August 6, 2009

- Streaked out BBa_K112808 (Enterobacteria Phage T4 Lysis Device) and 3 different colonies of BBa_K112022 (Lambda Phage Lysis Device) from our previous plates
- Grew in 37ºC incubator overnight

August 10, 2009

- Grew one liquid culture for each of the four plates
  - -each liquid culture contained 3mL LB, 3µL ampicillin, and 1 colony
  - -these cultures were grown in the 37ºC shaker overnight

August 11, 2009

- Centrifuged the liquid cultures at 13,000 rpm for 5 minutes to pellet the bacterial cells
- Removed the supernatant
- Purified the DNA from each culture according to the procedure on p. 22-23 of the QIAprep Miniprep Handbook

  -Resuspend pelleted bacterial cells in 250 µL Buffer P1 and transfer to a microcentrifuge tube.
  -Add 250 µL Buffer P2 and mix thoroughly by inverting the tube 4-6 times.
  -Add 350 µL Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times.
  -Centrifuge for 10 min at 13,000 rpm in a table-top microcentrifuge.
  -Apply the supernatants from step 4 to the QIAprep spin column by decanting or pipetting.
  -Centrifuge for 30-60 s. Discard the flow-through.
  -Recommended: Wash the QIAprep spin column by adding 0.5 mL Buffer PB and centrifuging for 30-60 s. Discard the flow-through.
  -Wash QIAprep spin column by adding 0.75 mL Buffer PE and centrifuging for 30-60 s.
  -Discard the flow-through, and centrifuge for an additional 1 min to remove residual wash buffer.
  -Place the QIAprep column in a clean 1.5 mL microcentrifuge tube. To elute DNA, add 50 µL Buffer EB or water to the center of each QIAprep spin column, let stand for 1 min, and centrifuge for 1 min.

August 17, 2009

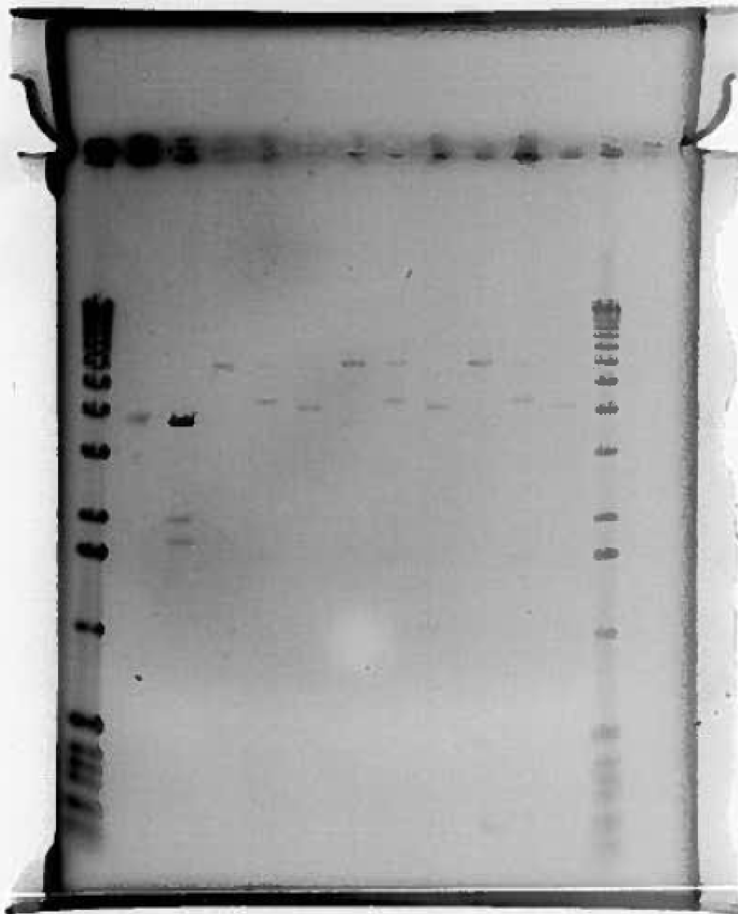
- Digested the four tubes of DNA with various enzymes

| 10 µL T4 DNA | 10 µL T4 DNA | 10 µL lambda DNA | 10 µL lambda DNA | 10 µL lambda DMA |
|---|---|---|---|---|
| 7 µL dd$H_2O$ | 6 µL dd$H_2O$ | 7 µL dd$H_2O$ | 6 µL dd$H_2O$ | 6 µL dd$H_2O$ |
| 2 µL Buffer 2 | 2 µL Buffer 2 | 2 µL Buffer 2 | 2 µL Buffer 2 | 2 µL Buffer 3 |
| 1 µL EcoRI | 1 µL PstI | 1 µL EcoRI | 1 µL EcoRI | 1 µL BamHI |
| | 1 µL XbaI | | 1 µL XbaI | 1 µL EcoRV |

August 18, 2009

- Ran a gel of the digestions from 8-17-09

    Lane 1 – 1 kb Invitrogen ladder
    Lane 2 – T4 cut w/ EcoRI
    Lane 3 – T4 cut w/ PstI and XbaI
    Lane 4 – lambda #1 cut w/ EcoRI
    Lane 5 – lambda #1 cut w/ EcoRI and XbaI
    Lane 6 – lambda #1 cut w/ EcoRV and BamHI
    Lane 7 – lambda #2 cut w/ EcoRI
    Lane 8 – lambda #2 cut w/ EcoRI and XbaI
    Lane 9 – lambda #2 cut w/ EcoRV and BamHI
    Lane 10 – lambda #3 cut w/ EcoRI
    Lane 11 – lambda #3 cut w/ EcoRI and XbaI
    Lane 12 – lambda #3 cut w/ EcoRV and BamHI
    Lane 13 – 1 kb Invitrogen ladder



September 14, 2009

- Grew a 25mL liquid culture of cells containing the plasmid pKD46 (which contains the arabinose-inducible promoter) using 25mL LB, 25µL ampicillin, and 1 colony

September 15, 2009

- Minipreped three 5mL samples of the liquid culture using the procedure used on 8-11-09

September 16, 2009

- Ran a PCR for each of the minipreped samples
  - 10 µL 10x PCR reaction buffer
  - 3 µL $MgCl_2$
  - 2 µL Primer (forward)
  - 2 µL Primer (reverse)
  - 2 µL dNTP mix
  - 10 µL template DNA
  - 71 µL $ddH_2O$
  - 0.5 µL taq DNA polymerase

September 17, 2009

- Digested the PCR product
  - 20 µL araC/araBp PCR product
  - 3 µL Buffer 4
  - 1 µL EcoRI
  - 1 µL NdeI
  - 5 µL $ddH_2O$
- Put in 37ºC incubator overnight

October 6, 2009

- An error was noticed in the primer for the araC/araBp piece, so I re-did the PCR using the new primer and procedure from 9-16-09 (2 tubes).

October 7, 2009

- Digested the 2 tubes of PCR product and 2 tubes of a vector (pLeuLP, prepared 6-11-09) using the procedure from 9-17-09

I plan on purifying the DNA from these two digestions, then ligating the arabinose-inducible promoter into the vector. Next, I can digest this new plasmid and the T4 lysis part with SpeI and PstI, and then ligate them together. At this point, I will be able to transform the plasmid into pseudomonas putida and test the killing strength of the T4 lysis device in this bacterium.