Friday 10/9/09

Charlie and Jungho helped with lab work today ☺

Protocol for Gel extraction (adapted from Jeremy's Protocol) used for extraction of PrXylR colony PCR

Running the Gel

1. Follow protocol on 7/27/09 for making a gel (50 mL TAE with .35 g or agarose) with following changes
    a. Use the larger comb to make the wells
    b. Mix the PCR product and blue juice in a 1:5 ratio
        i. 40 uL of PCR product (sample 2 from gel on 10/6/09 of PrXylR colony PCR)
        ii. 10 uL of 10X blue juice
    c. Mix ladder
        i. 2.5 uL of concentrated invitrogen 1 kb plus ladder
        ii. 17.5 uL of DI water
        iii. 5 uL of 10X blue juice
2. Before performing the electrophoresis make sure to use **FRESH TAE** so product does not become contaminated
3. Load gel with 25 ul of sample per well
4. Set to 85 V and run for 1:15 to 1:30

Gel extraction

1. Preheat water bath to 50 C
2. Weigh and label 1 eppendorf tube for each sample
3. Use clean razor blade to cut sample from gel and clean tweezers to transfer the gel slice into the eppendorf tube
    a. **BE CONSCIENCE OF ETHIDIUM BROMIDE CONTAMINATION!!**
        i. Do not touch the outside of the eppendorf tube with anything that has contacted the gel
        ii. Everything in this process should be disposed of in the ethidium bromide waste (column from gel extraction, eppendorf tube, ext.)
    b. When the gel is done running, drain of all excess buffer, pat dry and place on the transilluminator. When working with the gel wear UV glasses and try to keep the shield down if possible.
    c. Turn on the transilluminator and score the gel on each side of the band with a razor blade
    d. Turn off the transilluminator and cut out the band

e. Place the band back on the transilluminator and make sure the right part of the gel was cut
f. Place the band in the respective eppendorf tube

QIAgen kit

1. Cap and weight the eppendorf tubes
    a. Determine the weight of the band by subtracting the weight of the tube
    b. The maximum weight of the band is 300-400 mg
2. Add 3 volumes of buffer QG to 1 volume of gel
    a. 100 ug of gel is approximately 100 uL of QG buffer
3. Incubate at 50C in water bath for 10 minutes , or until completely dissolved. Every 2-3 minutes vortex to help the gel band dissolve
4. After the gel has dissolved the liquid should be yellow, if the solution is orange or purple the pH is too high. Add 10 uL of 3 M sodium acetate at a pH of 5
5. If DNA product is <500 bp or >4kb, add 1 gel volume isopropanol
6. Apply mixture to the spin column with 2 mL collection tube
7. Centrifuge at 13000 rpm for 1 minute
8. Discard flow through and place column back into the collection tube
9. Add 500 uL of QG buffer to wash off residual agarose, centrifuge at 13000 rpm for 1 minute
10. Add 750 uL of buffer PE and **LET SIT FOR 2-5 MINUTES.** Centrifuge at 13000 rpm for 1 minute
11. Discard flowthrough and centrifuge again at 13000 rpm for 1 minute
12. Place column into an eppendorf tube
13. Add 50 uL of ultra pure water **DIRECTLY TO THE MEMBRANE** to elute the DNA, **LET SIT FOR 1 MINUTE** and centrifuge at 13000 for 1 minute

Nanodrop of PrXylR gel extraction product

The nanodrop for each of the 2 PrXylR gel extraction samples was performed according to the protocol on 7/25/09

Gel of PrXylR gel extraction product

A gel was run according to the protocol on 7/27/09 except the amount added to each well was doubled for a total volume of 10 uL total.

Results

Gel Extraction of PrXylR

Weight of tube #1=1.0326 g
Weight of tube #2=1.0277 g

Weight of tube #1 plus sample=1.2505 g
Weight of tube #2 plus sample=1.2383 g
Weight of sample #1=.2279 g
Weight of sample #2=.2108 g
QG buffer for sample #1=684 uL
QG buffer for sample #2=633 uL

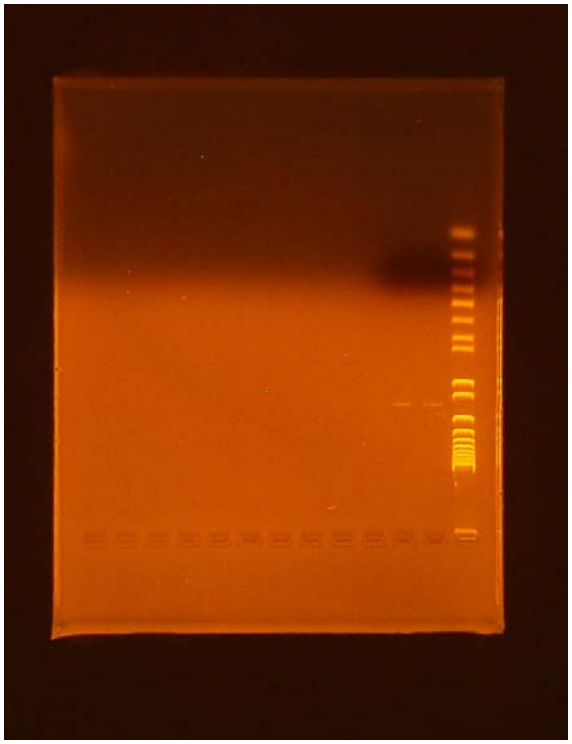
Nanodrop of PrXylR gel extraction product

10/9/2009 5:38 PM

Sample ID	ng/uL	A260	260/280	260/230	Constant	
PrXylR gel extraction 1		1.92	0.038	2.03	0.03	50
PrXylR gel extraction 2		2.10	0.042	1.30	0.03	50

There was no strong peak at 260 nm indicating there is probably no DNA in our sample. Since that nanodrop does not pick up DNA concentrations below 10 nm a gel will still be run the gel extraction samples to check for low concentrations of DNA. If any DNA is found in the gel, this DNA will be used as a template for a new PCR to get a pure product.

Gel of PrXylR gel extraction product



Lane 1 (far right): Invitrogen 1 kb plus ladder
Lane 2: PrXylR gel extraction #1
Lane 3: PrXylR gel extraction #2

The expected product length is 2338 bp

Since weak bands appeared at the expected length, the DNA from the gel extraction will be used as the DNA template for a second PCR reaction to amplify the fragments.