EDVOTEK®

The Biotechnology Education Company ®

EDVO-Kit

S-42-20

DNA GelSizer™

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this activity is to teach students to size DNA fragments which have been separated by agarose gel electrophoresis.

EDVOTEK, Inc. • 1-800-EDVOTEK • www.edvotek.com

EVT 100612AM

Table of Contents

Experiment Components

- Reusable DNA GelSizers™
- FlashBlue™ and Ethidium Bromide Gel Images

Requirements

- Optional white light visualization system

Background Information

Size determination of DNA fragments is essential to DNA mapping and analyzing restriction enzyme cleavage patterns. Restriction enzymes are endonucleases that cleave both strands of DNA at very specific sequences within DNA. Locations of their cleavage sites are important for DNA fingerprinting, determination of genetic diseases and for DNA analysis.

This exercise focuses on the first step for mapping DNA restriction sites, which is to determine the size of "unknown" DNA fragments generated after electrophoresis. The images are simulation of an electrophoretic separation that contains DNA fragments of unknown size and Standard DNA fragments. The unknown DNA fragments will migrate through the gel according to their respective sizes and relative to the Standard DNA fragments. The migration distances of the known and unknown fragments are measured and plotted on semi-log graph paper according to their size on the y-axis versus the migration distance on the x-axis. The size of the fragmentation the y-axis is expressed as the log of the number of base pairs. This allows the data to be plotted as a straight line. The DNA fragments of known size (Standard DNA fragments) are used to plot a standard curve. The migration distance of the unknown DNA fragments are estimated by extrapolation from the standard curve. After determining the size of the DNA fragments generated by single and combinations of restriction enzymes, a DNA map is constructed.

If you are unfamiliar with sizing DNA fragments, Edvotek® DNA GelSizer™ serves as a dry exercise for estimating the size of unknown DNA fragments from agarose gel before conducting the actual experiment. In this activity, students will learn how to accurately size DNA fragments using the Edvotek® GelSizer™ and simulated gel imprints. The GelSizer™ contains grids imprinted with ruler for sizing DNA fragment. It is reusable and can be preserved for future use (simple rinse and store in a plastic bag).

The first set of simulated gel imprints is the analysis of EcoRI cleavage patterns of Lambda DNA. Students will develop an understanding of the role of restriction enzymes that is used to digest Lambda DNA at specific nucleotide sequences. The second set is to introduce students to the concept of DNA Fingerprinting. Students will analyze PCR results obtained from different suspects and compare them to crime scene sample.

In both exercises, the DNA standards in Lane 1 makes it possible to measure the unknown" DNA fragments. In the restriction enzyme digestion, undigested Lambda DNA contains approximately 49,000 base pairs and has 5 recognition sites for EcoRI
When digested with Eco RI, it results in fragment sizes 21226, 7421, 5804, 5643, 4878, and 3530 in base pairs. In the DNA fingerprinting, the results of this analysis indicates an identical pattern in Lanes 2 and 4. This is strong evidence that the crime scene DNA and Suspect 2 match. In criminal investigations, several known variable regions in DNA are analyzed to match crime scene and suspect DNAs.

Experiment Procedure

Using DNA GelSizer™ - Size Determination of DNA Fragments

1. Measure and record the distance traveled in the agarose gel by each Standard DNA fragment (except the largest 23,130 bp fragment, which will not fit in a straight line in step 4).

In each case, measure from the lower edge of the sample well to the lower end of each band. Record the distance traveled in centimeters (to the nearest millimeter).

2. Label the semi-log graph paper:

   A. Label the non-logarithmic horizontal x-axis "Migration Distance" in centimeters at equal intervals.

   B. Label the logarithmic vertical y-axis "Log base pairs". Choose your scales so that the data points are well spread out. Assume the first cycle on the y-axis represents 100- 1,000 base pairs and the second cycle represents 1,000- 10,000 base pairs.

3. For each Standard DNA fragment, plot the measured migration distance on the x-axis versus its size in base pairs, on the y-axis.

4. Draw the best average straight line through all the points. The line should have approximately equal numbers of points scattered on each side of the line. Some points may be right on the line (see Figure 1 for an example).

5. Measure the migration distance of each of the "unknown" fragments from the remaining samples.

6. Using the graph of the Standard DNA fragments, determine the sizes in base pairs of each "unknown" fragment.

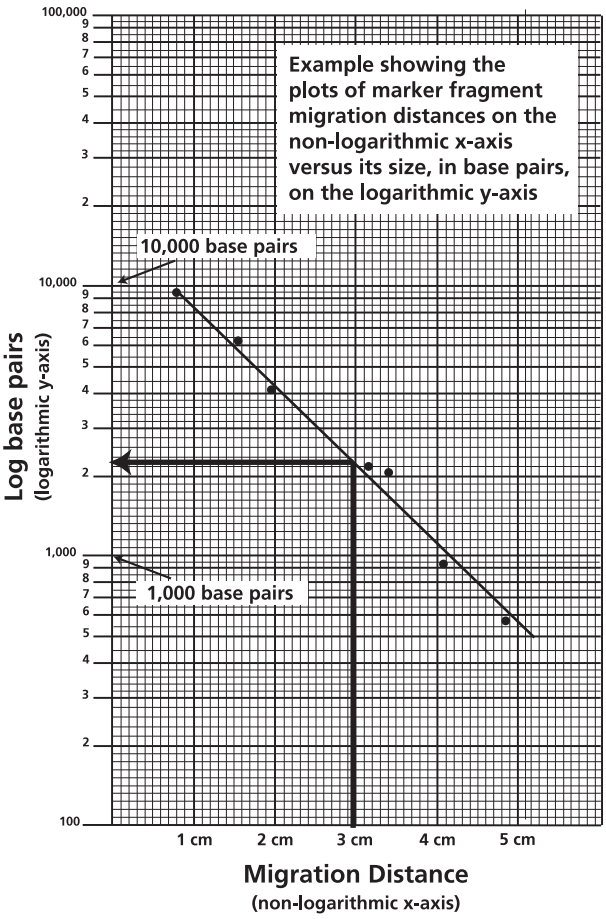
   - Find the migration distance of the unknown fragment on the x-axis. Draw a vertical line from that point until the standard graph line is intersected.
   - From the point of intersection, draw a second line horizontally to the y-axis and determine the approximate size of the fragment in base pairs (refer to Figure 1 for an example).

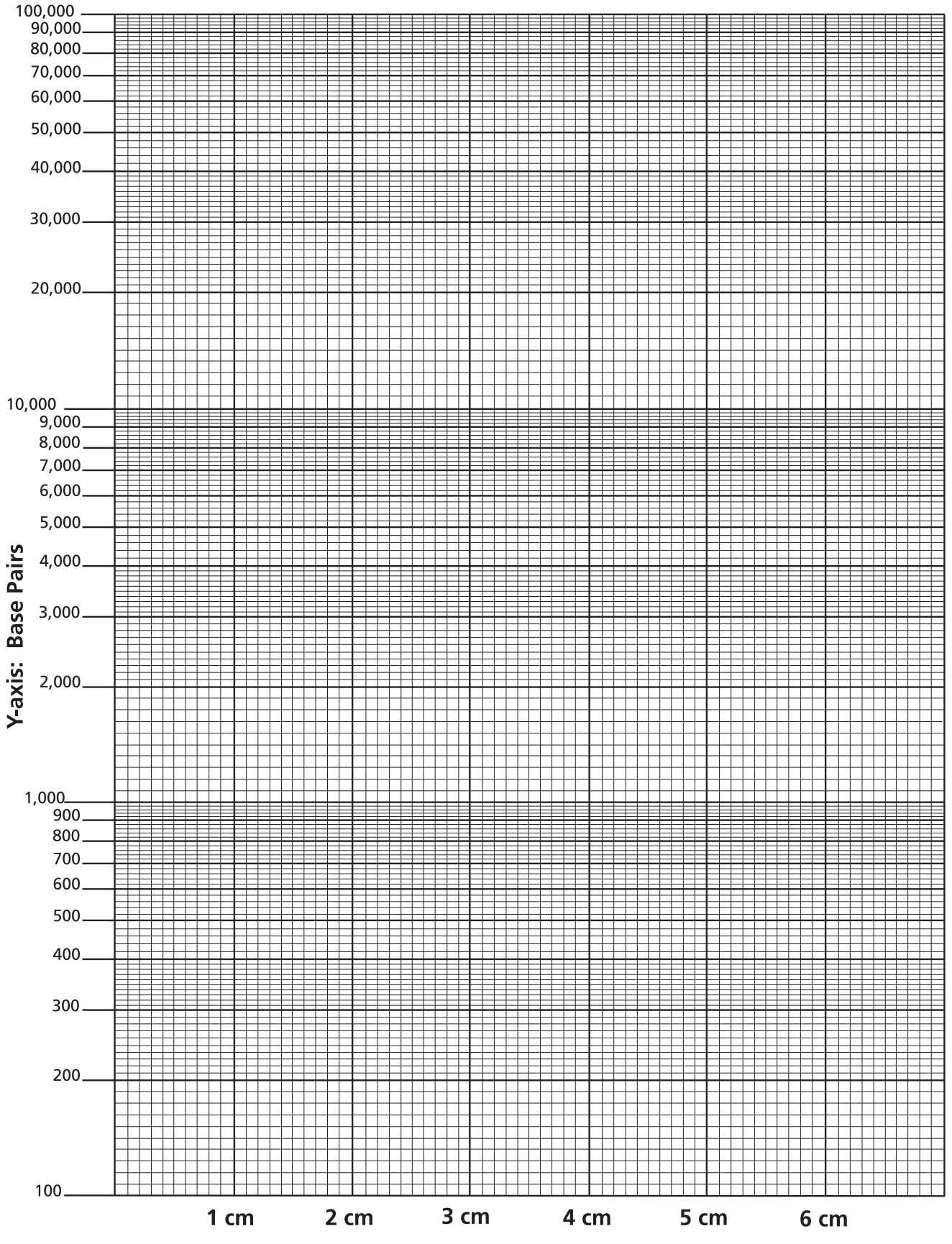
Quick Reference:

Standard DNA fragment sizes - length is expressed in base pairs.

23130	9416	6557
4361	3000	2322
2027	725	570

Figure 1




100,000
90,000
80,000
70,000
60,000
50,000
40,000
30,000
20,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
900
800
700
600
500
400
300
200
100
Y-axis: Base Pairs
1 cm
2 cm
3 cm
4 cm
5 cm
6 cm
X-axis: Migration distance (cm)

Care and Maintenance

- If the DNA GelSizer™ gets dirty over time, gently rinse it with tap water and let air dry.
- Do not use abrasive detergents to wash the DNA DuraGels™ since this will result in unwanted scratches on the surface of the gel.
- Store DNA Gelsizer™ and gel images in separate plastic bags.