Date 6/12/19

Name: Jiazi Tian, Sijia Qin, Justin and Chiara

Goal

1.:Perform Minipreps on Transformations done Monday 6/10/19

Protocol:

a. Perform Minipreps on Transformations done on Monday (PCB302) Centrifuge 3 mL of bacterial overnight culture in two separate Eppendorf tubes (1.5 mL in each) at 8,000 rpm for 3 minutes at room temperature.
a. Discard the supernatant and resuspend pelleted bacterial cells in one tube with 250 µL Buffer P1 and transfer to the other and resuspend until one eppendorf tube contains the pelleted cells resuspended in 250 µL Buffer P1.
b. Add 250 µL of Buffer P2 and invert 5 times.
c. Add 350 µL of Buffer N3 and immediately mix by inverting 5 times.
d. Centrifuge for 10 minutes at 13,000 rpm.
e. Micropipette 800 µL of the clear supernatant into a spin column and centrifuge for 60 seconds and discard the excess liquid.
f. Add 500 µL of PB and centrifuge the spin columns for 60 seconds. Discard the flow through.
g. Add 750 µL of PE to the spin columns, centrifuge for 60 seconds, and discard the flow through.
h. Centrifuge the spin columns again for 60 seconds to remove residual wash buffer and discard the flow through.
i. Transfer the spin columns to a clean eppendorf tube and add 50 µL of EB to the center of the spin column to elute the DNA.
j. Allow the spin column to stand for one minute and then centrifuge for one minute.
k. Record the concentrations for each sample.
1. Pellet 1-5 mL

Labels on tubes :

A- B1 Trial 3 (1)
B- B2 Trial 3 (2)
C- B2 Trial 1 (2)
D- B2 Trial 2 (1)
E- B2 Trial 2 (1)
F- B2 Trial 2 (2)

G- B2 (1)
H- B2 (2)

Results:

1. Performed miniprep on
   A- B1 Trial 3 (1) **.55(ng?/mL?)**
   B- B2 Trial 3 (2) **.75(ng?/mL?)**
   C- B2 Trial 1 (2) **.90(ng?/mL)**
   D- B2 Trial 2 (1) **.55(ng?/mL)**
   E- B2 Trial 2 (1) **.75(ng?/mL)**
   F- B2 Trial 2 (2) **1.10(ng?/mL)**
   G- B2 (1) **.75(ng?/mL)**
   H- B2 (2) **.65(ng?/mL)**

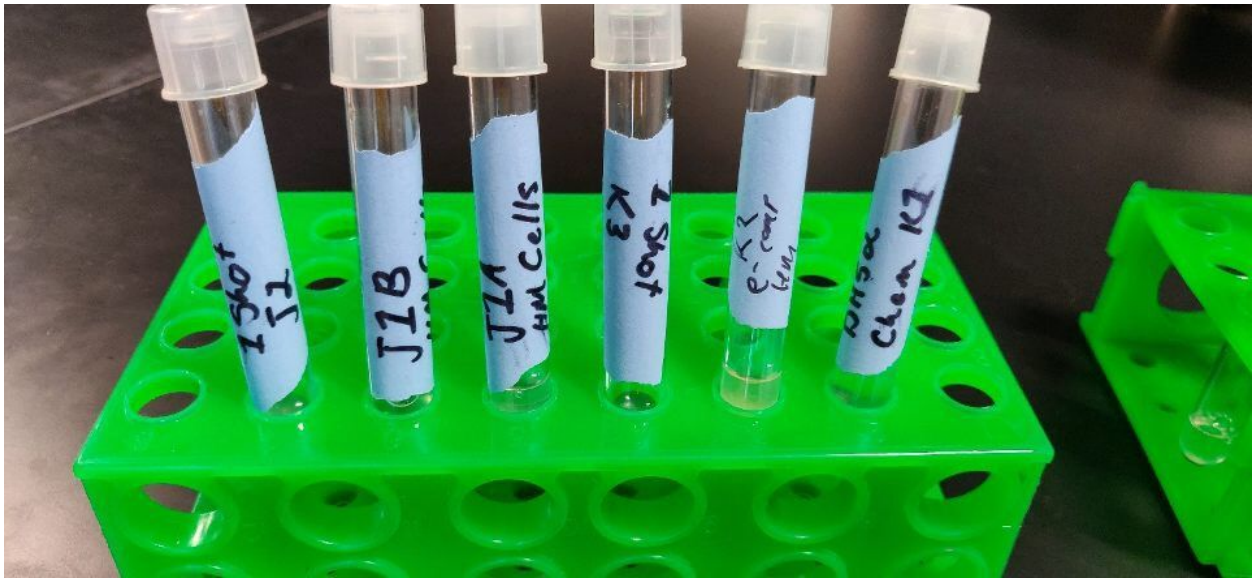
Date 6/12/19

Name Krithika and Chritina

Materials: Kanamycin plates that were made by Jiazi and Siija on 6/11/19

Goal: Replate Transformations with Parts from Registry

- The transformations were done 6/11/19 by Laura, Justin Chiara, Asma

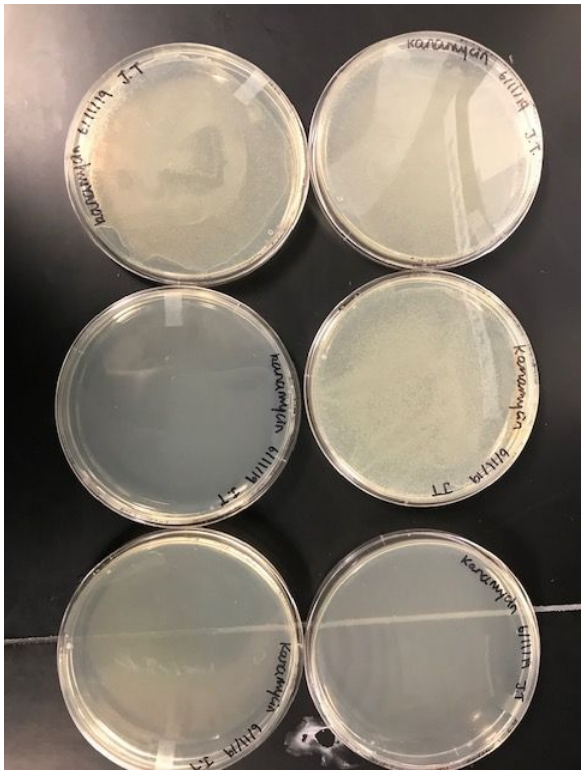


Purpose: Was to make sure that the plates served as a negative control. (No growth should appear on any of the plates)

Protocol:
Plated 6 plates that contained

__microLiters of J2
__ microLiters of J1B
__ microLiters pf J1A
__ microLiters of K3
__ microLiters of K2
__ microLiters of K1

Results:



3 plates had growth 3 plate had no growth. Something i growing with Kanamycin. Possibly faulty Kanamycin

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Date: 6/12/19

Name: Christina Clodomir

Goal: Redo Transformations for PCB302 (With One Shot) from 6/10/19 with “New” Kanamycin Plate made on 6/11/19

Materials:

Warm the vial of S.O.C. Medium made by Cara (6-6-17)

• Warm the selective plates in a 37°C incubator for 30 minutes (use 1 or 2 plates

for each transformation).• Place cuvettes on ice and set up your electroporator for bacterial transformation

as per the manufacturer’s instructions.

• One 15 ml snap-cap tube per transformation

Transformation Procedure

Use this procedure to transform One Shot® TOP10 Electrocomp™ E. coli. We

recommend including the pUC19 control plasmid DNA supplied with the kit

(10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8) in your transformation

experiment to verify the efficiency of the competent cells. Do not use these cells for

chemically competent transformation.

Protocol:

1. Thaw, on ice, one vial of One Shot® TOP10 Electrocomp™ cells for each

transformation.

2. Add 1 µl of the DNA (10 pg to 100 ng) into a vial of One Shot® cells and

mix gently. For the pUC19 control, add 10 pg (1 µl) of DNA into a separate

vial of One Shot® cells and mix gently.

3. Transfer the cells to the chilled electroporation cuvette on ice.

4. Electroporate the cells as per the manufacturer's recommended protocol.

5. Aseptically add 250 µl of pre-warmed S.O.C. Medium to each vial.

6. Transfer the solution to a 15 ml snap-cap tube and shake for at least 1 hour at 37°C to allow expression of the antibiotic resistance gene.

7. Spread 10 to 150 µl from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, dilute the transformation mix 1:50 into LB Medium (e.g. remove 20 µl of the transformation mix and add to 980 µl of LB Medium) and plate 20-100 µl.

8. Store the remaining transformation mix at +4°C. Additional cells may be plated out the next day, if desired.

9. Invert the selective plate(s) and incubate at 37°C overnight.

10. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

Additional Notes:

2 Trials were done. Eppendorf tube that was not labeled is PCB302A and the other was Labeled B.

There should be 3 plates for each trial (6 in total)

For Trial A: 1st plate was streaked. 2nd plate ahs 150 microliters and the 3rd has 50 microliters.

For Trial B: 1st plate was streaked. 2nd plate ahs 150 microliters and the 3rd has 50 microliters.

Results:

Name: Krithinka and Kat

Purpose: Redo Transformations with PCB302 with DH5aHomemade Cells (with Kanamycin plates done 6/11/19)

Materials:

Electroporation of DH5α Homemade Cells(made in Spring Semester)
SOC Medium made by Cara 6-6-17
Eppendorf Tubes
Cuvettes etc.

Protocol:

1. Combined 40 µL of electrically competent DH5α cells and 2 µL of ligated DNA to an Eppendorf tube.
2. Transferred the contents of the Eppendorf tube to a cuvette and lightly tapped the cuvette on the table to evenly distribute the contents and to get rid of air bubbles.
3. Placed the cuvette into the Bio-Rad MicroPulser and delivered an electric shock.
4. Immediately after, 900 µL of SOC medium was added to the cuvette and the solution was mixed via micropipette.
    a. DH5α A2 contained a newer SOC medium than DH5α A1 and DH5α B
5. Transferred the solution from the cuvette to a shaker tube and placed in the shaker at 37°C at 300 rpm for ~1 hour.
6. After shaking for ~1 hour, DH5α A1, A2, B, and Oneshot A and B were streaked and spread (100 µL) on kanamycin plates.
    a. DH5α A1 spread used 150 µl instead of 100 µ.
    b. 10 plates total
7. Plates left to incubate at 37 °C for 24 hours.

Additional Notes:

2 Trials were done labeled A and B. They are in culture tubes with Pink Tape on it.

Results:

For Trial A:1st plate was streaked. 2nd plate ahs 150 microliters and the 3rd has 50 microliters.
For Trial B: 1st plate was streaked. 2nd plate ahs 150 microliters and the 3rd has 50 microliters.

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Name: Chiara; Kennex

Purpose: **Running a PCR and Creating Overnight Cultures with the 3 Promotor Ampicillin Plates and 1 Coding Sequence Plate.**

*Chiara prepared LB Broth. Kennex ran the PCR reaction and completed overnight cultures.

Protocol:

PCR

To create the PCR mix, 10 uL of Master Mix, 1 uL of VF primer, 1 uL of VR primer, and 8 uL of water were needed. However, because we were picking 10 colonies, we performed 10 PCR reactions (10 PCR tubes). Created a cocktail mix of 12x (12x because you always want to make more than you should as the 10x may run out before pipetting the final tube.) Multiplied all the ingredients by 12: 120 uL Master Mix, 12 uL VF primer, 12 uL VR primer, and 96 uL of water.

Then each of the 10 PCR tubes had 20 uL of cocktail mix pipetted i to them.

The colonies picked and dipped the tip into each tube according to label. The same tip was then used for the overnight culture.

PCR machine left to run.

Overnight Cultures

10 mL of LB was placed into 10 tubes. (Ask chiara how much antibiotic I added to LB).

Tip of pipette touched a colony, dipped into corresponding PCR tube, and ejected into corresponding overnight culture tube.

Ken tested on Tatenda and Jessica's kanamycin solution to see if one of the kanamycin solutions was not working as kanamycin plates made previously were now Kanamycin resistant. M 2 tubes: 5 mL of LB and 5 uL of each Kanamycin solution was added into a tube (One tube contains Jessica's solution, other contains Tatenda's Kanamycin solution).

Left in shaker overnight.

Additional Notes:

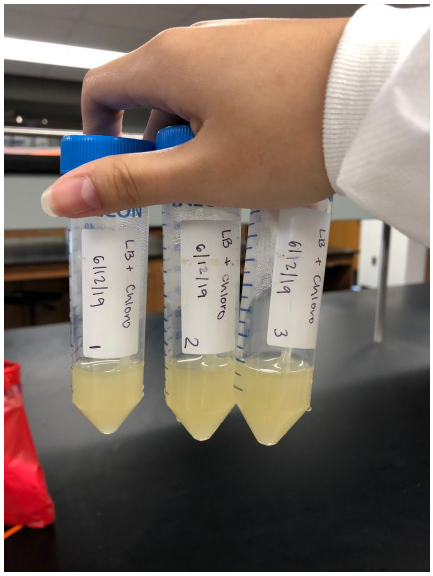
Labeled Tubes

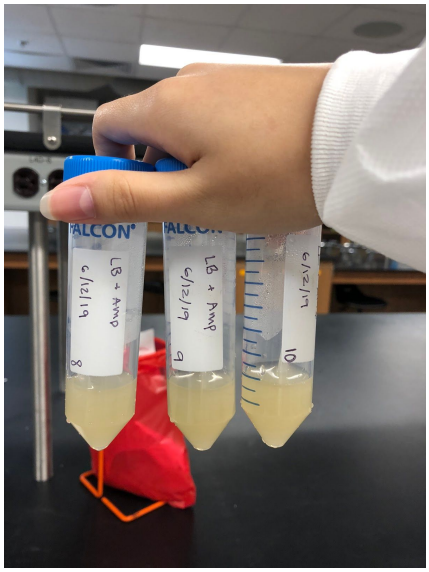
- 1-5 are chloramphenicol resistant coding sequence.
    - BBa_K592009 chlor K2 DH5a HM electrocomp
- 6-10 were ampicillin resistant promoter sequences.
    - 6 is BBA_J23102 amp J2 streak
    - 7 and 10 are BBa_J23102 50 mL amp
    - 8 and 9 are BBa_J23102 amp JIB

Results:

Overnight Cultures Results (from the transformation done on 6/11/19) for Promoter and Blue Chromoprotein

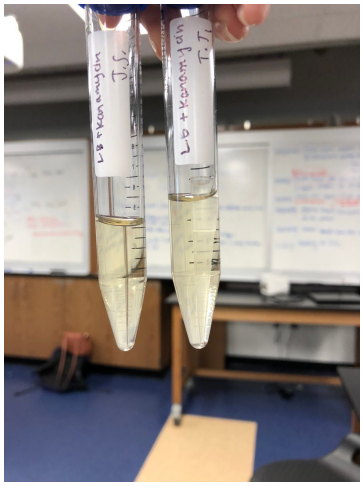
Approx. in there for 17 hours.





Every one of the 10 samples grew.

Results:



Nothing grew in the LB+Kanamycin samples.