Lab 9: Biotechnology: Restriction Enzyme Analysis of DNA

Answer Sheet

PreLab

Activity I: Restriction Enzymes

1. What is the sequence of the complementary DNA strand? Draw it.

2. Assume you cut this fragment with the restriction enzyme *EcoRI.* The restriction site for *EcoRI* is 5'-GAATTC-3', and the enzyme makes a *staggered* ("sticky end") cut between G and A on both strands of the DNA molecule. Based on this information, draw an illustration showing how the DNA fragment is cut by *EcoRI* and the resulting products.

Activity II: DNA Mapping Using Restriction Enzymes

1. Based on this information, can you make a prediction about the products of DNA from different sources cut with the same restriction enzyme?

2. Will the RFLP patterns produced by gel electrophoresis produced by DNA mapping be the same or different if you use just one restriction enzyme?

3. Do you have to use many restriction enzymes to find differences between individuals? Justify your prediction.

Activity III: Basic Principles of Gel Electrophoresis

1. Why do DNA fragments migrate through the gel from the *negatively* charged pole to the *positively* charged pole?

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PostLab

Analyzing Results

Examine your stained gel on a light box or other surface that helps visualize bands.

Take a picture of your gel results to attach to the lab.

1. What observations can you make?

2. What quantitative measurements can you make?

3. Using the ideal gel in Figure 5, measure the distance (in cm)that each fragment migrated from the origin (the well). For consistency, measure from the front end of each well to the front edge of each band, (i.e., the edge farthest from the well). Enter the measured distances into Table 1.

Table 1. DNA Fragment Migration Distance

*Hin*dIII		*Bam*HI		*Eco*RI	
Distance Traveled(cm)	**BP Length**	**Distance Traveled(cm)**	**BP Length**	**Distance Traveled(cm)**	**BP Length**
	*27,491				
	*23,130				
	9,416				
	6,557				
	4,361				
	2,322				
	2,027				
	**564				
	**125				

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Answer Sheet

***Plot the standard curve using the data from the DNA sample cut with *Hind*III. Use excel or the attached semi-log paper.**
***Use the standard curve to calculate the sizes of *Eco*RI and *PstI* fragments**.

4. Using a ruler, how can you use the standard curve to calculate the sizes of unknown fragments?

Evaluating Results

***Plot the standard curve using the data from the your experimental DNA sample cut with *Hind*III. Use excel or the attached semi-log paper.**
***Use the standard curve to calculate the sizes of *Eco*RI and *Bam*HI fragments.**

Table 2. Experimental DNA Fragment Migration Distance

*Hin*dIII		*PstI*		*Eco*RI	
Distance Traveled(cm)	**BP Length**	**Distance Traveled(cm)**	**BP Length**	**Distance Traveled(cm)**	**BP Length**

1. What are some possible challenges you had in performing your investigation?

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Answer Sheet

2. What are some possible sources of error in the electrophoresis procedure?

3. How can you minimize any potential sources of error?

Thinking About Your Results

1. Social and Ethical Implications

a. Who should have access to your genetic profile? (justify)

b. What issues about confidentiality are raised by genetic testing? (give example)

c. Who owns your DNA and its information? (justify)

2. Predicting Disease

a. Who should have access to this information? (justify)

Lab 9: Biotechnology: Restriction Enzyme Analysis of DNA

Answer Sheet

b. Would you want to know this information?(explain)

3. Wrongful Convictions

a. What social and ethical issues are raised by using DNA evidence to reexamine old court decisions? (explain)

b. What other arguments can you make (or find) against using DNA evidence for court cases?

4. Bioengineering

a. Should countries where native plants are located benefit from the use of that plant in bioengineering? (justify)

b. Who owns the information in DNA? (defend)

c. Who can profit from that information?(explain)

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***Don't forget to attach your standard curve graphs**
***Don't forget to attach a picture of your gel results**