EXERCISE 9B - Lab Procedures

I. Examine your DNA gel in order to determine which unknown plasmid sample had no plasmids, which had non-recombinant pUC18 plasmids, and which had recombinant pUC18 plasmids

1. Place a piece of plastic wrap over the top of a light box and gently slide your gel onto the plastic wrap. Cover your gel with another piece of plastic wrap.

2. Orient your gel so the triangular notch is in the lower right corner. Remember that the wells were assigned numbers counting from left to right.

   Several of the lanes should contain blue bands. The blue bands are areas where DNA has been stained with methylene blue. Because the migration rate of the DNA fragments through the gel is based on size, all of the fragments in a single band should have the same size. Also, because you did not load any sample into well 6, this lane should have no bands.

3. How many bands are visible in the lane that was loaded with unknown sample A + *EcoR*I? _____

   How many bands are visible in the lane that was loaded with unknown sample B + *EcoR*I? _____

   How many bands are visible in the lane that was loaded with unknown sample C + *EcoR*I? _____

4. Based on your observations, which unknown sample contained no plasmids? Explain your answer.

   _____

   _____

   _____

   Which unknown sample contained plasmid pUC18 with no "foreign DNA" insert (non-recombinant plasmids)? Explain your answer.

   _____

   _____

   _____

   Which unknown sample contained plasmid pUC18 with the phage λ DNA insert (recombinant plasmids)? Explain your answer.

   _____

   _____

   _____

II. Measure the migration distance of the DNA fragments in your gel

1. "Marker DNA" is a commercially available preparation that contains many DNA fragments of known length. The marker DNA that you used contained DNA fragments with the following lengths:

784 bp(base pairs)
1120 bp
2040 bp
3621 bp

Which fragments do you expect to travel farthest from the well? _____

Which fragments do you expect to travel the least distance from the well? _____

Explain:

_____

_____

2. Using a transparent millimeter ruler, measure the distance from the middle of each well to the middle of each DNA band on your gel. There should be four bands in the two "DNA marker" lanes and fewer bands in the A, B, and C lanes.

Distance each DNA fragment traveled (mm)

Lane 1 Sample A	Lane 2 Marker DNA	Lane 3 Sample B	Lane 4 Marker DNA	Lane 5 Sample C

III. Use size and migration distance of the 4 DNA markers to prepare a standard curve

1. Using either a sheet of graph paper or a computer with spreadsheet program, make a scatter diagram that shows the relationship between log of bp and migration distance for the 4 DNA markers. Plot migration distance on the x-axis and log of bp on the y-axis.

Make sure your scatter diagram is big enough so that it fills an entire 8.5" x 11" sheet of paper. See Appendix E for more detailed descriptions of graphing techniques.

2. Using either a hand-held calculator or a computer with spreadsheet program, carry out linear regression to determine the equation for the "best fit" straight line for your data points. Write the equation on your graph. Next to the equation, write down the linear correlation coefficient. If the absolute value of the linear correlation coefficient is less than 0.95, ask your instructor for help.

If you used a calculator, do not clear its memory at this point. You will use the stored data in Part IV.

IV. Estimate the size of the fragments that were produced when EcoRI digested the DNA in your 3 unknown samples

1. Use your linear regression equation to estimate the size of the DNA fragments listed below. Make sure you include appropriate units of measure:

   plasmid pUC18 _____

   phage λ DNA _____

V. Observe your *E. coli* cultures

1. Retrieve the Petri dishes that you inoculated with *E. coli.*

2. Record the color of the colonies (if any) in each plate:

   Plate A _____

   Plate B _____

   Plate C _____

Clean up

Dispose of any materials that had contact with *E. coli* cells in a **biohazard bag**.

Dispose of your excess solutions as directed by instructor.

Remove label tape and any marks made with a marking pen from all glassware. Wash and rinse all glassware, give it a final rinse with dH_2O, and leave it inverted at your work area in order to drain.

All disposable glassware goes into the special glass disposal receptacle.

All instruments should be turned off and unplugged.

Wipe off your workspace with disinfectant.

Make sure everything that you have used is clean, put away, or discarded. Leave your work area in the same order that you found it in.

Wash your hands and ask your instructor to check your work area before you leave.