Name: Laura Das Neves, Chiara Brust, Rehmat Babar, Kennex Lam, Krithika Karunakarran
Date: 7/10/2019

Goals:

1. PCR for PCB302 straight from papers 1 & 2
    - Primers 3 & 4 at 48 degrees Celcius
2. Overnight cultures of codon-optimized-RFP from transformations done 7/9/19
3. Transformations
    - K1357009 (New Blue chromoprotein)
4. Gel Electrophoresis of PCR pcb302 from today

Name: Laura Das Neves
Date: 7/10/19

Goals:

1. PCR of pcb302 straight from original papers
    a. Primers 3 & 4 at 48 degrees Celsius

Materials:
DreamTaq Green PCR Master Mix (2x) Lot 00603571
pcb302 from original papers

Protocol:

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.

- 1 reaction with primers 3 & 4

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2. 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. 48° 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

The PCR was analyzed on a gel electrophoresis gel

Name: Rehmat Babar
Date: 7/10/19

Goal:

1. Run gel of pcb302 PCR from 7/10/19

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

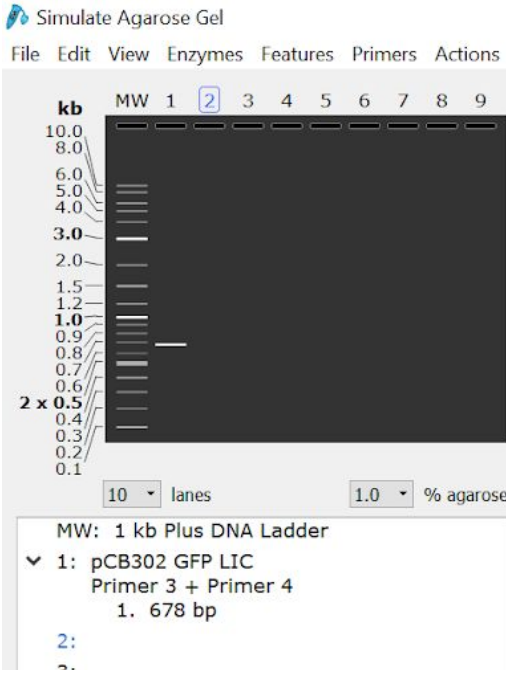
Loading

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

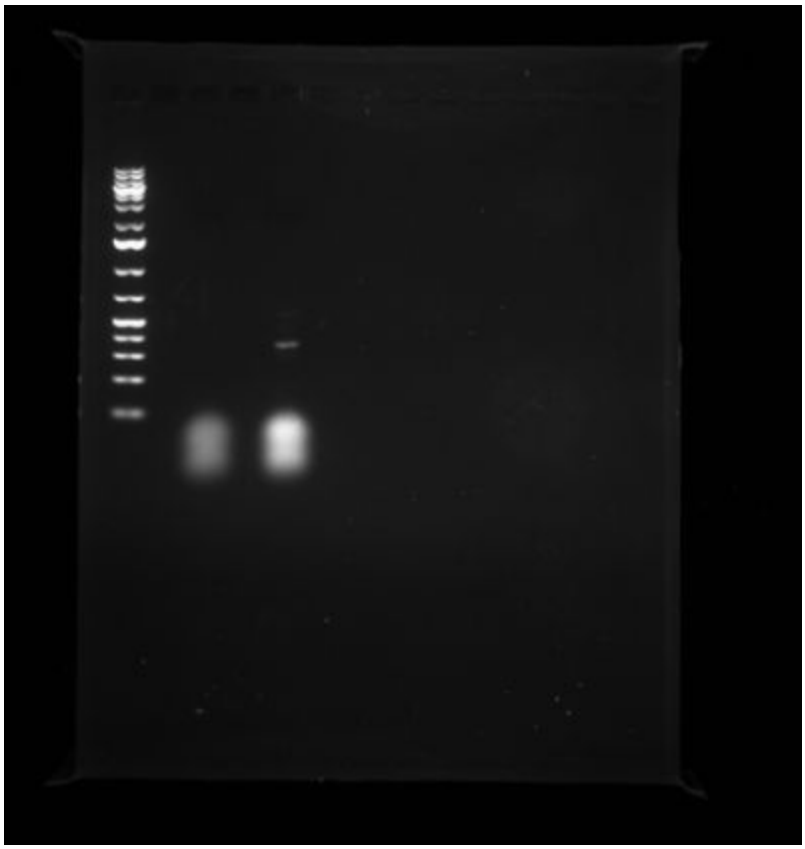
Running

1. Once the gel had been loaded, slid on the cover making sure the negative electrode was closest to the DNA and the positive electrode was at the bottom of the gel
2. Ran for about 45 minutes at 117 V

Expected Results:



Results:



Key
Lane 1: 1 kb Plus Ladder
Lane 2: Empty
Lane 3: 1 pCB302 primers 3
Lane 4: Empty
Lane 5: 2 pCB302 primers 3 and 4

Conclusion:

The faint band in lane 5 appears to be placed correctly, therefore proving primers 3 and 4 are capable of amplifying a part of the pCB302 plasmid. However, the major band is still an apparent primer dimer. We will do a PCR reaction at a higher annealing temperature to see if the primer dimers are reduced or removed altogether.

Name: Chiara Brust
Date: 7/10/19

Goal:

1. Transformations
    a. K1357009 (New blue chromoprotein with promoter, RBS, & terminator)

Materials:

- One Shot TOP10 chemically competent cells

Protocol:

Heat Shock Transformation

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** added 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. Chloramphenicol
8. Incubated plates at 37°C for at least 24 hours.

Name: Krithika Karunakarran
Date: 7/10/19

Goal:

1. Overnight cultures of codon-optimized RFP from transformations done on 7/9/19

Protocol:

Overnight Cultures

1. 5 colonies on each plate (2 total) were identified and circled
2. 10 falcon tubes were labeled with colony numbers 1-10 that correspond with said colonies (“RFP C.O.” with colony number circled)
3. A cocktail mix of 60mL LB and 60µl ampicillin was made
4. Each falcon tube was filled with 5mL of the cocktail mix
5. A p10 was used to touch a selected colony and the tip was dropped into the corresponding falcon tube
6. All tubes were placed in incubator at 3:45pm (back bench) at 225 rpm and 37°C overnight.