EDVOTEK®

The Biotechnology Education Company ®

EDVO-Kit

S-43

DNA DuraGel™

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective is for students to learn both the critical skill of pipeting samples into agarose gels and the ability to size DNA fragments which have been separated by agarose gel electrophoresis.

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Experiment Components

- DNA DuraGels™
- Gel loading samples
- Plastic pipets
- FlashBlue™ and InstaStain® Ethidium Bromide Gel Images

Requirements

- Micropipets are recommended
- Optional white light visualization system

Background Information

PIPETING

Pipeting is a critically important technique in life science experiments to ensure accurate experimental results. In typical biotechnology experiments, biologicals and reagents such as DNA, enzymes and buffers are transferred (by pipeting) into small microcentrifuge tubes which serve as reaction vessels. For these types of reactions, microliter volumes are typically used. There are 1,000 microliters in 1 milliliter of a solution. To put it in perspective, a 50-microliter sample is approximately equal in size to a single raindrop. A raindrop-sized sample is relatively large when compared to experimental samples which often are 10 to 25 microliters in volume.

Accurate sample delivery technique ensures the best possible gel results. If you are unfamiliar with loading samples in agarose gels, Edvotek® DuraGels™ teach practice sample delivery techniques before conducting the actual experiment.

In this activity, students will learn how to accurately pipet gel loading sample into a simulated agarose gel. The Edvotek® DuraGel™ is reusable and can be preserved for future use (simple rinse and store in a plastic bag). The reusability of DNA DuraGels™ eliminates the preparation time, expense, and waste of pouring actual agarose practice gels.

SIZE DETERMINATION OF DNA FRAGMENTS

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.

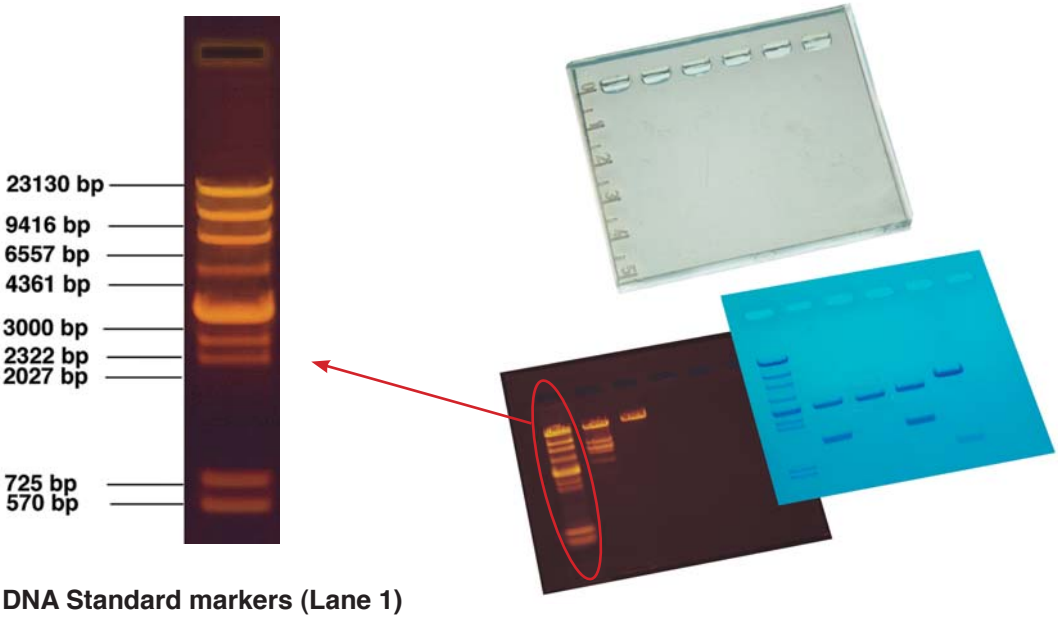
This exercise focuses on the first step for mapping DNA restriction sites, which is to determine the size of "unknown" DNA fragments generated after electrophoresis. The images are simulation of an electrophoretic separation that contains DNA fragments of unknown size and Standard DNA fragments. The 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® DuraGels™ 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® DuraGels™ and simulated gel imprints. DuraGels™ contain 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).

Background Information

The first set of simulated gel imprints is the analysis of *Eco*RI 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 *Eco*RI. 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.



DNA Standard markers (Lane 1)

Using the DNA DuraGel™ - Sample Delivery Methods

Although the variable automatic micropipet is the preferred instrument for delivering accurate, reproducible volumes of sample, other less expensive equipment alternatives such as fixed volume micropipets or disposable transfer pipets can be used effectively.

VARIABLE AUTOMATIC MICROPIPETS

To measure microliter volumes, a special instrument called a micropipet is used. The variable automatic micropipet is the preferred instrument for delivering accurate, reproducible volumes of sample. These instruments are manufactured to deliver samples in various ranges (e.g., 0.5-10 µl, 5-50 µl, 200-1000 µl, etc.) and usually can be adjusted in one-microliter increments. Typically, these instruments have an ejector button for releasing the tip after sample delivery. Variable automatic micropipets can also be multi-channeled, designed to uniformly deliver several samples at the sample time. However, for this experiment, only one sample will be delivered at a time.

TO USE

1. Place the gel in a clear shallow dish (large Petri plate or clear glass vessel). Add water to just cover the gel (several mm over the gel).

2. Set the micropipet to the appropriate volume and place a clean tip on the micropipetor. Press the top button down to the first stop and hold it in place while placing the tip into the gel loading sample tube (Figure 1.1).

3. Once the tip is immersed in the sample, release the button slowly to draw sample into the tip (FIgure 1.2).

4. To dispense, position the pipette tip into the wells of the DNA DuraGels at 2-3 mm immersion depth.

   Be careful not to puncture or damage the well with the pipet tip.

5. Deliver the sample by pressing the button to the first stop – when loading gels, never press to the second stop (Figure 1.3).

   Note: After delivering the sample, do not release the top button until the tip is out of the tube or vessel to which the sample is delivered. Otherwise, the sample could be drawn back up into the pipet tip.

6. Press the ejector button to discard the tip. Continue loading with the next well (Figure 1.4).

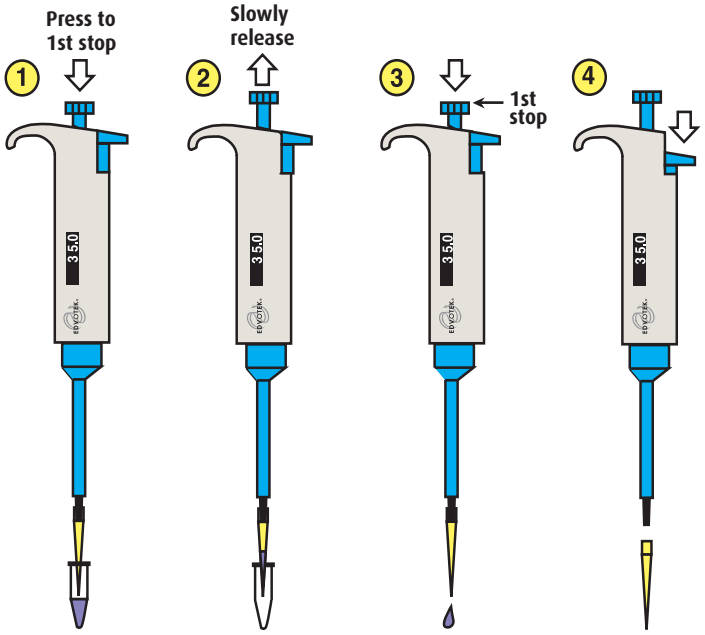


Figure 1

Experiment Procedure

Using the DNA DuraGel™ - Sample Delivery Methods

FIXED VOLUME MICROPIPETS

Accurate pipetting can also be achieved using fixed volume micropipets. These types of micropipets are preset to a specific volume. Although the volume of each individual micropipet cannot be changed, fixed volume micropipets operate similarly to the variable automatic micropipets. Most fixed volume pipets do not have ejector buttons, so the tips must be removed manually.

EDVOTEK® offers the widest selection of fixed volume micropipets which can be used to deliver samples with volumes raging from 5 to 200 µl. Edvotek® Minipipets™ are accurate, easy to use and cost effective micropipets that use standard micropipet tips. Since the wells of DNA DuraGels™ can hold up to 50 µl of liquid sample, it is recommended that Minipets™ Cats # 585, 586, 586-1, 587, 587-1, 587-2, 588, and 588-1 are to be used among this selection.

TRANSFER PIPETS

With EDVOTEK DNA DuraGels™, an alternative sample delivery method can be used if you do not have automatic micropipets. Disposable plastic transfer pipets can be used, but they are not precise. Because their volumes cannot be accurately controlled, their use can result in significant sample waste. To help control the delivery of small sample volumes with transfer pipets, gently squeeze the pipet stem, instead of the bulb. When using transfer pipets for sample delivery, load each sample well until it is full. Clean by flushing the transfer pipet with distilled water several times after delivering each sample and before loading a new sample.

Care and Maintenance

- If the DuraGel™ 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 DuraGels™ and gel images in separate plastic bags.

Size Determination of DNA Fragments Using DuraGels™ and Gel Images

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.

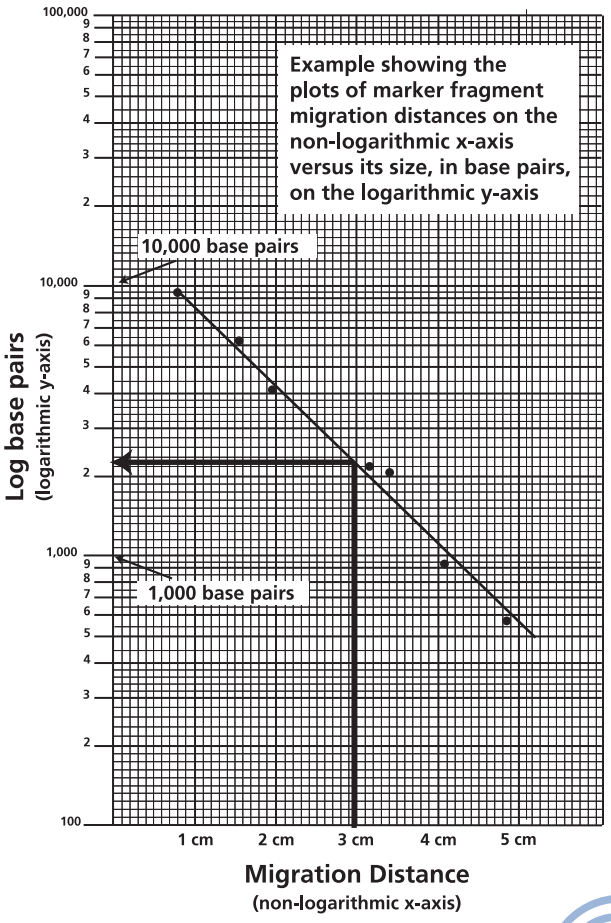
   - Find the migration distance of the unknown fragment on the x-axis. Draw a vertical line from that point until the standard graph line is intersected.
   - From the point of intersection, draw a second line horizontally to the y-axis and determine the approximate size of the fragment in base pairs (refer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

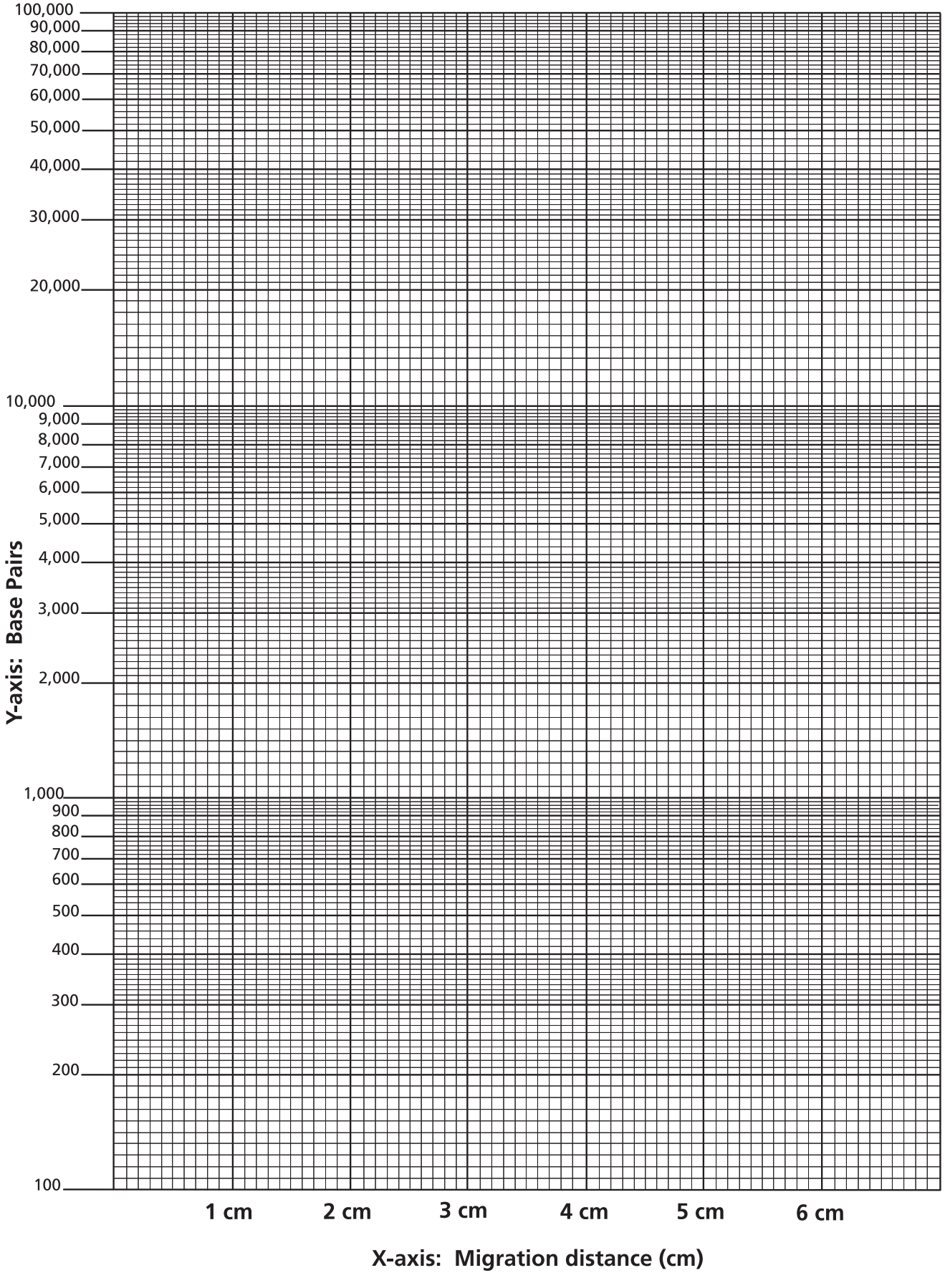
Quick Reference:

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

23130	9416	6557
4361	3000	2322
2027	725	570

Figure 1

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Y-axis: Base Pairs
1 cm
2 cm
3 cm
4 cm
5 cm
6 cm
X-axis: Migration distance (cm)