Name: Justin, Kennex,Christina
Date: 6/13/19

Purpose: Running Gel Electrophoresis on PCR that Kennex and Chiara ran from yesterday.

Justin and Kennex ran gel electrophoresis. Justin made the gel. Kennex and Christina loaded and ran the gel.

Shakira made new kanamycin plates (about 40).

Jessica, Sijia and Jiazi did the miniprep of 10 samples.

1% Agarose Gel and Gel Red for Dye

Protocol:

Purpose: To separate DNA based on size (base pairs)

Preparing, Loading, and Running a 1% Agarose Gel

Preparing

1. Add 1 g of Agarose in 100 mL of 1X TBE in an Erlenmeyer flask
2. Heat in the microwave until fully dissolved (usually about 45 seconds to 1 minute)
    a. Solution should be completely clear
3. Allow the solution to cool until comfortable to touch
4. Add 10 µL GelRed Nucleic Acid Gel Stain and mix
5. Insert casting tray, make sure the rubber on the sides is not overlapping
6. Carefully pour the agarose into the tray and place the comb to create the wells
7. Allow the gel to solidify
8. Once solidified, change the orientation of casting tray where the rubber sides are not in contact with the sides of the system.
9. Pour in 1X TBE into the gel electrophoresis system to the fill line, be sure to submerge the gel, and remove the comb

Loading

1. Load ~5 µL of the ladder in the first well
2. Prepare your samples to load by adding in 1 µL of 6X Loading dye for every 5 µL of DNA and load

Running

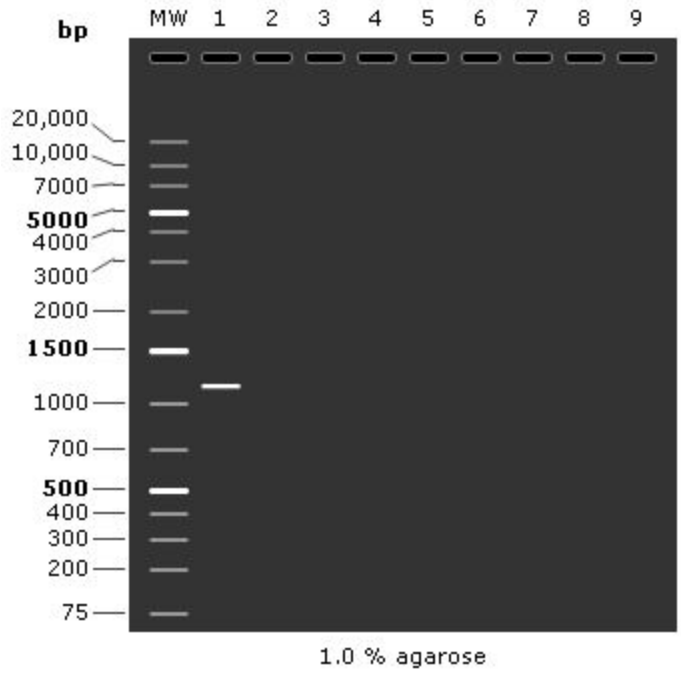
1. Once the gel has been loaded, slide on the cover making sure the negative electrode is closest to the DNA and the positive electrode is at the bottom of the gel
2. Run for about 45 minutes to an hour

14 wells
10 samples

Lane 1 - Generuler 1 kb Plus Lot 00516263
Lane 2 - Sample 1C (BBa__K592009 k2 Chloro DH5)
Lane 3 - Sample 2C (BBa_K592009 k2 Chloro DH5)
Lane 4 - Sample 3C (BBa_K592009 k2 Chloro DH5)
Lane 5 - Sample 4C (BBa__K592009 k2 Chloro DH5)
Lane 6 - Sample 5C (BBa__K592009 k2 Chloro DH5)
Lane 7 - Sample 6A BBa_J23102 j2 Amp Streak)
Lane 8 - Sample 7A (BBa_J23102 j2 Amp)
Lane 9 - Sample 8A (BBa_J23102 J1B Amp)
Lane 10 - Sample 9A (BBa_J23102 J2 Amp)
Lane 11 - Sample 10A (BBa_J23102 J2 Amp)
Lane 12, 13, 14 - Blank

Each lane contains 5 uL of sample and 1 uL of dye.
*Sample contains about 5-7 uL of DNA sample plus dye. (Tube 8A total volume may the less than others)

Gel ran at approx. 1hr @ 120 volts
For Jpart (Full J23102 palsmid with VF primer and VR primer)

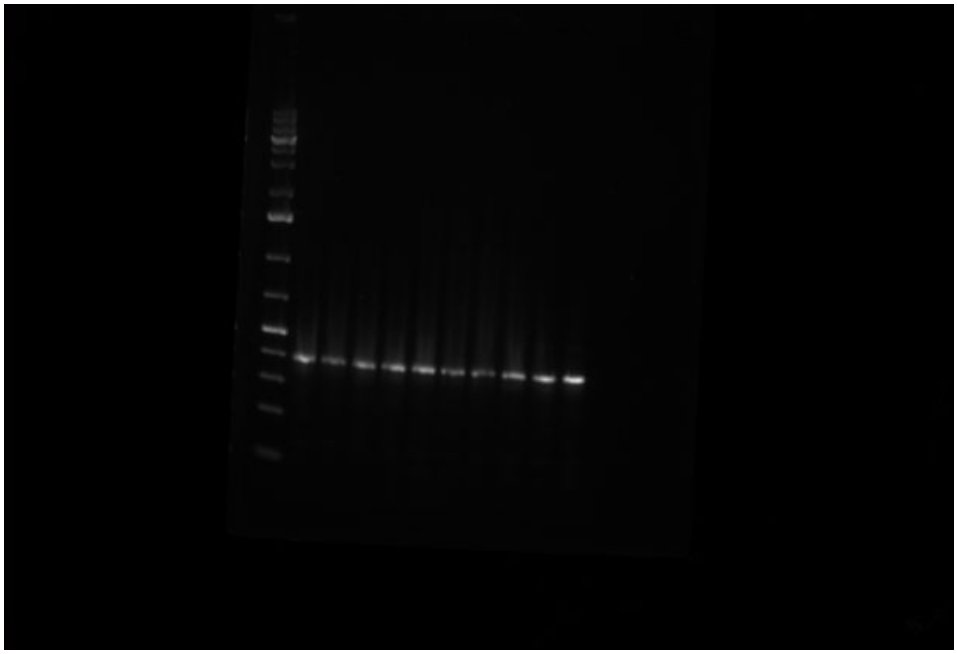


Ladder is 1kb Plus DNA Ladder

VF2+VR

1. 1143 bp

Our Results:



Miniprep of Overnight Cultures for Transformations done
(glycerol stock of 10 samples on 6/12 done)

a. Perform Minipreps on Transformations. Centrifuge bacterial overnight culture as 4mL and 5mL two groups in total 20 tubes at 8,000 rpm for 3 minutes at room temperature. (Jessica did samples of 1(LB+chlo) and 8(LB+amp). Sijia and Jiazi did other 8 samples.)
a. Discard the supernatant and resuspend pelleted bacterial cells in one tube with 250 µL Buffer P1 and transfer to Eppendorf tubes 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 1000 µL of the clear supernatant of the first group into a spin column and centrifuge for 60 seconds and discard the excess liquid. And then micropipette 800 µL of the clear supernatant of the second group 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.

Our results:

2 (LB+chl) concentration: 155 ng/µL

3 (LB+chl) concentration: 173 ng/µL

4 (LB+chl) concentration: 105 ng/µL

5 (LB+chl) concentration: 143 ng/µL

6 (LB+amp) concentration: 303 ng/µL

7 (LB+amp) concentration: 433 ng/µL

8 (LB+amp) concentration: 348 ng/µL

9 (LB+amp) concentration: 358 ng/µL

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Date 6/13/2019

Name: Christina Clodomir

Purpose: Overnight Cultures for Transformations done on 6/12/19 (PCB302)

Protocol:

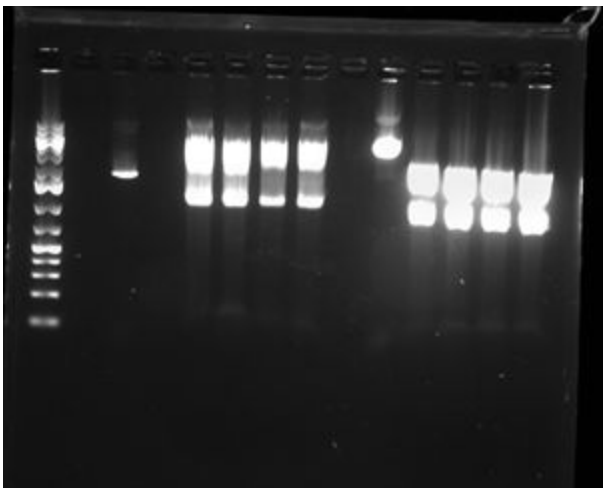
1. Collected 10 50mL Falcon Tubes
    a. Labeled 5 Plate A “Kanamycin Plates 6/11/” by K.S. and K.K. Colony (Number) C.C. and A.H.
    b. Orange Stickers meant that Tatenda’s Kanamycin was added.
    c. Yellow Stickers meant that Jessica’s Kanamycin was added.
2. Used a p20 tip to touch colonies on the plate of the transformation that KK and KS did.
3. Released tip in tube
4. Put them in bath of 37C at 220rpm
5. After 16-18 hrs colonies frew in broth.

Results are as follows:

Date: 6/13/19

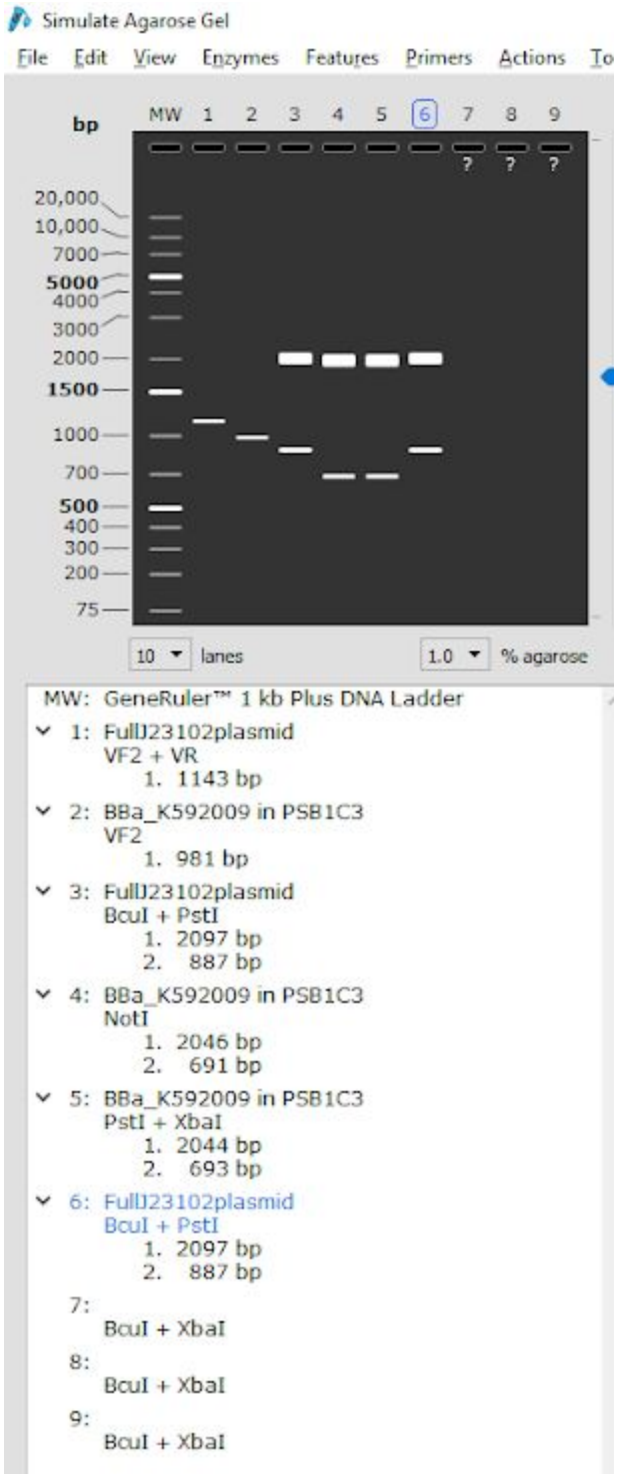
Purpose: Running Restriction digests and gel on colonies

Protocol:

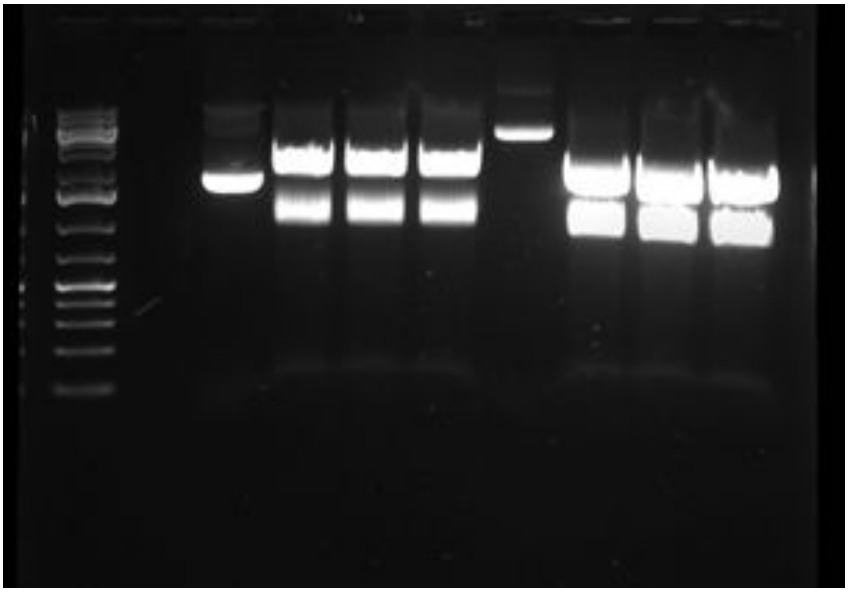
- Did analytical restriction digests on Bluechromoprotein (K592009) and RFP promotor (J23102)
    - K592009
        - Digested with not1
    - J23102
        - Digested with PST1 and BCU1
- Ran gel of restriction digest
    - Gel:
        - 100ml 1x TBE
        - 1g agarose
        - 10ul gel red
        - Ran at 120V until we got good separation of bands
    - 
    - Gel Key
        - 1-1 kb ladder
        - 2-
        - 3-undigested colony 8
        - 4
        - 5- colony 2
        - 6- colony 3

- 7- colony 4
- 8- colony 5
- 9-
- 10- undigested colony 1
- 11- colony 6
- 12- colony 7
- 13- colony 9
- 14- colony 10

It looks like it worked but the bands all fall a little higher up than expected from simulation gel:



- Ran another gel with digested colony 8 and 1
    - Gel
        - 100ml 1x TBE
        - 1g agarose
        - 10ul gel red
        - Ran at 120V until we got good separation of bands

- 
- Gel Key
  - Lane 1- m1 kb ladder
  - Lane 2 undigested K592009
  - Lanes 3-5 K592009 (cut with XbaI and PstI)
  - Lane 6 undigested J23102
  - Lanes 7-10 J23102 (cut with BcuI and PstI)
- Dna was cut/extracted from gels
  - For K we took bottom fragments
  - For J we took top fragments
- Fragments were placed in 15ml falcon tubes and stored @ 4 degrees C