

Running

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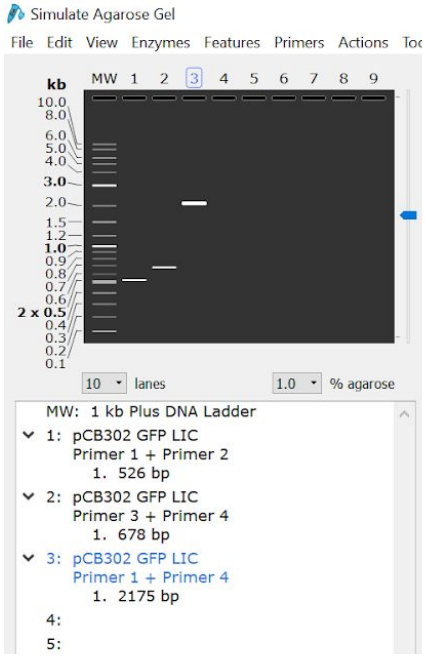
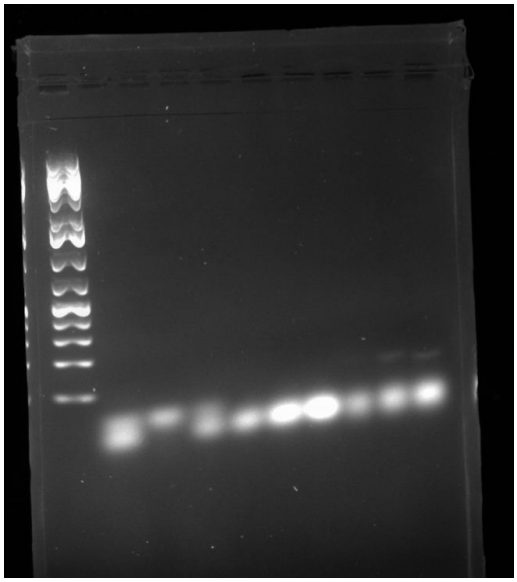
Expected Results:



Results:



Gel Key

Lane #	**Sample**
1	1 Kb Plus MW DNA Ladder
2	200 µL, colony 2, Primers 1 & 2
3	200 µL, colony 7, Primers 1 & 2
4	100 µL, colony 3, Primers 1 & 2
5	200 µL, colony 2, Primers 3 & 4
6	200 µL, colony 7, Primers 3 & 4
7	100 µL, colony 3, Primers 3 & 4
8	200 µL, colony 2, Primers 1 & 4
9	200 µL, colony 7, Primers 1 & 4
10	100 µL, colony 3, Primers 1 & 4

Conclusion:

The primers formed dimers again. We need to design new primers.