Name: Jiazi Tian, Sijia Qin, Justin, Asma
Date: 6/24/2019

Goal:

1. Make PII Trace Metal mix
2. Gel electrophoresis on PCR samples from:
    a. pcb302 in E. Coli from papers A & B minipreps done on 6/20/19
    b. Ligations (K592009 + J23102)
3. O. Marina
    a. Fed 5 mL of D. Tertiolecta

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1. Make PII Trace Metal mix

Protocol:

PII TRACE METAL (1x 1L)

1. Dilute 48mg CoSO4.7H2O in 100ml H2O to get 100x CoSO4.7H20 solution.
2. Use the table below to make 1x 1L PII TRACE METAL stock.

COMPONENTS	MW	1X STOCK (1L)
CoSO4.7H2O	281.12	10mL 100x CoSO4.7H2O
EDTA.2Na	372.2	1.107g
FeCl3.6H2O	270.3	0.049g
H3BO3	61.8	1.14g
MnSO4.4H2O	223	0.164g
ZnSO4.7H2O	287.5	0.022g

Name: Justin Benton and Asma Khimani
Date: 6/24/2019

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    a. pcb302 in E.coli
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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 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 about an hour at 90 V

Gel Keys

Gel 1 Ligations

Lane	Contents
1.	*1kb Plus Ladder*
2.	*--*
3.	*Ligation1, 100µl #7*
4.	*--*
5.	*Ligation1, 150µl #7*
6.	*--*
7.	*Ligation1, 150µl #11*
8.	*--*
9.	*Ligation2, 100µl #7*
10.	*--*
11.	*Ligation2, 100µl #12*
12.	*--*
13.	*Ligation2, 150µl #7*
14.	*--*

Gel 2 pCB302

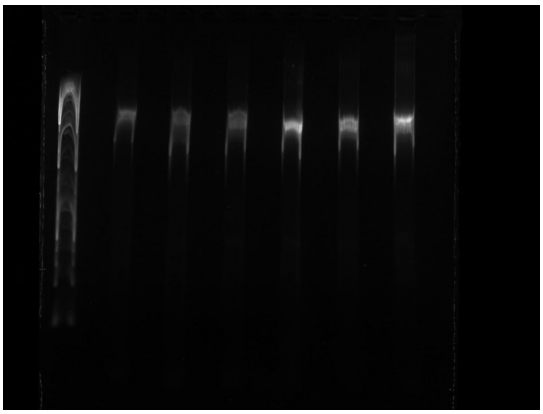
Lane	Contents
1.	*1kb Plus Ladder*
2.	*#7 with primers 1/2*
3.	*#8 with primers 1/2*
4.	*#9 with primers 1/2*
5.	*#10 with primers 1/2*
6.	*#11 with primers 1/2*
7.	*#12 with primers 1/2*
8.	*#7 with primers 3/4*
9.	*#8 with primers 3/4*
10.	*#9 with primers 3/4*
11.	*#10 with primers 3/4*
12.	*#11 with primers 3/4*
13.	*#12 with primers 3/4*
14.	*--*

Gel 3 pCB302

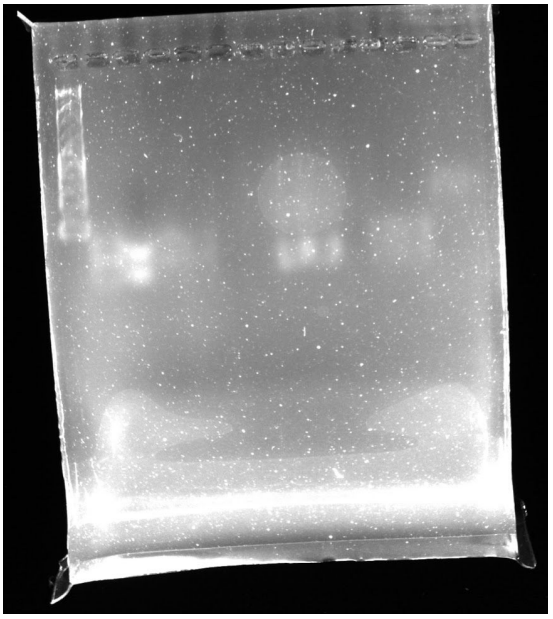
Lane	Contents
1.	*1kb Plus Ladder*
2.	*--*
3.	*#7 with primers 1/4*
4.	*--*
5.	*#8 with primers 1/4*
6.	*--*
7.	*#9 with primers 1/4*
8.	*--*
9.	*#10 with primers 1/4*
10.	*--*
11.	*#11 with primers 1/4*
12.	*--*
13.	*#12 with primers 1/4*
14.	*--*

Results

Gel 1 (Ligation of k592009 + J23102 PCR with Vf and Vr primers)



Gel 2 or 3 (Pcb302 PCR with different primers)



Conclusion

The gels are too blurry to analyze. New gels should be run.