Laboratory Questions and Analysis

1. Compare the DNA fingerprints produced by restriction digestion of the PCR products generated from the children's DNA and the DNA fingerprints created by digestion of the PCR products from the control CF mutant and wild-type DNA.

   a. Which pattern does the DNA fingerprint from each child's DNA match—the pattern from the mutant DNA, the pattern from the wild-type DNA, or a combination of both?

   b. What does this tell you about the genotype of the children with respect to this CF mutation?

2. Remember that this particular mutation in the cystic fibrosis gene destroys a restriction enzyme site. If you did not know about the destroyed restriction site, what about the fragment patterns on your gel would indicate to you that a restriction enzyme site had been destroyed?

3. Being able to calculate the molecular weight of DNA fragments on a gel is an integral part of using electrophoresis as a tool. Calculate the size of the DNA fragments in the lanes containing the samples generated from the children's DNA.

   In general, linear, double-stranded DNA fragments like the ones used in this lab migrate at rates inversely proportional to the $\log_{10}$ of their molecular weights. Because of this relationship, the molecular weight of a DNA fragment can be interpolated from the distance the DNA fragment moves into the gel. In order to interpolate the molecular weight, the marker, the sample containing multiple DNA bands of known molecular weights, is used to create a standard curve. You will create a standard curve, in this case actually a straight line, by graphing the relative mobility (R_f) of each DNA fragment versus the $\log_{10}$ of its molecular weight.

   The step-by-step instructions below describe how to create a standard curve and how to use it to determine the base-pair size of the fragments of unknown size. In this exercise, as is often done for simplicity's sake, base-pair length is substituted for molecular weight.

   a. Examine your stained gel on a light box or overhead projector.

      Using your gel or a to-scale representation of your gel, measure the distance (in cm) that each DNA marker band migrated from the well. Also measure the distance traveled by the bands in the lanes containing the children's samples. Measure from the front edge of the well to the front edge of the band (the edge farthest from the well). Enter the distances into the table below.

   b. Calculate the relative mobility (R_f) for each of the DNA fragments. For this exercise

$$R_f = \frac{\text{Distance DNA band travels into the gel}}{\text{Distance the bromphenol blue dye travels into the gel}}$$

      Distance traveled by the bromphenol blue load dye: _____ cm

      Enter the data in the data table.

Data Table

HindIII/EcoRI Lambda Marker			Sample from Child 1				Sample from Child 2			
Distance Traveled	R_f	Actual bp	Distance Traveled	R_f	Interpolated bp	Actual bp	Distance Traveled	R_f	Interpolated bp	Actual bp
		21,226								
		5148								
		4973								
		4268								
		3530								
		2027								
		1904								
		1584								
		1375								
		947								
		831								
		564								
		125								

c. Plot the standard curve

   i. Set up the semilog paper with R_f as the arithmetic (x-axis) and the base-pair length as the (logarithmic) y-axis. Note: You do not need to plot $\log_{10}$ of the base pairs since you are using semilog graph paper.

   ii. Now, plot R_f versus the base-pair length for each EcoRI/HindIII lambda DNA fragment on your gel.

d. Connect the data points with a best-fit line. The point for the 21,226-bp or the 564-bp band may be an outlier. If this is the case, these points (but not any of the other points) may be excluded from the line. This best-fit line is the standard curve.

e. Use the standard curve to calculate the size of the DNA fragments in the sample generated from Child 1 and from Child 2.

   i. Locate on the x-axis the R_f of the first fragment in the lane with the sample generated from Child 1. Using a ruler, draw a vertical line from this point to its intersection with the standard curve (the best-fit line you just drew).

   ii. Extend a horizontal line from the point where your vertical line intersects the standard curve to the y-axis. The point where the horizontal line intersects the y-axis indicates the base-pair size of the DNA fragment.

   iii. Repeat steps e.i. and e.ii. for each DNA fragment in the samples and place the answers under "Interpolated bp" in the Data Table. After completion, your teacher will provide the actual base-pair (Actual bp) data for comparison. (Your interpolated values will not be exact.)