Tuesday 8/4/09

Megalign Pu promoter from registry that is available with primers

Want to determine if the part we received from the registry can be used as a positive control for the Pu promoter colony PCR

Colony PCR Pu Promoter

1. Take a stab of the frozen stock of *P. putida* pWW0 from the IGEM -80C frozen stock (not too much because too much template will lead to unspecific replication) and resuspend it in 50 uL of DI water
2. Turn on PCR machine to instant incubate at 95 C for the first initial denaturation step
3. Place PCR tubes for run on ice
4. PCR reaction KEEP EVERYTHING ON ICE
    a. Vortex all tubes before starting to make sure everything is well mixed
    b. For a single reaction mixture
        i. 36.275 uL Ultra pure water (37.75 uL was added for this experiment by accident)
        ii. 10 uL of 5x phusion master mix
        iii. 1 uL 10 mM dNTP
        iv. 0.625 uL of primer A (0.5 uM concentration from 40 uM stock)
        v. 0.625 uL of primer B (0.5 uM concentration from 40 uM stock)
        vi. 1 uL of DNA template (from step 1)
        vii. 0.5 uL Phusion DNA polymerase
    c. Combine all ingredients but polymerase for 5 samples
        i. 181.25 uL of ultra pure water (188.75 uL was added for this experiment accidentally)
        ii. 50 uL of 5x phusion master mix
        iii. 5 uL 10 mM dNTP
        iv. 3.125 uL of primer A
        v. 3.125 uL of primer B
        vi. 5 uL of DNA template (from step 1)
    d. Chill mixture for 15 minutes
    e. Add 2.5 uL Phusion DNA polymerase with chilled pipette tip
    f. Transfer 50 uL of sample to each PCR tube with chilled pipette tip

5. PCR cycle
    a. 95 C for 6 minutes
    b. 98 C for 10 seconds
    c. 52 C to 62 C for 30 seconds
        i. Sample 1: column 1 @ 52C
        ii. Sample 2: column 4 @ 53.7C
        iii. Sample 3: column 6 @ 56.3C
        iv. Sample 4: column 7 @ 58C
        v. Sample 5: column 9 @ 60.5 C
    d. 72 C for 15 seconds
    e. Goto step b four times
    f. 98 C for 10 seconds

g. 69 C for 30 seconds
h. 72 C for 15 seconds
i. Goto step f twenty nine times
j. 4C forever
k. End

Meeting with Pablo about using the HPLC

- C-18 column: reverse polarity coated with 18 chain carbon
  - 3 micron particles
  - Approximately 10 inches long
  - Organic substances move through slowly
  - Polar substances pass through quickly
- UV detector
- Share with grad student in Dr. Wang's Lab
- Purchase solvents/column

Gel of the Pu promoter colony PCR products

A gel was run to see if the Pu promoter was amplified according to the protocol on 7/27/09.

Results

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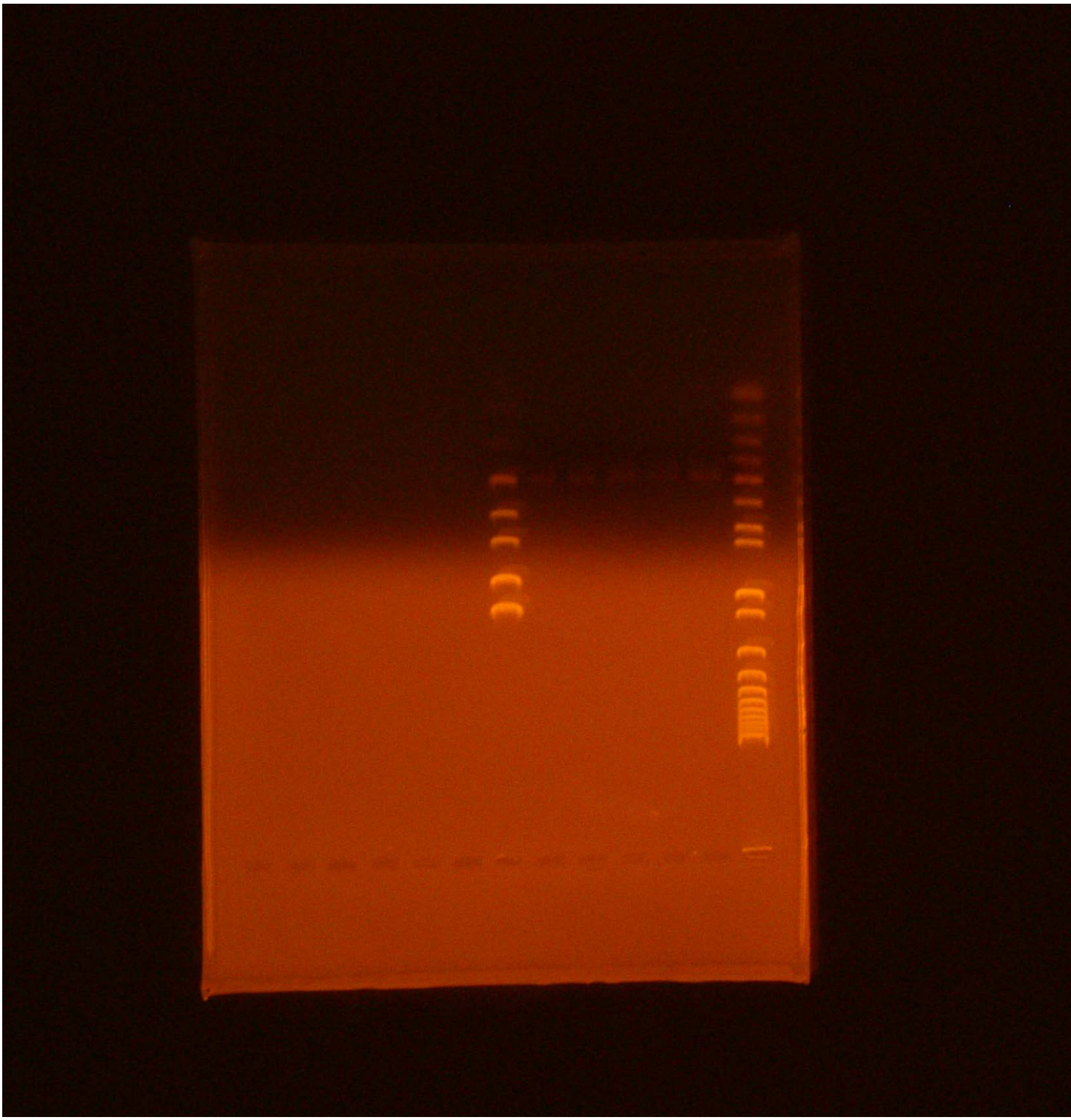
After looking at the DNA sequence the Glasgow 2007 IGEM team submitted, no significant overlap between either of the primer was found for the Pu-luciferase part. This means there will be no positive control for the Colony PCR of the Pu promoter

Gel of the Pu promoter colony PCR products



Lane 1: Invitrogen 1 kb plus ladder (far right)
Lane 2: Sample 1-initial annealing at 52C
Lane 3: Sample 2-initial annealing at 53.7 C
Lane 4: Sample 3-initial annealing at 56.3 C
Lane 5: Sample 4-initial annealing at 58 C
Lane 6: Sample 5-initial annealing at 60.5 C

Lane 7: Exact gene low range DNA ladder

Expected length: Approximately 500 bp

Faint bands with expected length!