To close the yellow note, click once to select it and then click the box in the upper left corner.
To open the note, double click (Mac OS) or right click (Windows) on the note icon.

Determining Cutting Site Locations

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INTRODUCTION

Description

The following activity introduces DNA restriction mapping. Students cut pieces of paper into lengths representing those produced when specific enzymes are used to cut a strand of DNA.

Student Audience

This activity is appropriate for use as early as seventh-grade life science, but is more appropriate for high school. The activity is effective with kinesthetic learners, as well as visual and auditory learners.

Goals for the Activity

Students will

- comprehend the effect of enzymes on strands of DNA,
- determine the cutting locations of enzymes on a specified piece of DNA,
- diagram (map) the cutting sites on a piece of DNA, and
- give a basic description of electrophoresis.

Recommended Placement in the Curriculum

The student should have a working knowledge of the metric system. This activity should be included as part of the human biology/health unit and introduced after studying DNA, genes, and chromosomes.

STUDENT HANDOUT
Determining Cutting Site Locations

Purpose

To determine the locations of the restriction enzyme cutting sites on a 1,650-base-pair piece of DNA using two different enzymes *(Eco*R I and *Bam*H I) alone and in combination.

Scenario

The goal of the Human Genome Project is ultimately to develop a map of all genes in the human DNA sequence for human biology and medicine purposes. A genome map describes the order of the genes or other markers and spacing between them on each chromosome. The technology used to determine the locations of the cutting sites is essential to this project.

Materials

Per student or group

- 3, 16.5-cm strips of colored paper (one red, one orange, and one yellow; the width of the strips is not important)
- metric ruler
- scissors
- marking pen

Procedure

1. When measuring the paper strips, use the scale 1 cm = 100 base pairs. The strips begin at a length of 16.5 cm since they each represent a piece of DNA with 1,650 base pairs.

2. Use the following paper strip colors to represent the following digestions: red for *Eco*R I, yellow for *Bam*H I, and orange for *Eco*R I + *Bam*H I.

3. Using the scale in step 1, cut the paper strips into lengths indicated in Figure 1 to show how each enzyme cuts the DNA into fragments. (See the caption in Figure 1.) Label each piece with the name of the enzyme (or combination) and the length of the fragment in base pairs.

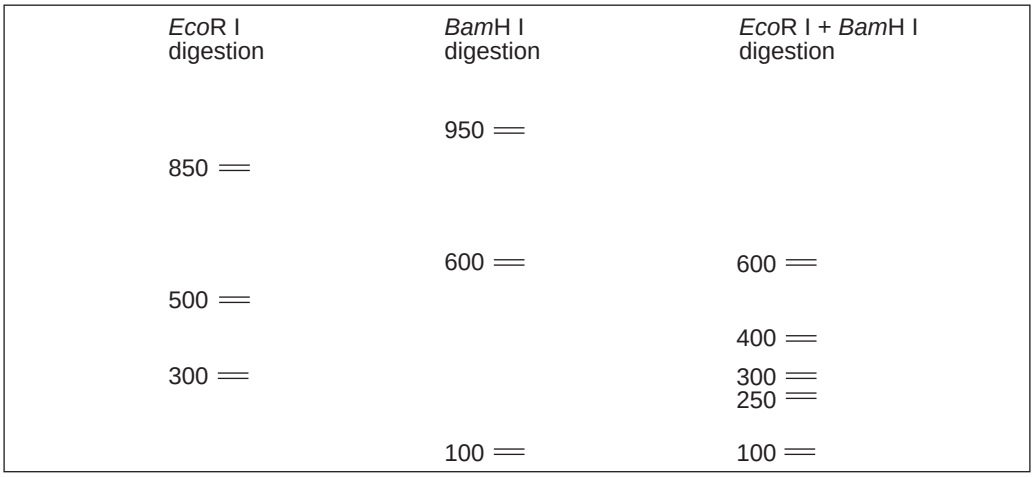


Figure 1: Electrophoresis: When an electrical charge is applied to DNA fragments loaded into small wells in an agarose gel, the fragments migrate from the well toward the positive charge. The smaller fragments move faster than the larger ones. These labeled bands represent the fragments produced by adding the two enzymes alone and in combination with the DNA. They become visible when stained with a dye that binds to the DNA molecule. The numbers next to the bands indicate how many base pairs long the fragments are.

4. To figure out the exact locations of the restriction enzyme cutting sites, place the paper strips flat on your desk and put each colored DNA strip back together. Construct the paper strips parallel to each other, but do not mix the colors. Since the orange strip represents *Eco*R I + *Bam*H I, put the orange strip between the red and yellow strips. Most importantly, arrange the pieces until you have them in an order where the cutting sites of the orange strip (representing *Eco*R I + *Bam*H I digestion) align with either the cutting sites of the red strip (representing *Eco*R I digestion) or the cutting sites of the yellow strips (representing *Bam*H I digestion).

5. Draw a diagram of the 1,650-base-pair-length section of DNA and show the cutting sites for the two different enzymes.

6. If you have extra time, devise a way to determine mathematically where the cutting sites are for each of the enzymes.

Questions

1. Which direction (electrically) do the fragments migrate when a charge is applied?

2. Which fragments move faster, the larger or the smaller?

3. What is the largest fragment in the *Eco*R I digestion? What is the smallest fragment?

4. What is the largest fragment in the *Bam*H I digestion? What is the smallest fragment?

Suggested Reading

Watkins, P.A. *Holt Life Science;* Holt, Rinehart and Winston: Austin, TX, 1994; pp 127–133, 166, 167.

Genentech, Inc. Home Page. www.gene.com (accessed August 31, 2000).

Johns Hopkins Medical Institutions Web Site. Division of Biomedical Information Sciences. www.bis.med.jhmi.edu (accessed August 31, 2000).

INSTRUCTOR NOTES
Determining Cutting Site Locations

Time Required
This activity requires approximately 40 minutes to complete.

Group Size
Students should work individually or in pairs for this activity.

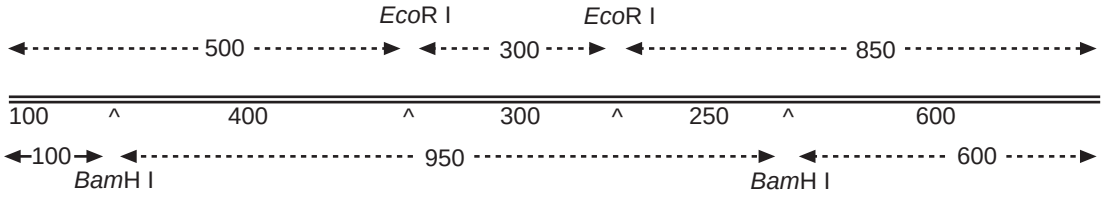
Materials
Per student or group
- 3, 16.5-cm strips of colored paper (one red, one orange, and one yellow; the width of the strips is not important)
- metric ruler
- scissors
- marking pen

Points to Cover in the Pre-Lab Discussion
- electrophoresis
- enzymes and cutting sites

Procedural Tips and Suggestions
1. A quick review of metric measuring may be necessary.

2. When the students are arranging the pieces of paper, recommend that they align the pieces parallel to each other and with the orange strip between the red and yellow strips.

Sample Results



Plausible Answers to Questions
1. Which direction (electrically) do the fragments migrate when a charge is applied?
   When an electrical charge is applied to the DNA fragments, they migrate (move) toward the positive electrode.

2. Which fragments move faster, the larger or the smaller?
   The smaller fragments move faster, and the larger fragments move slower.

3. What is the largest fragment in the *Eco*R I digestion? What is the smallest fragment?
   850 base pairs (bp); 300 bp.

4. What is the largest fragment in the *Bam*H I digestion? What is the smallest fragment?
   950 bp; 100 bp.

Extensions and Variations

Ethical questions such as the following may be raised regarding how this information (the Human Genome Project) should be used to benefit humankind:

- Should we do prenatal screenings?
- Should we clone humans in part or in whole?

Reference

Bloom, M.V.; Freyer, G.A.; Micklos, D.A. *Laboratory DNA Science: An Introduction to Recombinant DNA Techniques and Methods of Genome Analysis;* Benjamin/Cummings: Menlo Park, CA, 1996.