Name: Kennex Lam, Laura Das Neves, Rehmat Babar, Chiara Brust
Date: 7/11/19

Goals:

1. Pick colonies from pcb302 agrobacterium transformations from papers 1 & 2 and start overnight cultures
2. Minipreps of pcb302 in A. Tume from papers 1 & 2 glycerol stocks made on 7/8/19
3. Restriction digest of codon-optimized-RFP transformed on 7/9/12
4. Run gel of restriction digest of codon-optimized-RFP
5. Colony PCR on K1357009 (Blue chromoprotein)
6. Overnight cultures
    a. Pcb302 in A. Tume from papers 1 & 2 from 7/9/19 transformations

Name: Chiara Brust
Date: 7/12/19

Goal:

1. Colony PCR on K1357009 (Blue Chromoprotein)

Protocol:

Colony PCR Protocol

20 µL Reaction

1. Prepared a PCR concentration cocktail with the following proportions: 7 µL of diH2O, 10 µL PCR Mastermix, 1 µL of the forward primer, and 1 µL of the reverse primer.
2. Added 19 µL of the concentration cocktail into a PCR tube.
3. Using a 10 µL micropipette, touched the tip onto the selected colony and swirled around in the PCR tube.
4. Placed PCR tube in the thermocycler at the following generic settings:
    1. 95° C for 3:00 minutes
    2. 95° C for 1:00 minute
    3. 52° 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

Results:

N/A

Conclusion:

N/A

Name: Rehmat Babar
Date: 7/12/19

Goal:
Extract pCB302-gfp-MBD plasmid from agrobacterium overnights

Protocol:

Mini Preps for Agrobacterium tumefaciens

1. Centrifuged 10 mL of overnight for 15 minutes at 3500 rpm and resuspended in 250 µl buffer P1 containing 0.1 mg/ml RNase A.
2. Added 250 µl lysis buffer P2 to the tube and inverted gently 6 times to mix.
3. Added 350 µl neutralization buffer N3 to the tube and inverted immediately but gently 6 times.
4. Centrifuged the lysate for 10 min at 13,000 rpm
5. Placed a QIAprep Spin Column in a 2 mL collection tube.
6. Transferred the cleared lysates from step 4 to the QIAprep Spin Column by decanting or pipetting.
7. Centrifuged for 60 seconds at 13,000 rpm and discarded flow-through.
8. Washed the QIAprep Spin Column by adding 500 µL of Buffer PB and centrifuged for 60 seconds at 13,000 rpm and discarded flow-through.
9. Washed the QIAprep Spin Column by adding 750 µL of Buffer PE and centrifuging at 60 seconds at 13,000 rpm and discarded the flow through.
10. Centrifuged for an additional 1 min to remove residual wash buffer at 13,000 rpm.
11. Placed the QIAprep Spin Column in a clean 1.5 mL microcentrifuge tube.
12. Added 50 µL of Buffer EB to the center of each QIAprep Spin Column, let stand for 1 min, and centrifuged for 1 min.

Results:

Colony 7 - too low
Colony 2 - 2.5 ng/µL
Colony 3 - too low

Conclusion:

This round of transformations was not successful, we will do mini preps on the overnights from the cultures prepared today from the transformations done on Tuesday.

Name: Rehmat Babar
Date: 7/12/19

Goal:
Start overnight cultures for the pcb302 in A. Tume transformations done on 7/9/19

Protocol:

Overnight Cultures

1. Added about 7 mL of LB with kanamycin to a 15 mL Falcon tube
2. Dipped a p10 tip into the selected colonies and drop into the tube
3. Incubate in the water bath at 28° C at 200 rpm over the weekend


Kan + LB Alt 6/19/19
1A LB pCB302
7/19/19 150mL
RS
01
02
03
04
05
07
08
10
40
LB+Kan 6/19/2019 S.T.
1A LB PCB302
7/19/19
250mL
RS
01
02
04
05
07
08
Kan+LB 6/19/2019 S.T.
PCB302-2A (LB)
250mL
7/19/19 RS
01
02
04
05
08
10

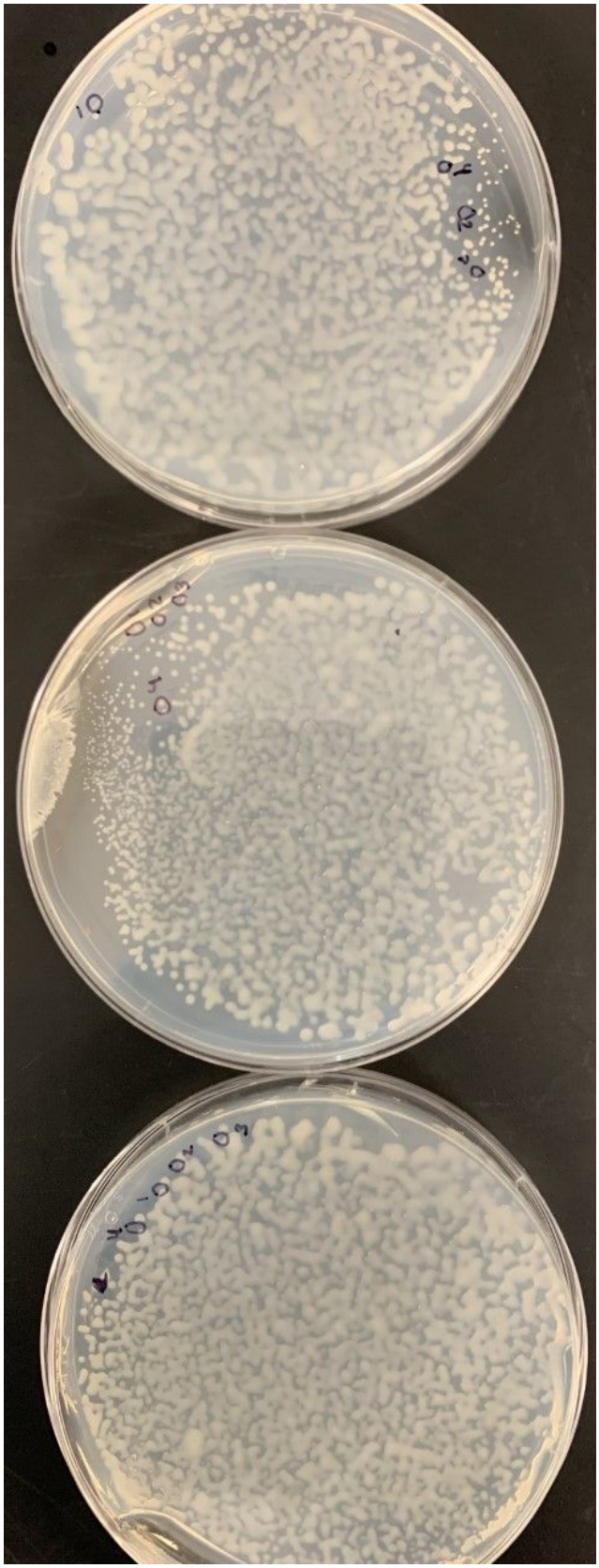


Plate 1B (top 200, middle 300, bottom 400)

Plate 2A (top 200, middle 300, bottom 400)

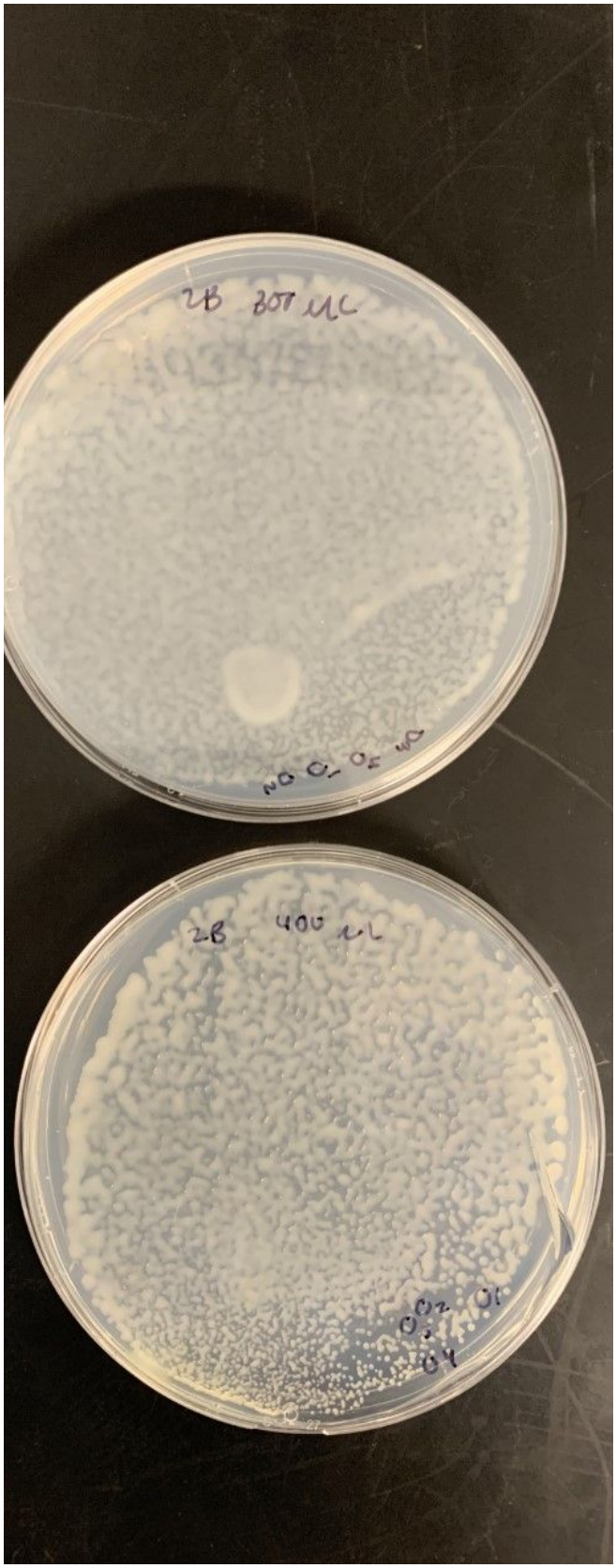


Plate 2B (top 200, middle 300, bottom 400)

Name: Chiara Brust
Date: 7/12/19

Goal:

1. Restriction Digest on codon-optimized-RFP from miniprep samples done on 7/11/19

Protocol:

Restriction Digest Protocol

30 µL Fast Digest Restriction Digest

1. Prepare a Fast Digest concentration cocktail with the following proportions: 1 µL Restriction Enzyme XbaI, 1 µL Restriction Enzyme BglII, 3 µL of 10X Fast Digest Buffer, and 15 µL of diH2O.
2. Add 20 µL of this cocktail to a clean 1.5 Eppendorf tube and then add 10 µL of DNA
3. Incubate at 37° C for 30 minutes.

Results:

N/A

Conclusion:

N/A

Name: Chiara Brust
Date: 7/12/19

Goal:

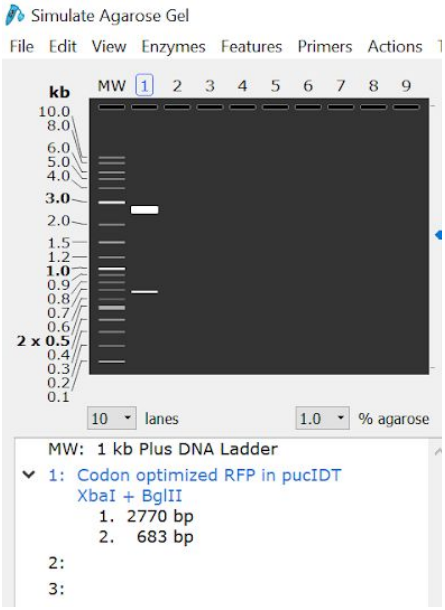
1. Run gel on restriction digest of codon-optimized-RFP from minipreps done on 7/11/19

Protocol:

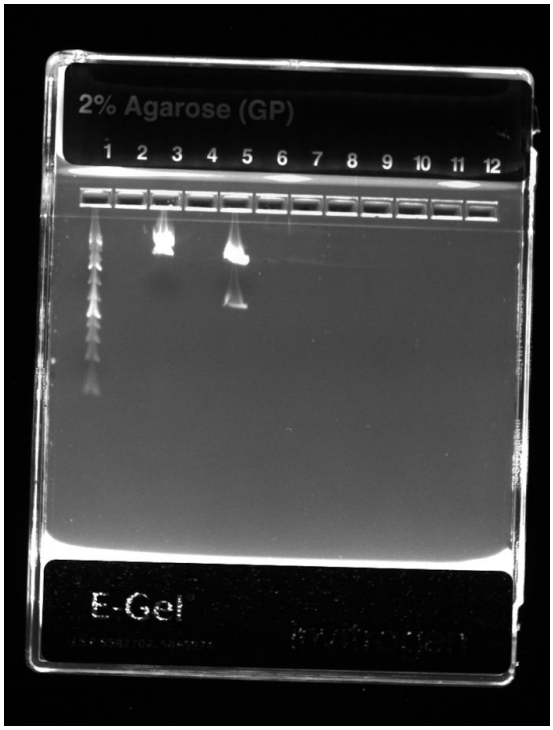
E-gel 2 % Agarose

1. Inserted E-gel into tray box
2. Loaded 5 µL of MW ladder and DNA into each well
    a. Colonies 7 and 10
3. Ran for 25 minutes

Expected Results:



Results:



Gel Key
Lane 1- MW 1 Kb Plus DNA ladder
Lane 2- Blank
Lane 3- Colony7
Lane 4- Blank
Lane 5-Colony 10

Conclusion:

The digest was only successful for colony 10. Perhaps something went wrong when colony 7 was being digested.