Name: Kennex, Rehmat, Chiara
Date: 7/4/19

Goals:

1. Restriction digest of pcb302 in E. Coli from papers 1 & 2
    a. Restriction enzyme KpnI
2. Gel electrophoresis analysis of pcb302 in E. Coli papers 1 & 2 digest
3. Make culture for S. Microadriaticum in L1 and L1+F2
4. Prepare ASP-8A media with filtered seawater

Name: Chiara Brust
Date: 7/4/19

Goals:

1. Restriction digest of pcb302 in E. Coli
    a. Enzyme: KpnI

Protocol:

Restriction Digest Protocol

30 µL Fast Digest Restriction Digest

1. Prepared a Fast Digest concentration cocktail with the following proportions: 2 µL Restriction Enzyme KpnI , 3 µL of 10X Fast Digest Buffer, and 15 µL of diH2O.

2. Added 20 µL of this cocktail to a clean 1.5 Eppendorf tube and then add 10 µL of DNA

3. Incubated at 37° C for 30 minutes.

Name: Chiara Brust
Date: 7/4/19

Goals:

1. Gel electrophoresis analysis of pcb302 in E. Coli digest

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

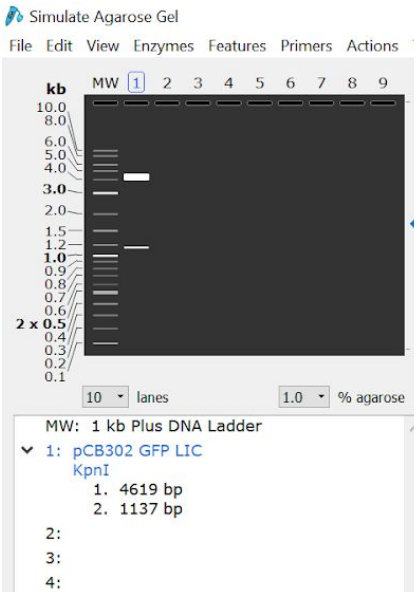
Loading

1. Loaded ~5 µL of the Molecular Weight Ladder 1 Kb plus in the first well
2. Prepared our samples to load by adding in 1 µL of 6X Loading dye for every 5 µL of DNA and loaded

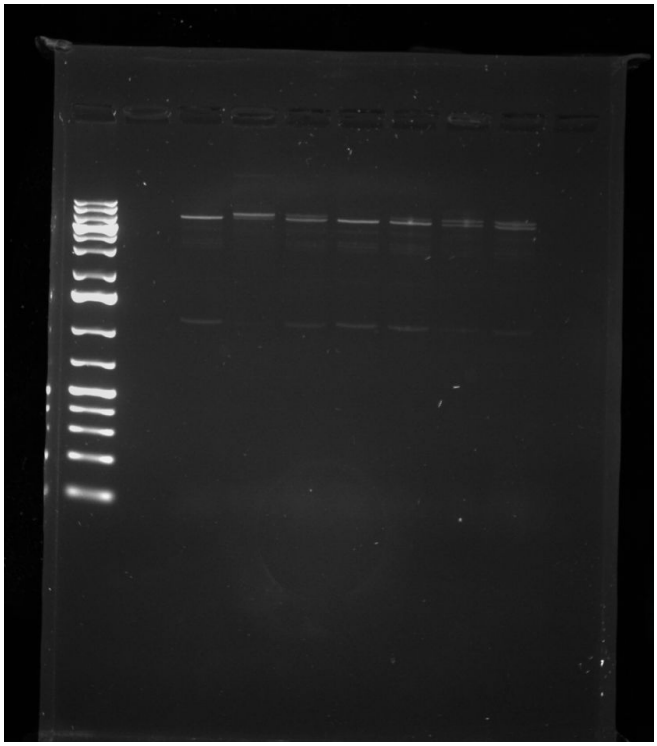
Running

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 118 V

Expected Results:



Results:



Gel Key

Lane #	1	2	3	4	5	6	7	8	9
Sample	1 Kb plus MW ladder	Blank	Pcb302,150 µL, colony 7	Pcb302, 150 µL, colony 3	Pcb302 ,75 µL, colony 1	Pcb302, 75 µL, colony 7	Pcb302, 150 µL, colony 2	Pcb302, 75 µL, colony 4	Pcb302, 150 µL, colony 1

Conclusion:

The largest band on the gel is of the correct size. However, the second band is smaller than expected by a few hundred base pairs. This does not coincide with the last restriction digest performed on pcb302, which revealed the plasmid to be significantly larger than anticipated. Perhaps another digest should be performed. Either way, the plasmid should be sent off for sequencing.

Name: Kennex Lam
Date: 7/4/19

Goal:

1. Prepare L1 and L1& F/2 cultures

Materials: L1 media, F/2 media, pipet filler gun, S. Microadriaticum

Protocol:

Preparation of L1 Cultures

1. Using sterile technique,
    a. 9 mL of L1 media and 1 mL of S. micradriaticum was added into a 15 mL falcon tube.
    b. 25 mL of L1 media and 2 mL of S. micradriaticum was added into a 50 mL falcon tube.
    c. 75 mL of L1 media and 3 mL of S. micradriaticum was added into a 75 mL flask.
2. The cultures were then left by the window.

Preparation of L1+F/2 Cultures

1. Using sterile technique,
    a. 4.5 mL of both the L1 and F/2 media and 1 mL of S. microadriaticum was added into a 15 mL falcon tube.
    b. 12.5 mL of both the L1 and F/2 media and 1 mL of S. microadriaticum was added into a 50 mL falcon tube.
    c. 37.5 mL of both the L1 and F/2 media and 1 mL of S. microadriaticum was added into a 75 mL flask.
2. The cultures were then left by the window.

Name: Rehmat Babar
Date: 7/4/19

Goal:

1. Prepare ASP-8A media with filtered seawater

Protocol:

1. Dissolved 25 grams in 800 mL of filtered seawater.
2. Added 10 mL of 1 M KCl, 20 mL 1.8 M of MgSO47H2O, 10 mL of 0.75 M of CaCl22H2O, 1 mL of 0.58 M NaNO3, 1 mL of 0.073 M KH2PO4, 1 mL of 0.157 M NTA, 10 mL of 0.825 M Tris Base pH 9, 1 mL of 12.5 mM NH4NO3, 10 mL of the prepared PII Metal Mix, 0.25 mL of the 8A Vitamin Mix, and 2.5 mL of 9.5 mM GeO2.
3. This solution was brought up to 1000 mL and was autoclaved for 45 minutes.

Conclusion:

This will be used to culture the S. microadriaticum.