Big Idea 3

Genetics and Information Transfer

Baker's Dozen Lab 9: DNA Fingerprinting

Objectives

Before doing this lab you should understand how gel electrophoresis separates DNA molecules present in a mixture and how restriction endonucleases function.

After doing this lab you should be able to explain how restriction endonucleases are used in genetic engineering and determine unknown DNA fragment sizes when given DNA fragments of known sizes.

Introduction

Restriction enzymes (**restriction endonucleases**) are essential tools in recombinant DNA methodology. Several hundred have been isolated from a variety of prokaryotic organisms. Scientists hypothesize that bacteria use these enzymes during DNA repair and as a defense against infection by bacteriophages. Restriction endonucleases are named according to the organism from which they came and in what order they were isolated from that organism. See the examples below.

Examples:

***Hind*III:** H = *Haemophilus* (genus)
ind = *influenzae* (species)
I = strain I
II = second endonuclease isolated
Recognition sequence = A/AGCTT

***Eco*RI:** E = *Escherichia* (genus)
co = *coli* (species)
R = strain R
I = first endonuclease isolated
Recognition sequence = G/AATTC

***Pst*I:** P = *Providencia* (genus)
st = *stuartii* (species)
I = first endonuclease isolated
Recognition sequence = CTGCA/G

Restriction enzymes recognize specific DNA sequences in double-stranded DNA (usually a four to six base pair sequence of nucleotides) and digest (cut) the DNA at these sites. The result is the production of fragments of DNA of various lengths. Some restriction enzymes cut cleanly through the DNA helix at the same position on both strands to produce fragments with blunt ends. Other endonucleases cleave each strand off-center at specific nucleotides to produce fragments with "overhands," or sticky ends. (See Figure 9.1) By using the same restriction enzyme to "cut" DNA from two different organisms, complementary "overhands," or sticky ends, will be produced and can allow the DNA from two sources to be "recombined." Digestion with *Eco*RI or *Hind*III will produce DNA fragments with sticky ends.

When any molecule enters an electrical field, the direction it will move is influenced by the charge of the molecule. The mobility or speed at which it will move is influenced by the strength of the electrical field, the size and shape of the molecule, and the density of the medium (gel) through which it is migrating. When all molecules are positioned at a uniform starting site on a gel and the gel is placed in a chamber containing a buffer solution and electricity is applied, the molecules will migrate and appear as bands. Nucleic acids, like DNA and RNA, move because of the charged phosphate groups in the backbone of the molecule. Because the phosphates are negatively charged at neutral pH, the DNA will migrate through the gel toward the positive electrode. In this experiment, we will use an agarose gel. In agarose the migration rate of linear fragments of DNA is inversely proportional to their size; the smaller the DNA fragment, the faster (farther) it migrates through the gel.

Figure 9.1a

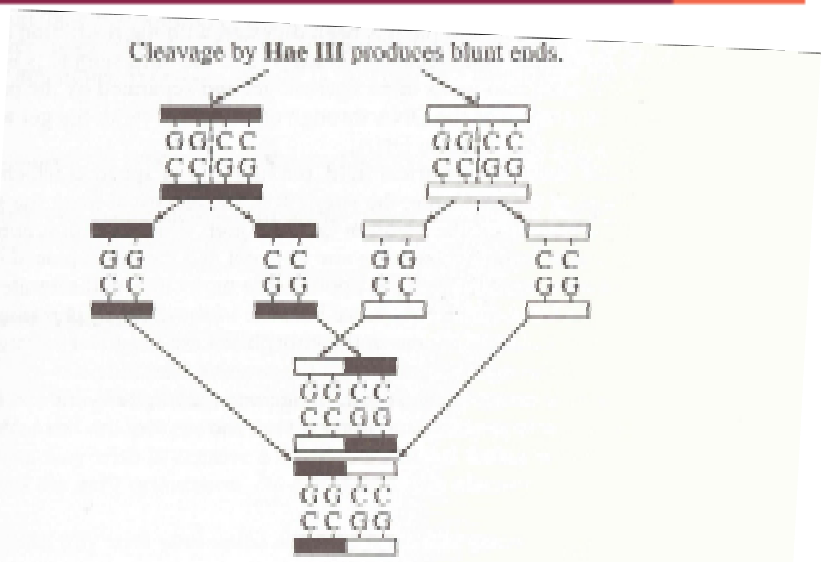
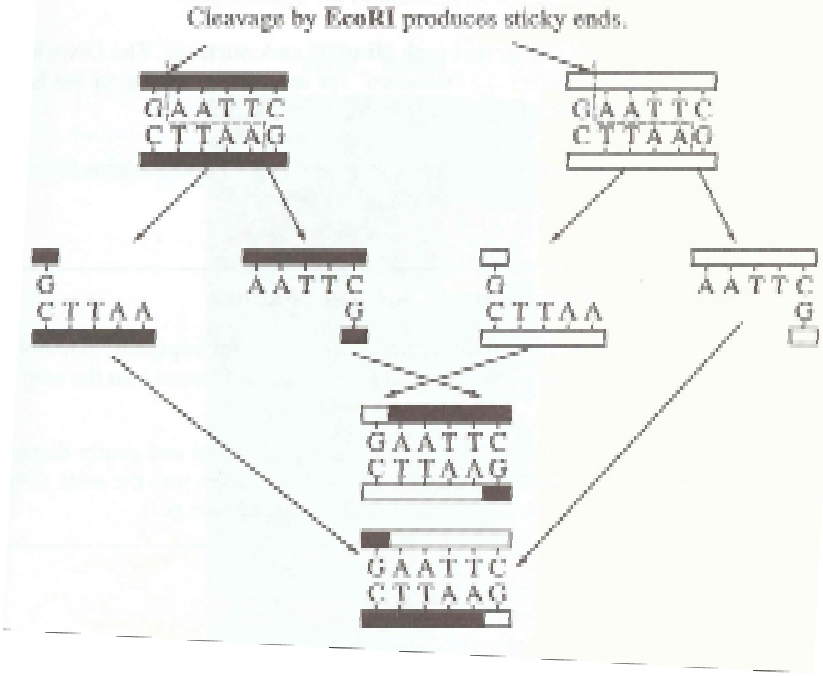


Figure 9.1b



Procedure:

1. Place the tube containing the restriction enzyme mix, labeled ENZ, on ice.
2. Label the Styrofoam holder with the micro test tubes with your group #/names and period.
3. Using a fresh tip for each sample, pipet 10 µl of each DNA sample from the stock tubes and transfer to the corresponding colored micro test tubes. Make sure the sample is transferred to the bottom of the tubes.
4. Pipet 10 µl of enzyme mix (ENZ) into the very bottom of each tube. Use a fresh tip to transfer the ENZ sample to each tube.

5. Tightly cap the tubes and mix the components by gently flicking the tubes with your finger. If a microcentrifuge is available, pulse-spin in the centrifuge to collect all the liquid in the bottom of the tube. Otherwise, gently tap the tube on the table top.
6. Place the tubes in the foam micro tube hold and incubate for 45 minutes at 37°C or overnight at room temperature in a large volume of water heated to 37°C.
7. After the incubation period, remove the tubes from the water bath and place in the refrigerator until the next laboratory period. IF there is sufficient time to continue, proceed directly to step 9.
8. Remove the digested DNA samples from the refrigerator (if applicable).
9. If a centrifuge is available, pulse-spin the tubes in it to bring all of the liquid into the bottom of the tube or gently tap on the table top.
10. Using a separate tip for each sample, add 5 µl of loading dye "LD" into each tube. Cap the tubes and mix by gently flicking the tube with your finger. Collect the sample at the bottom of the tube by tapping it gently on the table or by pulse-spinning in a centrifuge.
11. Remove the agarose gel from the refrigerator (if applicable) and remove the plastic wrap.
12. Place an agarose gel in the electrophoresis apparatus. Fill the electrophoresis chamber with 1x TAE buffer to cover the gel, using approximately 275 ml of buffer.
13. Check that the wells of the agarose gel are near the black (-) electrode and the bottom edge of the gel is near the red (+) electrode.
14. Using a separate tip for each sample, load the indicated volume of each sample into 7 wells of the gel in the following order:
    a. Lane 1: **M,** DNA size marker, 10 µl
    b. Lane 2: **CS,** green, 20 µl
    c. Lane 3: **S1,** blue, 20 µl
    d. Lane 4: **S2,** orange, 20 µl
    e. Lane 5: **S3,** violet, 20 µl
    f. Lane 6: **S4,** red, 20 µl
    g. Lane 7: **S5,** yellow, 20 µl
15. Carefully place the lid on the electrophoresis chamber. The lid will attach to the base in only one orientation. The red and black jacks on the lid will match with the red and black jacks on the base. Plug the electrodes into the power supply, red to red and black to black.
    Turn on the power and electrophorese your samples at 100 V for 30 minutes.

Visualization of DNA Fragments

16. When the electrophoresis run is complete, turn off the power and remove the top of the chamber. Carefully remove the gel and tray from the gel box. Be careful – the gel is very slippery. Slide the gel into the staining tray.
17. Add 120 ml of **1x** Fast Blast DNA stain to the staining tray (2 gels per tray).
18. Let the gels stain overnight, with gentle shaking for best results. No destaining is required.
19. Pour off the stain into a waste beaker.
20. Place an overhead transparency on top of the gel. Using a marking pen (thin-tipped Sharpie is preferable), trace the outlines of the sample wells and the location of the bands.
21. Air-dry the gel on gel support film and tape the dried gel into your laboratory packet.

Analysis of Results

The size of the fragments produced by a specific endonuclease (***Eco*RI/*Pst*I** in this experiment) can be determined by using standard fragments of known size (fragments produced by ***Hind*III**, in this case). When you plot the data on semilog graph paper, the size of the fragments is expressed as the log of the number of base pairs they contain. This allows data to be plotted on a straight line. The migration distance of the unknown fragments, plotted on the x-axis, will allow their size to be determined on the standard curve.

1. Using a ruler, measure the distance (in mm) that each of your DNA fragments or bands traveled from the well. Measure the distance from the bottom of the well to the center of each DNA band and record your numbers in the table on the next page. The data in the table will be used to construct a standard curve and to estimate the sizes of the crime scene and suspect restriction fragments.
2. Using your semilog graph paper, plot distance versus size for bands 2-6 of the known HindIII size marker. Draw a line of best fit through the points. Extend the line all the way to the right-hand edge of the graph.
3. To estimate the size of an unknown crime scene or suspect fragment, find the distance that fragment traveled. Locate that distance on the x-axis of your standard graph. From that position on the x-axis, read up to the standard line, and then follow the graph line over to the y-axis. Where the graph line meets the y-axis is the approximate size of your unknown DNA fragment. Do this for all crime scene and suspect fragments and record their approximate base pair sizes in the table below.

Data Table

	HindII Marker		**Crime Scene**		**Suspect 1**		**Suspect 2**		**Suspect 3**		**Suspect 4**		**Suspect 5**	
Band	Distance (mm)	Size (bp)	Distance (mm)	Size (bp)	Distance (mm)	Size (bp)	Distance (mm)	Size (bp)	Distance (mm)	Size (bp)	Distance (mm)	Size (bp)	Distance (mm)	Size (bp)
1		23,130												
2		9,416												
3		6,557												
4		4,361												
5		2,322												
6		2,027												

Interpretation of Results

1. Do any of your suspect samples seem to be from the same individual as the DNA from the crime scene? Describe the scientific evidence that supports your conclusion.

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Analysis Questions

1. Discuss how each of the following factors would affect the results of electrophoresis.

   a. Voltage used _____

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   b. Running time _____

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   c. Amount of DNA used _____

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   d. Reversal of polarity _____

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2. What are restriction enzymes? How do they work? (Be sure to explain recognition sites in your answer).

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3. What is the source of restriction enzymes? What is their function in nature?

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4. Describe the function of electricity and agarose gel in electrophoresis.

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5. A certain restriction enzyme digest results in DNA fragments of the following sizes: 4,000 bp, 2,500 bp, 2,000 bp, and 400 bp. Sketch the resulting separation by electrophoresis. Show starting point, positive and negative electrodes, and the resulting bands.

6. What are the functions of the loading dye in electrophoresis? How can DNA be prepared for visualization?

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7. How can a mutation that alters a recognition site be detected by gel electrophoresis?

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