Size Determination of DNA Restriction Fragments

Laboratory BIOT 3250

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Experiment Objective

The objective of this experiment module is to develop an understanding of principles involved in estimating the size of unknown DNA fragments by agarose gel electrophoresis.

Materials

DNA SAMPLES FOR ELECTROPHORESIS

The DNA samples for electrophoresis in experiment 104 are packaged in one of the following ways:

- Pre-aliquoted Quickstrip™ connected tubes, which contain:

A and D	Standard DNA Fragments
B and E	Unknown DNA 1
C and F	Unknown DNA 2
G and H	blank

OR

- Individual 1.5 ml (or 0.5 ml) microcentrifuge tubes, which contain:

A	Standard DNA Fragments
B	Unknown DNA 1
C	Unknown DNA 2

Materials

REAGENTS & SUPPLIES

- UltraSpec-Agarose™ powder
- Concentrated electrophoresis buffer
- FlashBlue™ DNA Stain
- InstaStain® Blue cards
- Practice Gel Loading Solution
- 1 ml pipet
- Microtipped Transfer Pipets

Requirements

- Horizontal gel electrophoresis apparatus
- D.C. power supply
- Automatic micropipets with tips
- Balance
- Microwave, hot plate or burner
- Pipet pump
- 250 ml flasks or beakers
- Hot gloves
- Safety goggles and disposable laboratory gloves
- Small plastic trays or large weigh boats (for gel destaining)
- DNA visualization system (white light)
- Distilled or deionized water

Concepts

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.

Concepts (cont…)

Agarose gel electrophoresis is a convenient analytical method for determining the size of DNA molecules in the range of 500 to 30,000 base pairs. Samples of DNA are delivered in wells made in an agarose gel, which is placed in an electrophoresis chamber containing a buffer solution and electrodes. Direct current (D.C.) is applied from a power source. Since DNA is negatively charged at neutral pH, it will migrate through the gel towards the positive electrode. The agarose gel consists of microscopic pores that act as a molecular sieve that separates DNA molecules according to their size and shape. The migration rate of DNA molecules of the same shape is inversely proportional to their size. This results in smaller DNA molecules to migrate faster through the gel. The charge to mass ratio is the same for different sized DNA molecules.

Concepts (cont…)

Nucleotides in DNA are linked together by negatively charged phosphodiester bonds. For every base pair (average molecular weight of approximately 660) there are two charged phosphodiester linkages. Therefore, negative charges in DNA is accompanied by approximately the same mass. The absolute amount of charge in DNA is not a critical factor in the separation process. Separation occurs because smaller molecules pass through the gel pores more easily than larger ones (i.e., the gel is sensitive to the physical size of the molecule). DNA fragment migration rate is inversely proportional to the log10 of its size in base pairs.

Concepts (cont…)

In this experiment, DNA fragments of unknown size and Standard DNA fragments are submitted to electrophoretic separation. The unknown DNA fragments will migrate through the gel according to their respective sizes and relative to the Standard DNA fragments. After electrophoresis, the gel is stained and the DNA bands are visualized. 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 fragments on the y-axis are 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.

Concepts (cont…)

Quick Reference:

Standard DNA fragments, which were generated by restriction enzymes are provided in this experiment. A standard curve will be plotted on semi-log graph paper. The following Standard DNA fragment sizes are expressed in base pairs.

23130	9416	6557
4361	3000	2322
2027	725	570

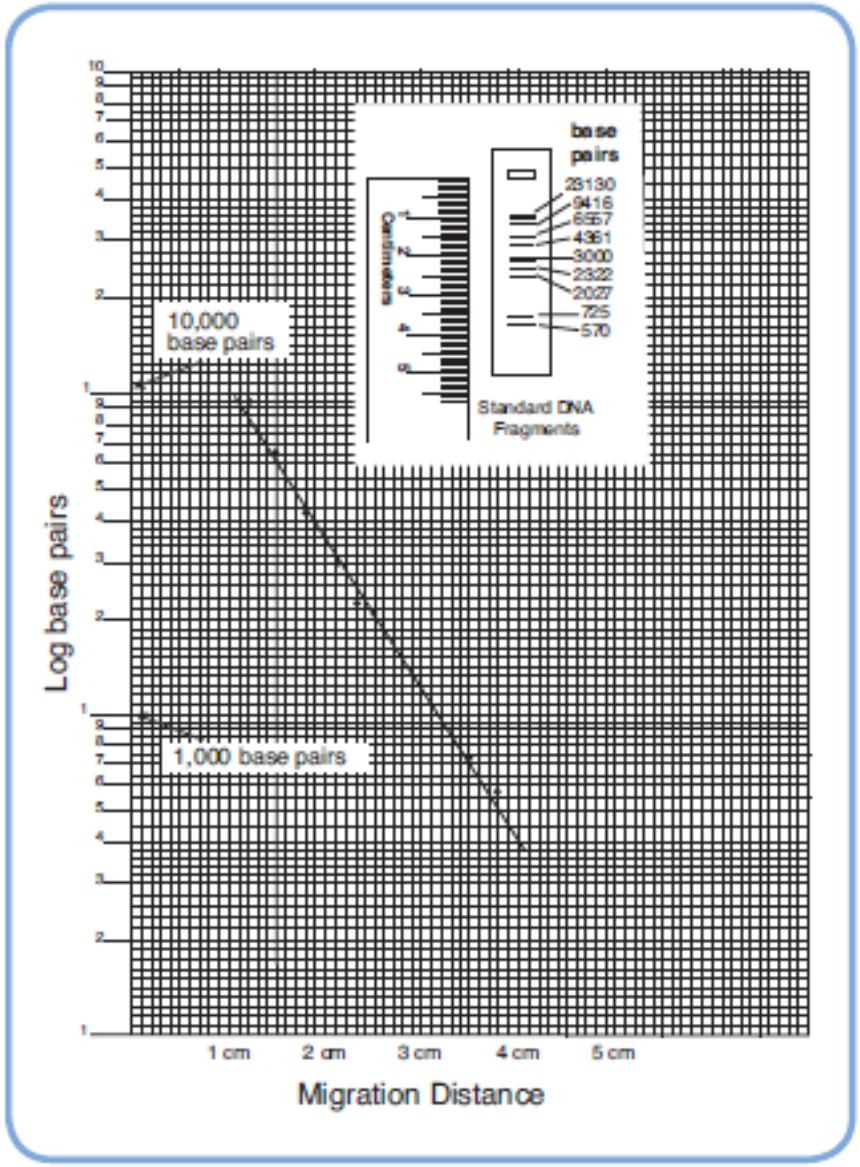
Concepts (cont…)

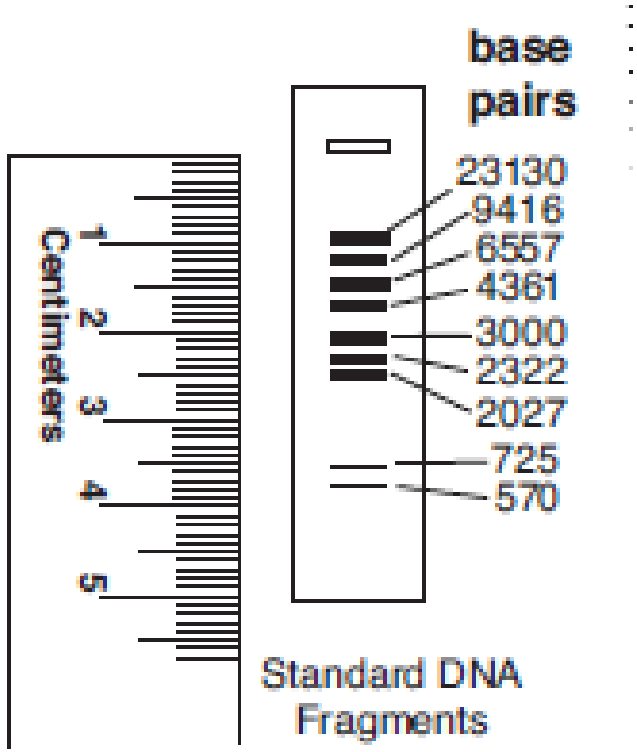
The standard fragments are used to make a standard curve by plotting their size on the y-axis versus the migration distance on the x-axis. The size of the fragments on the y-axis are expressed as the log of the number of base pairs they contain or the log of their molecular weight. Most of the plotted data obtained from the markers will yield a straight line. The migration distance of the unknown DNA fragment(s) are found on the X-axis and their size is estimated 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 as previously described.

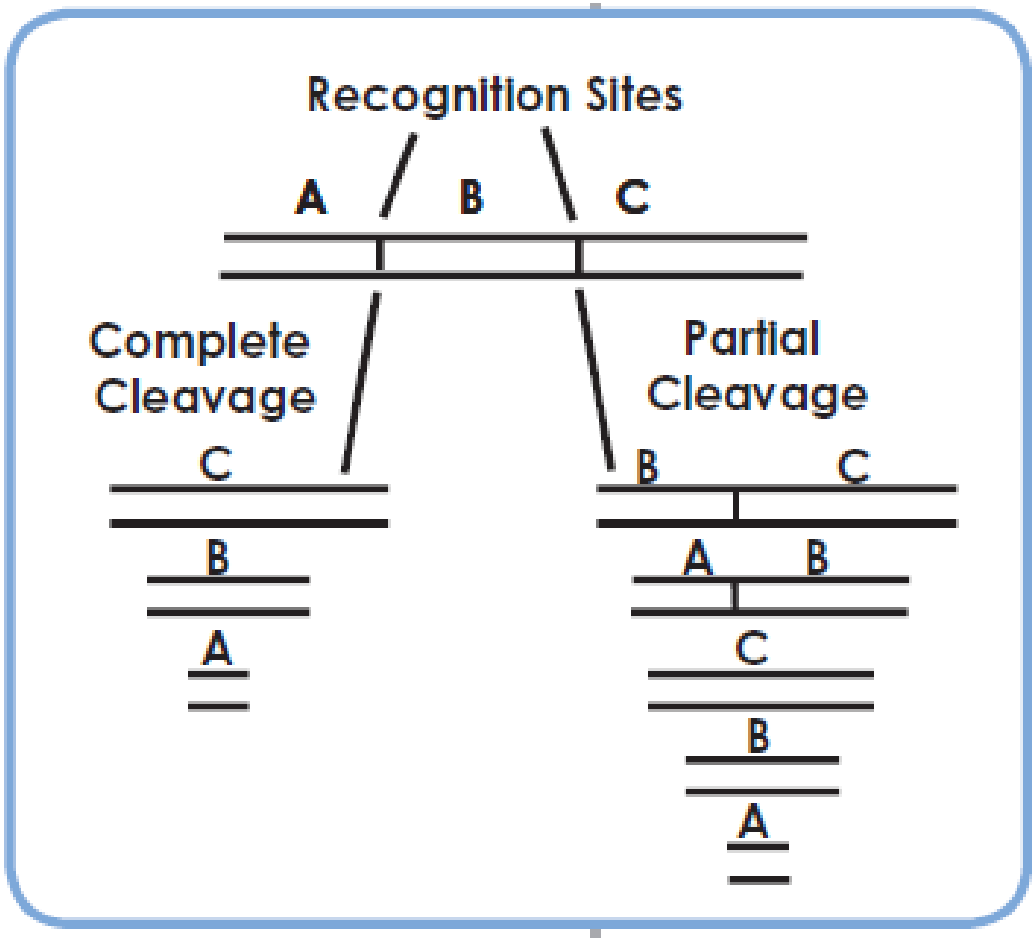
In this experiment, you will determine the relative locations of three restriction enzyme cleavage sites on a circular plasmid DNA. The plasmid has been cleaved with two restriction enzymes. Enzyme 1 cleaves the plasmid once. Assume that the Enzyme 1 site is at position 0. Enzyme 2 cuts the plasmid twice. The objective is to calculate the distances in base pairs between the points of cleavage and to determine whether the Enzyme 1 site is in between the Enzyme 2 sites.

Concepts (cont…)

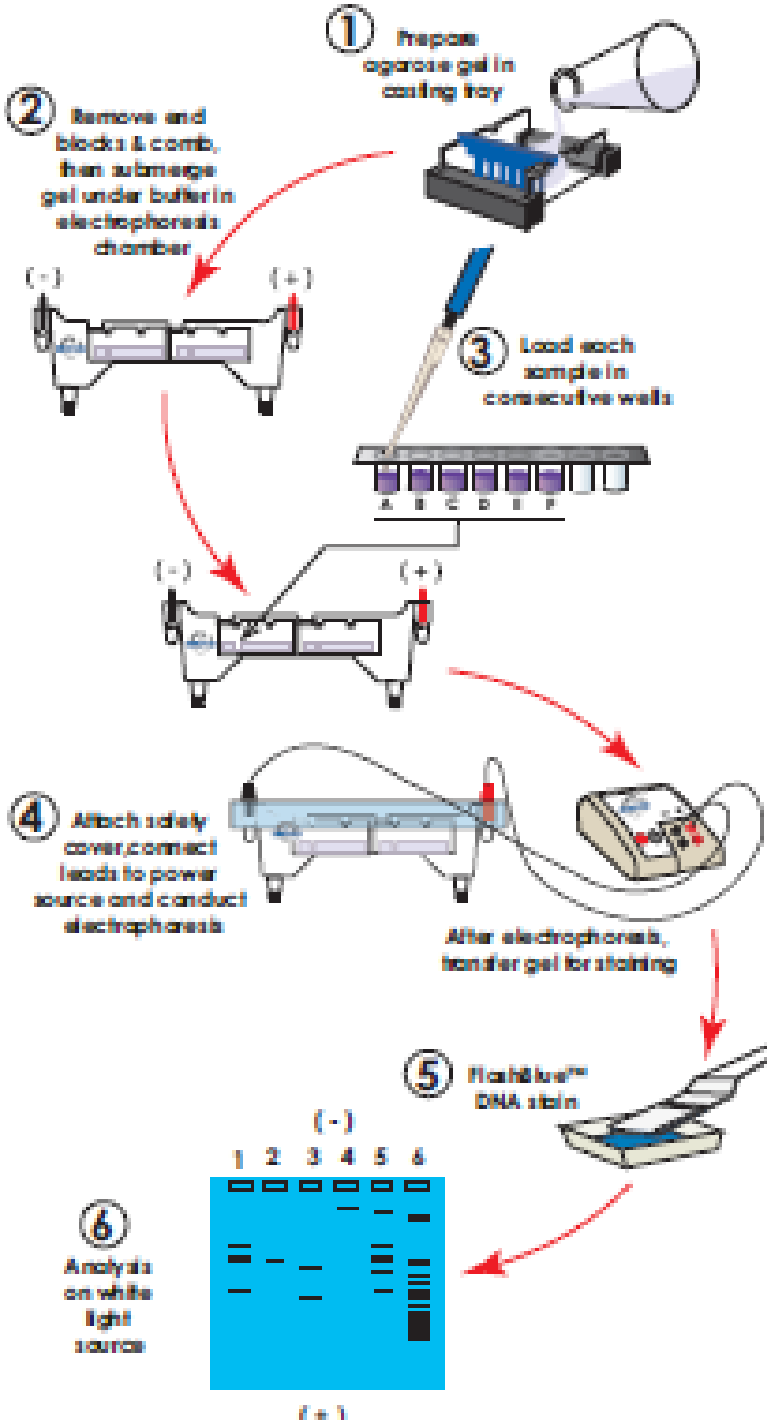




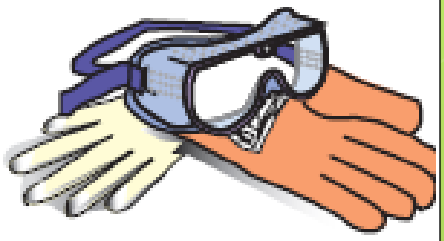
Concepts (cont...)



Flow Chart


1
Prepare agarose gel in casting tray
2
Remove end blocks & comb, then submerge gel under buffer in electrophoresis chamber
(-)
(+)
3
Load each sample in consecutive wells
A B C D E F
(-)
(+)
4
Attach safety cover, connect leads to power source and conduct electrophoresis
After electrophoresis, transfer gel for staining
5
FlashBlue™ DNA stain
(-)
1 2 3 4 5 6
6
Analysis on white light source
(+)

LABORATORY SAFETY



1. Gloves and goggles should be worn routinely as good laboratory practice.
2. Exercise extreme caution when working with equipment that is used in conjunction with the heating and/or melting of reagents.
3. DO NOT MOUTH PIPET REAGENTS - USE PIPET PUMPS.
4. Exercise caution when using any electrical equipment in the laboratory.
5. Always wash hands thoroughly with soap and water after handling reagents or biological materials in the laboratory.

LABORATORY NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the Experiment:

- Write a hypothesis that reflects the experiment.
- Predict experimental outcomes.

During the Experiment:

- Record (draw) your observations, or photograph the results.

Following the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

EXPERIMENT

Agarose Gel Electrophoresis

Prepare the Gel

1. Prepare an agarose gel with specifications summarized below.

• Agarose gel concentration required:	0.8%
• Recommended gel size:	7 x 10 cm or 7 x 14 cm
• Number of sample wells required:	6
• Placement of well-former template:	Middle set of notches (7 x 10 cm) Middle set of notches (7 x 14 cm)

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Load the Samples

2. Load the DNA samples in tubes A - C (or D - F) into the wells in consecutive order.

   - For gels to be stained with FlashBlue™ or InstaStain® Blue, load wells with 35 - 38 µl.
   - For gels to be stained with InstaStain® Ethidium Bromide, load wells with 18 - 20 µl.

Lane	Tube	
1	A or D	Standard DNA Fragments
2	B or E	Unknown 1
3	C or F	Unknown 2

Run the Gel

3. After DNA samples are loaded, connect the apparatus to the D.C. power source and set the power source at the required voltage.

4. Check that current is flowing properly - you should see bubbles forming on the two platinum electrodes. Conduct electrophoresis for the length of time specified by your instructor.

5. After electrophoresis is completed, proceed to DNA staining and visualization. Refer to Appendix E, F, G, or H for the appropriate staining instructions.

6. Document the results of the gel by photodocumentation.

   Alternatively, place transparency film on the gel and trace it with a permanent marking pen. Remember to include the outline of the gel and the sample wells in addition to the migration pattern of the DNA bands.

Results

Conclusions

Questions

Reference

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FAX: (301) 340-0582 • email: info@edvotek.com