Name: Asma, Sijia, Jiazi, Krithika, Chiara , Laura
Date: 6/25/19

Goal:

1. Redo gel electrophoresis for PCR products
    a. Ligations (K592009 & J23102); Vf, Vr
    b. Pcb302 in E. Coli from papers A & B
2. Redo PCR on ligations (40 µL reaction)
    a. K592009 & J23102
3. Perform restriction digest on pcb302 from minipreps in E. Coli from papers A & B (colony 7) (6/20/19)
    a. Digested with KpnI
4. Make ASP-8A medium
5. Transformation on K592009 /J23102 ligation mix from 06/17
6. O.Marina
    a. Fed 3 mL of D. Tertiolecta

Date: 7/30/19

Goal:

1. Redo gel electrophoresis for PCR products
    a. Ligations (K592009 & J23102); Vf, Vr
    b. Pcb302 in E. Coli

Protocol:

**Gel electrophoresis**

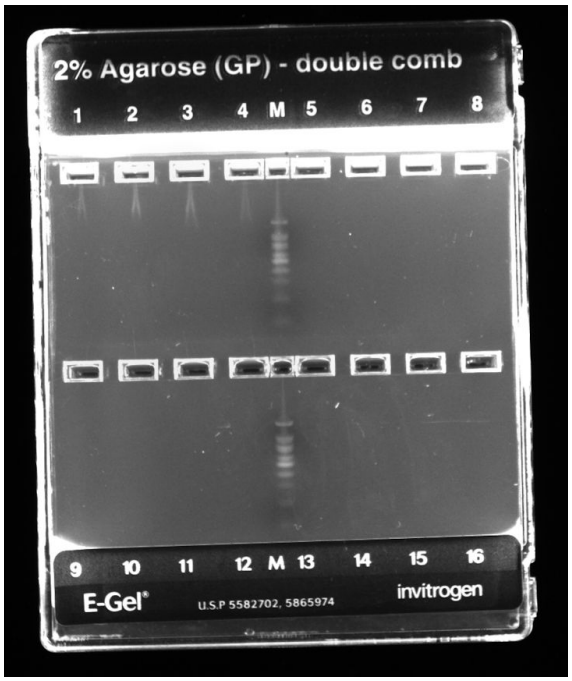
1. Loaded 2 µL of DNA into each well
2. Loaded 4 µL of 1 kb plus DNA ladder
3. Ran on 2 % double comb fast E-gel for 25 minutes

Results:

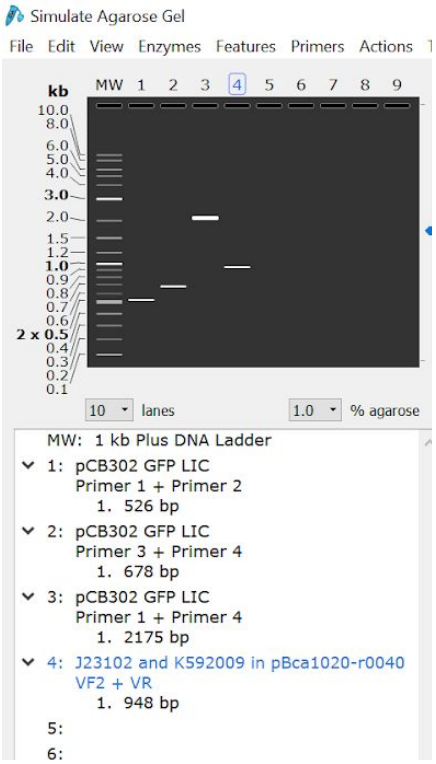
**Gel Key (Ligations and pcb302)**

| **Lane #** | **Sample** |
|---|---|
| 1 | Ligation 1, 150 µL, colony 11 |
| 2 | Ligation 2, 100 µL, colony 7 |
| 3 | Ligation 2, 100 µL, colony 12 |
| 4 | Ligation 1, 100 µL, colony 9 |
| M | 1 kb plus DNA ladder |
| 5 | Pcb302, colony 7, Primers 1 & 2 |
| 6 | Pcb302, colony 8, Primers 1 & 2 |
| 7 | Pcb302, colony 9, Primers 1 & 2 |
| 8 | Pcb302, colony 10, Primers 1 & 2 |

| Lane # | Sample |
|---|---|
| 9 | Pcb302, colony 7, Primers 3 & 4 |
| 10 | Pcb302, colony 8, Primers 3 & 4 |
| 11 | Pcb302, colony 9, Primers 3 & 4 |
| 12 | Pcb302, colony 10, Primers 3 & 4 |
| M | 1 Kb plus DNA ladder |
| 13 | Pcb302, colony 7, Primers 1 & 4 |
| 14 | Pcb302, colony 8, Primers 1 & 4 |
| 15 | Pcb302, colony 9, Primers 1 & 4 |
| 16 | Pcb302, colony 10, Primers 1 & 4 |



Expected Results:




Conclusion:

This gel did not produce any solid bands.

Date: 6/25/19

Goal:

1. Redo PCR on ligations (40 µL reaction)
    a. K592009 & J23102

Protocol:

**40 µL PCR of Ligated BCP Samples**

1. A 5x cocktail was created with the following proportions: 70µl diH2O, 100µl PCR MasterMix, 10µl Vr Primer, and 10µl Vf Primer
2. Each PCR tube was loaded with 38µl of the cocktail mix and 2µl of the “Ligation” DNA
3. The samples were:
    a. Ligation 1, 150µl, colony 11
    b. Ligation 1, 100µl, colony 7
    c. Ligation 2, 100µl, colony 7
    d. Ligation 2, 100µl, colony 12
4. The samples were then put in the PCR Thermocycler in the Vf/Vr cycle and were left overnight

Date: 6/25/19

Goal:

1. Perform restriction digest on pcb302 from minipreps in E. Coli (colony 7) (6/20/19)
    a. Digested with KpnI

Protocol:

**30 µL Fast Digest Restriction Digest**

1. Prepared a Fast Digest concentration cocktail with the following proportions: 1 µL Restriction Enzyme KpnI, 3 µL of 10X Fast Digest Buffer, and 16 µL of diH2O.
    a. Let enzyme thaw on ice
2. Added 20 µL of this cocktail to a clean 1.5 Eppendorf tube and then added 10 µL of DNA
3. Incubated at 37° C for 30 minutes.

Date: 6/25/19

Goal:

1. Make ASP-8A medium

Protocol:

**Make ASP-8A medium**

1. **Vitamin 8A Mix 2X 1L**

| **COMPONENTS** | **2X STOCK (1L)** |
|---|---|
| p-aminobenzoic acid | 0.0172g |
| biotin | 0.001g |
| B12 | 0.001g |
| Choline diH2 citrate | 1g |
| Folic acid | 0.005g |
| Folinic acid, Ca salt | 0.0004g |
| Inositol | 2g |
| Nicotinic acid | 0.2g |
| Orotic acid | 0.036g |
| D-Pantothenic,Ca salt | 0.4g |
| B6 | 0.12g |
| Riboflavin(B2) | 0.01g |
| Thiamine,HCl(B1) | 0.4g |
| Thymine | 1.6g |

**2.ASP-8A MEDIUM 1L**

| **COMPONENTS** | **1L** |
|---|---|
| NaCl | 25g |
| KCl (1M) | 10ml |
| MgSO4 7H2O(1.8M) | 20ml |
| CaCl2 2H2O(0.75M) | 10ml |
| NaNO3(0.58M) | 1ml |
| KH2PO4(0.073M) | 1ml |
| NTA(0.157M) | 1ml |
| Tris Base pH 9 | 10ml |
| Adjust pH 8.5 | |
| NH4NO3 | 1ml |
| PII Metal Mix | 10ml |
| 8a Vitamin Mix | 0.25ml |
| Vitamin B12 | 100ul |
| GeO2 | 2.5mg |

3. Autoclaved for almost 46 minutes.

Date: 6/25/19

Goal:

1. Transformation on K592009 /J23102 ligation mix from 06/17

Protocol:

**Heat Shock**

1. Thawed One Shot TOP10 chemically competent cells on ice.
2. Added 2 µL of DNA sample into competent cells
3. Incubated the cells on ice for 35 minutes.
4. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
5. **Quickly** took them out and **immediately** added 250µL of SOC medium
6. Placed the samples into a 37° C shaking water incubator for 1 hour at 200 rpm.
7. After shaking for 1 hour, streaked 150 µL of the solution onto an agar plate with the respective antibiotics.
    a. Ampicillin
8. Incubated plates at 37°C for at least 24 hours.