Name**:** Krithika Karunakaran, Shakera Thomas, Kennex Lam, Sijia Qin, Jiazi Tian, and Saleh Alhassan, Rehmat
Date: 6/21/2019

Goal:

1. Prepare ASP-8A media
2. Restriction digest on mini prep samples (J23102 in K592009) done on 6/20/19
    - Digested with PstI and EcoRI
3. PCR for PCB302 in E. Coli from papers A & B
4. Run gel for restriction digest on ligation

Name: Kennex, Saleh, Krirthika
Date: 6/21/19

Goal:

1. Restriction digest on Ligations 1 & 2 minipreps from 6/20/19
    a. K592009 + J23102
    b. Digested with PstI and EcoRI

Protocol:

Restriction Digest Protocol: (for BCP Ligation samples)

A 20 uL FastDigest Restriction was performed on 7 “Ligation 1” miniprep samples, 2 controls, and 9 “Ligation 2” miniprep samples.

1. A cocktail mix of the following components was made:
    a. 22µl PstI & 22µl EcoRI
    b. 44µl 10x FastDigest Buffer
    c. 110µl di water
    *Calculations for the cocktail mix:
    The 10 uL restriction digest cocktail mix required 1 uL Pst1, 1uL EcoRi, 2 uL 10X fast digest buffer, and 5 uL di water.
    We originally planned to digest 19 samples, so we made a cocktail mix of x22. However, there were only 10 wells per gel, so Krithika and Kennex omitted Ligation 1 100 uL #?1 and Ligation 2 150 #11, 12.
    Everything x22 because a cocktail mix usually runs out when you make an exact amount.
2. Each new Eppendorf tube was loaded with 10µl of the DNA sample and 10µl of the cocktail mix
3. All 16 samples were then incubated for 30 minutes in a 37° hot water bath

Name: Kennex and Krithika
Date: 6/21/19

Goal:

1. Run gel on ligation digests
    a. J23102 with K592009 in pcba1020-r0040

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 were 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

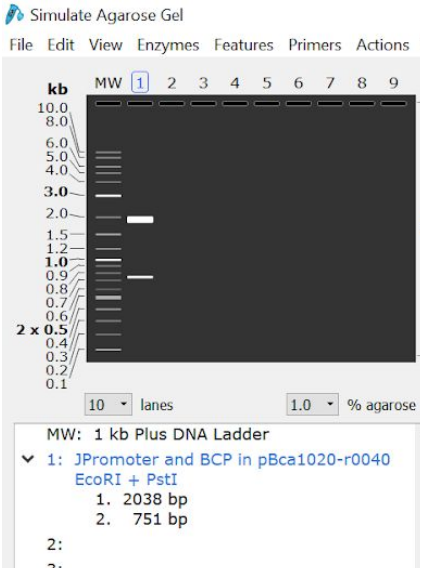
Loading

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

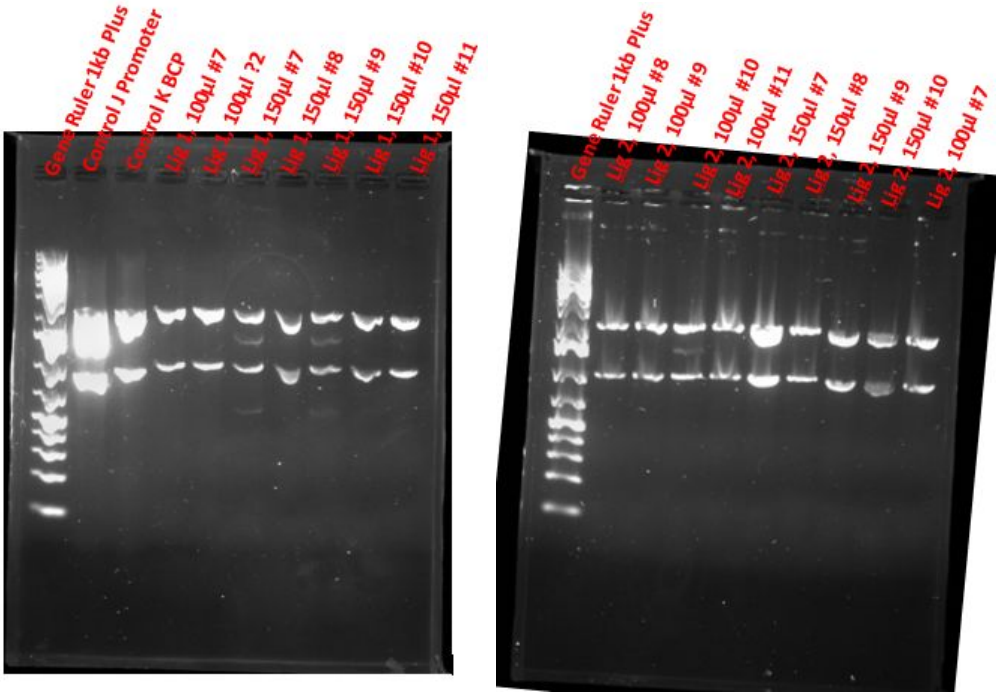
Running

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 127 V

Expected Results:



Results



Conclusion:

These results show that the transformation was successful.

Name: Sijia, Jaizi, Shakera
Date: 6/21/19

Goal:

1. PCR
    a. Pcb302 in E. Coli with primers 1-4 from miniprep done on 6/20/19
    b. Ligation (J23102 with K592009 in pcba10020-r0040

Protocol:

PCR Protocol (for ligation samples)

1. Added 7µL of diH2O, 10 µL PCR Mastermix(2x), 1 µL of the forward primer, 1 µL of the reverse primer, and 1 uL sample into PCR tubes.
2. Placed PCR tubes 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

PCR Protocol For pCB302 plasmid miniprep

1. Diluted primers 1-4
    a. 10 µL of 100 µM primers and 90 µL IDTE
    b. Named them 10 µM pCB302 primer 1-4
2. Set up 3 PCR rxn for each pCB302 miniprep
    a. Primers 1&2
    b. Primers 3&4
    c. Primers 1&4
3. Added in PCR tubes
    a. 7 µL H2O
    b. 10 µL MasterMix
    c. 1 µL of sample
    d. 1 µL of primer combination (1&2, 3&4, 1&4)
4. Placed PCR tube in the thermocycler at the following generic settings:
    a. 95° C for 3:00 minutes
    b. 95° C for 1:00 minute
    c. 52° C for 1:00 minute
    d. 72° C for 1:00 minute
    e. 30X (Go to Step 2)
    f. 72° C for 5:00 minutes
    g. Lid Temperature: 105° C

5. PCR tubes were then stored for later

Date: 6/21/19

Goal:

1. Prepare ASP-8A media as per the recipe

Protocol:

ASP-8A STOCK 1L

NaCl 25 g

KCl 1 M - 35 g/500 ml 10 mL

$MgSO_4 7H_2O$ 1.8 M-450 g/ 1 L 20 mL

$CaCl_2 2H_2O$ 0.75 M-55 g/500 ml 10 mL

$NaNO_3$ 0.58 M-2.5 g/50 ml 1 mL

KH_2PO_4 0.073 M-0.5 g/50 ml 1 mL

NTA 0.157 M-1.5 g/50 ml 1 mL

Tris Base pH 9 0.825 M-100g/1 L 10 mL （ADJUST pH 8.5）

NH_4NO_3 12.5 mM-.05g/50 ml 1 mL

PII Metal Mix 10 mL

8A Vitamin Mix (x2)
0.25mL

Vitamin B_{12} 10 µg/mL 100 µL

***GeO_2** 9.5 mM-50 mg/50 mL 2.5 mL

Autoclave 45 min.