Saturday 10/10/09

PCR amplification of gel extraction PrXylR

Sample Preparation

1. Vortex all tubes before starting to make sure everything is well mixed
2. Make two reaction mixtures for the 2 PrXylR gel extraction DNA templates
    a. 37.5 uL Ultra pure water
    b. 10 uL of 5x phusion master mix
    c. 1 uL 10 mM dNTP
    d. 0.500 uL of primer A
    e. 0.500 uL of primer B
    f. 1 uL DNA template (from gel purification)
    g. 0.5 uL Phusion hot start DNA polymerase
3. Transfer 50 uL of sample to each PCR tube

Cycling conditions

1. 98 C for 30 seconds
2. 98 C for 10 seconds
3. 66.7 C for 30 seconds
4. 72 C for 35 seconds
5. Goto step 2 for 34 cycles
6. 72 C for 5 minutes
7. 4C forever
8. End

Colony PCR Pu promoter from P. putida *mt-2(modified from protocol on 8/4/09)*

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. Mix the primers by making a 50 uM stock. Multiply the nmoles of DNA in each primer by 20, and resuspend in that amount of ultra pure water
    a. Upstream
        i. 6.5nmoles
        ii. 130 uL of ultra pure water
    b. Downstream
        i. 44.2 nmoles
        ii. 884 uL of ultra pure water
3. Turn on PCR machine to instant incubate at 98 C for the first initial denaturation step
4. Place PCR tubes for run on ice, place tips in -20C freezer
5. 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. 37.5 uL Ultra pure water

ii. 10 uL of 5x phusion master mix
iii. 1 uL 10 mM dNTP
iv. 0.500 uL of primer A
v. 0.500 uL of primer B
vi. 1 uL of DNA template (from step 1)
vii. 0.5 uL Phusion DNA polymerase

c. Combine all ingredients but polymerase for 6 samples
   i. 225 uL of ultra pure water (217.65 uL of ultra pure water was added to this reaction
   ii. 60 uL of 5x phusion master mix
   iii. 6 uL 10 mM dNTP
   iv. 3uL of primer A
   v. 3 uL of primer B
   vi. 6 uL of DNA template (from step 1)
d. Chill mixture for 15 minutes
e. Add 3 uL Phusion DNA polymerase with chilled pipette tip
f. Transfer 50 uL of sample to each PCR tube with chilled pipette tip

6. PCR cycle

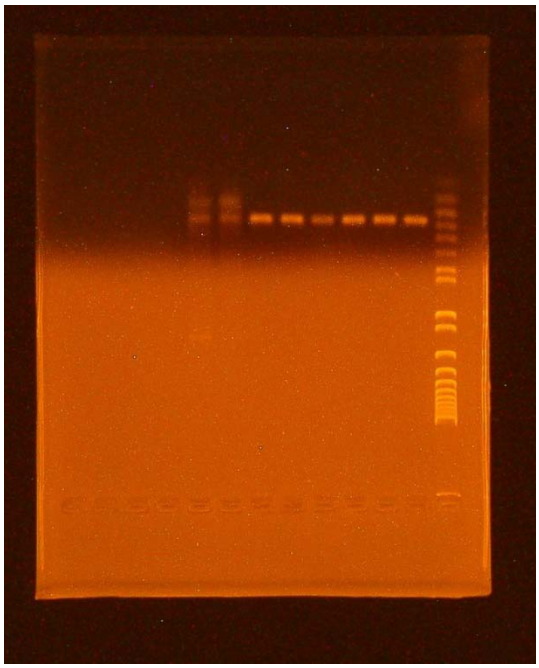
   a. 98 C for 30 minutes
   b. 98 C for 10 seconds
   c. 53 C to 58 C for 30 seconds
   d. 72 C for 15 seconds (15 seconds per 1 kb for plasmid DNA)
   e. Goto step b four times
   f. 98 C for 10 seconds
   g. 69.5 C for 30 seconds
   h. 72 C for 15 seconds
   i. Goto step f twenty nine times
   j. 72 C for 5 minutes
   k. 4C forever
   l. End

Gel of PrXylR amplification and Pu promoter colony PCR

A gel was run according to the protocol on 7/27/09 for the 6 Pu promoter colony PCR samples and the PrXylR PCR amplification

Results

Gel of PrXylR amplification and Pu promoter colony PCR



Lane 1 (far right): Invitrogen 1 kb plus ladder
Lane 2: Pu 1
Lane 3: Pu 2
Lane 4: Pu 3
Lane 5: Pu 4
Lane 6: Pu 5
Lane 7: Pu 6
Lane 8: PrXylR 1
Lane 9: PrXylR 2

When loading the gel only 3 uL were loaded into the Lane 5 for Pu 4.

The expected length of PrXylR is 2338 bp
The expected length of Pu is 370 bp

The re-amplification of the PrXylR part was unsuccessful so the gel extraction purification will be used for the digestion.

The Pu promoter amplification was successful and will be purified for digestion