Name: _____ Date: _____

PEARSON EDUCATION

Virtual Student Guide

http://www.phschool.com/science/biology_place/labbench/index.html

AP® Biology Laboratory 6
Part II
DNA Electrophoresis

Introduction

In this laboratory you will use some basic tools of molecular biology to gain an understanding of some of the principles and techniques of genetic engineering. In the second part, you will use gel electrophoresis to separate fragments of DNA for further analysis.

1. Log onto the Pearson LabBench web page at http://www.phschool.com/science/biology_place/labbench/index.html
2. Select Lab 6 – Molecular Biology
3. Select 6-II – DNA Electrophoresis.
4. Follow the instructions carefully on each screen – be sure to review all animations.
5. Answer the lab packet questions as you proceed through the virtual lab.

1. Read *"Key Concepts II: Electrophoresis"*. What are restriction enzymes? What is the source of restriction enzymes?

_____

_____

_____

_____

   a. How do restriction enzymes work?

   _____

   _____

   b. In what way did the discovery of restriction enzymes make genetic engineering possible?

   _____

   _____

   c. What is our goal in this part of the laboratory?

   _____

   _____

2. Read "*How do Restriction Enzymes Work?"*. Click on each of the "cut" options and watch the animation. Describe below how restriction enzymes accomplish cutting up DNA into fragments:

_____

_____

_____

_____

_____

   a. What does *palindromic* mean?

      _____

      _____

3. Read "*Cutting DNA with Restriction Enzymes*". Briefly describe how this is accomplished:

_____

_____

_____

_____

4. Read "Gel Electrophoresis". Click the "run" tab to view the animation. What is gel electrophoresis?

_____

_____

   a. What affects the direction of movement in gel electrophoresis?

      _____

      _____

   b. What affects the rate of movement in gel electrophoresis?

      _____

      _____

   c. How is DNA charged? In which direction will it move?

      _____

      _____

   d. Which fragments will move the farthest? Why?

      _____

      _____

   e. How is the actual length of each fragment of DNA measured?

      _____

      _____

5. Read "*Design of Experiment II*". In order to understand each step, you will need to progress through the next few screens. BE SURE TO CLICK ON AND WATCH ALL OF THE AVAILABLE ANIMATIONS! Describe below the steps involved in the procedure:
    a. Prepare the Gel:

    _____

    _____

    b. Obtain Prepared DNA Samples:

    _____

    _____

    c. Load Samples into Gel:

    _____

    _____

    d. Separate Fragments by Electrophoresis:

    _____

    _____

    e. Stain DNA and Measure Fragments:

    _____

    _____

    f. Determine Fragment Sizes:

    _____

    _____

6. Read "*Analysis of Results II*". How do researchers determine the size of DNA fragments produced with particular restriction enzymes?

    _____

    _____

7. Read "*Making a Standard Curve for HindIII DNA Fragments*". Describe how fragment size is determined by this method...then use this information to complete the "measured distance" chart.

    _____

    _____

Actual Base Pairs (bp)	Measured Distance (mm)
23,130	
9416	
6557	
4361	
2322	
2207	
564	

8. Read "*Making a Standard Curve".* Watch the animation. Describe the following:
   a. Semilog Graph Paper:

9. Read "*Practice Problem I*". Place your answers in the chart below.

	Distance Migrated (mm)	Interpolated Fragment Size (in base pairs)
Fragment 1		
Fragment 2		
Fragment 3		

10. Read "*Practice Problem II*". Place your answers in the chart below.

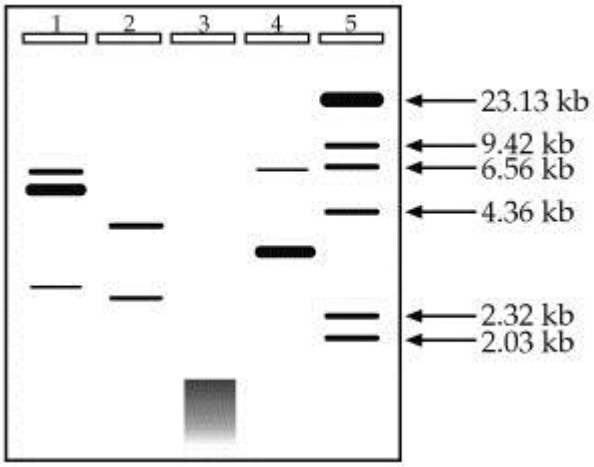
	Distance Migrated (mm)	Interpolated Fragment Size (in base pairs)
Fragment 1		
Fragment 2		

11. Take "*Lab Quiz II*". You may write your responses below:

- ☐ Question #1 _____
- ☐ Question #2 _____
- ☐ Question #3 _____
- ☐ Question #4 _____
- ☐ Question #5 _____
- ☐ Question #6 _____
- ☐ Question #7 _____
- ☐ Question #8 _____

Lab Analysis Questions

You have performed Restriction Digestion and Agarose Gel Electrophoresis on a plasmid you purified, using 3 different Restriction Enzymes, and the gel is shown below. Unfortunately, you forgot to label your tubes or keep good records, and the only things you can remember about the experiment are that your standards are in Lane 5 and your uncut control is in Lane 1, and that you loaded roughly the same amount of total DNA in your sample lanes (1-4). Hey, at least you remembered that much!



1. Given no other information and using no math, approximately how big is your original plasmid?

- 35 kb
- 15.5 kb
- 6.5 kb
- 5.0 kb
- 3.0 kb

2. How many times did the enzyme used in Lane 2 digest the plasmid? Does the data seem reasonable? What is the likely number of base pairs this enzyme recognizes?

3. When DNA appears as a messy, continuous band as it does at the bottom of Lane 3, rather than independent, discreet bands, the effect is known as smearing. What are some likely explanations for the smearing detected in Lane 3? You should be able to come up with at least two.

4. How many times did the enzyme used in Lane 4 digest the plasmid? Does the data seem reasonable?