iGEM Notes 4/10/09

Clean up the lacI PCR product (obtained originally from pleu repressilator) digested (by Amrit) with XbaI and PstI using the Qiagen PCR purification kit following the manual instructions.

Running a Gel!

1. Make 1% Agarose Solution
    a. 1 g of Aarose Low EEo powder (2nd shelf)/100 mL TAE
    b. TAE solution
        i. 20 mL of tris acetate 50X (below microwave)
        ii. Fill with DI/distilled water to 1L mark
2. Make Gel
    a. Choose a mold
        i. Longer gels=better resolution
    b. Choose comb for # of samples
    c. Black bars to prevent agar from leaking
    d. Microwave 1% Agarose Solution so dissolved (approximately 2-3 minutes)
    e. Let 1% Agarose cool
    f. Pour Agarose into mold, do not let it overflow
    g. Wait 5-10 minutes to solidify
3. Pour TAE into machine so it will cover the gel
4. Place the gel into the TAE buffer
    a. Make sure it is aligned next to the wall so it runs straight
5. Preparing Samples
    a. Mix samples on tape
        i. 1.5 µL of dye
        ii. Add 4.5 µL of sample to the dye, mix by pipetting
        iii. Add into gel, record the lane each sample goes into
        iv. Add 1.5 µL of ladder directly to the gel because already has dye (smaller amount because the ladder is concentrated)
6. Turn on the machine
    a. Make sure the voltage is on minimum
    b. Turn on
    c. Hit DC start
    d. Start running the gel at 50 V and after it has started turn up to 100V
        i. Start running at 50 for better resolution
7. Developing gel
    a. Let the sample run ¾ down the gel
    b. Remove and place in container of ethidium bromide for 15 minutes
    c. Use spatula to take gel out
        i. Put on the tray to transilluminator
        ii. Wipe excess liquid with paper towel so the gel doesn't move
    d. If the program isn't open

i. Click Quantity one
ii. Hit okay
iii. Choose basic
iv. File
v. Gel doc XR
vi. Hit auto expose
    1. wait til you see the gel than hit freeze
vii. Save to Desktop
    1. Ninfa
        a. Probj.
viii. Print

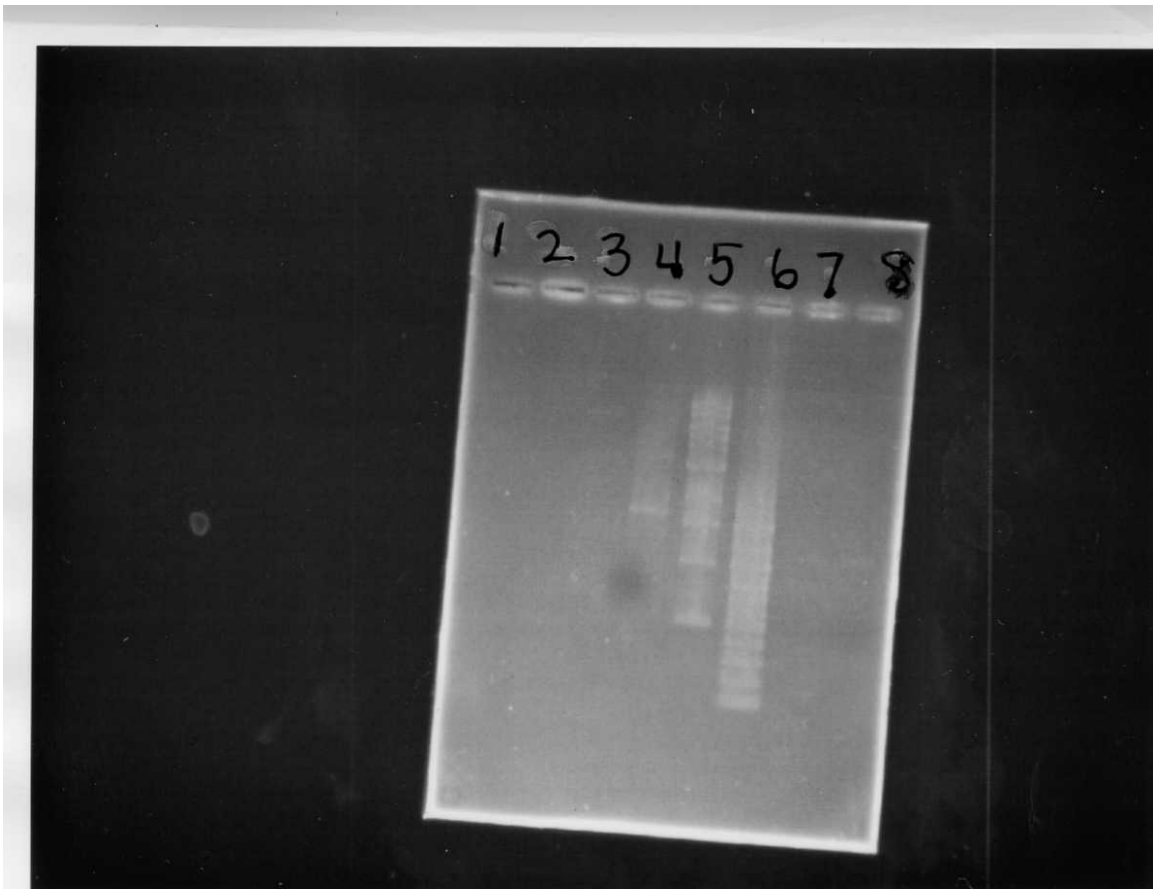
e. Take gel out
f. Wipe with chemical wipes and DI water
g. Place gel in red bucket in gel room for proper disposal with ethidium bromide

Ligation

- Restriction enzymes in freezer 2 in box labeled restriction enzymes
- KEEP ENZYMES COLD IN COLD BOX OR ON ICE!
- In freezer 2 Stephan's DNA box #1 contains ligase and ligase buffer, also Taq polymerase and PCR buffer
- Must check correct buffer online
    - New England Biolabs

1. Check amount of DNA in sample with spectophotometer
    a. Press UV
        i. Lower case u will appear
        ii. Press ABS
            1. type 260 (for DNA, 280 for protein)
        iii. hit λ
        iv. wait 30 minutes for UV lamp to warm up, u will become upper case when ready (U)
        v. Read Amount of DNA in microcell
            1. add 50µL ddH20 to wash by pipetting
                a. Don't touch clear ends on the side
                b. Repeat
                c. Remove ddH2O remove with pipet
            2. add 50 µL of ddH20
                a. make sure you have a meniscus
            3. Place in machine so red dot is facing you
            4. Hit calibrate CALB to blank
            5. Add the sample in a proportion to ddH2O
                a. High concentration 1 µL sample : 49 µL ddH2O
                b. Low concentration 5 µL sample: 45 µL ddH2O
            6. Take measurement in reader
        vi. Repeat for next sample, must blank every sample
2. To Be Continued...

Agarose Gel with LacI digests and nifa PCR purification



Lane	Sample
1	LacI 1 (PCR sample from pelu repressilator digested with XbaI and PstI, expected 1300 bp)
2	LacI 2 (PCR sample from pelu repressilator digested with XbaI and Pst expected 1300 bp)
3	p clock 2 (repressor module for Sequestilator, control to make sure the enzyme cut, know the pattern these digestion enzyme make on the gel, 2000 and 5000bp band)
4	nifa (make sure PCR purification worked, band should show up at 1600)
5	100 bp ladder
6	1 kb ladder
7	LacI 2 (same as lane 2, problems loading)
8	LacI 1 (same as lane 1, problems loading)

Analysis: Digest was not efficient. The digest on the control did not work and only faint bands showed up where expected on the digested LacI samples in lanes 7 and 8. nifa was purified successfully!

Spectrophotometer Readings

Spectrophotometer Reading

Sample	OD 260	DNA ng ((OD(260))x(Dilution factor)x(50(constant))
LacI 1	0.02	10
LacI 2	0.013	6.5
Tetr	0.019	9.5

The dilution for the samples was made with 5 μL of sample for 45 μL of ddH2O