DNA Fingerprinting Lab Activity

88 W 8400
QUIKView DNA Stain

88 W 9900
Ethidium Bromide DNA Stain

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WARD'S is working with educators to design our literature to meet the many challenges of today's teachers. Included in every activity are correlations to National Science Education Content Standards and easy to follow instructions, as well as diversified assessments that cater to students of all levels and learning styles.

MATERIALS INCLUDED IN THE KIT

This lab activity is designed for eight groups of students

Materials Checklist

- □ 1 Bottle, 0.8% Agarose, 200 mL
- □ 1 Bottle,TBE running buffer 5X, 500 mL
- □ 1 Bottle, WARD'S DNA stain concentrate, 60 mL (88 W 8400)
- □ 1 Tube, Ethidium Bromide concentrate, 50 µL (88 W 9900)
- □ 8 Gel staining trays
- □ 32 Microcentrifuge tubes
- □ 1 Redemption coupon for the following:
  - 1 Vial, DNA marker standard, 80µL
  - 1 Vial, Crime scene DNA, 80µL
  - 1 Vial, Suspect 1 DNA, 80µL
  - 1 Vial, Suspect 2 DNA, 80µL

MATERIALS NEEDED BUT NOT PROVIDED

- Electrophoresis chambers
- Power supplies
- Waterbath or Microwave
- Lab markers
- Micropipets with tips
- Metric ruler
- Calculator
- Goggles
- Aprons
- Gloves
- UV Transilluminator (88 W 9900)
- UV goggles (88 W 9900)
- Biohazard bag (88 W 9900)

Consult your WARD'S catalog for additional products you may need

SPECIAL HANDLING INSTRUCTIONS

- Redeem the coupon included in the kit for the DNA sample set.
- The specially-treated DNA samples are stable at room temperature. However, if you do not plan to use them within one month, they should be stored frozen.

NATIONAL SCIENCE EDUCATION CONTENT STANDARDS

Standard	Content
Standard K-12: Unifying Concepts and Processes	Evidence, models, and explanation
	Form and function
	Change, constancy, and measurement
Standard A: Science as Inquiry	Abilities necessary to do scientific inquiry
	Understandings about scientific inquiry
Standard C: Life Science	The cell
	Molecular basis of heredity
Standard E: Science & Technology	Understandings about science and technology
Standard F: Science in Personal & Social Perspectives	Personal and community health
	Science and technology in local, national, and global challenges

** This lab activity can be used to fulfill the requirements of AP Biology Lab #6B.*

OBJECTIVES

- Learn the process of agarose gel electrophoresis
- Perform the electrophoresis procedure
- Identify the guilty suspect in a criminal investigation
- Determine the size of unknown DNA molecules

TIME REQUIREMENTS

- Casting gels and diluting buffer: 30 minutes
- Loading and running gel: 60 minutes
- Staining and analyzing gel: 60 minutes

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BACKGROUND

Restriction enzymes, one of the primary tools in molecular biology, can be used to cut DNA molecules into precisely sized fragments. A more accurate name for this class of enzymes is restriction endonucleases because they break DNA molecules at internal (endo) positions. Enzymes that degrade DNA by digesting the molecules from the ends of the DNA strand are called exonucleases ("exo" meaning "outside").

Restriction endonucleases are frequently named using the following convention: The first italicized letter indicates the genus of the organism from which the enzyme was isolated. The second and third italicized letters indicate the species. An additional letter indicates the particular strain used to produce the enzyme. The Roman numerals denote the sequence in which the restriction endonuclease enzymes from that particular genus, species, and strain of bacteria have been isolated.

DID YOU KNOW?
More than 900 restriction enzymes have been isolated from more than 230 strains of bacteria.

Examples of Restriction Endonuclease Names

*Eco*R I
E = genus *Escherichia*
co = species *coli*
R = strain RY 13
I = first RE isolated from this species

*Bam*H I
B = genus *Bacillus*
am = species *amyloliquefaciens*
H = strain H
I = first RE isolated from this species

*Hin*d III
H = genus *Haemophilus*
in = species *influenzae*
d = strain Rd
III = third RE isolated from this species

Some restriction enzymes cut cleanly through the DNA molecule by cleaving both complementary strands at the same nucleotide position within the recognition sequence, which is generally four to six base pairs long. These nucleotide recognition sites are also termed palindromic sequences because both strands have the same sequence running in opposite directions. The restriction endonuclease scans the length of the DNA molecule and stops to cut the molecule only at its particular recognition site. For example, the endonuclease *Hin*d III will cut a double strand of DNA in the following way:

The *Hin*d III enzyme recognizes the sequence

A|A G C T T
T T C G A|A

and will cut the DNA at a specific point (between the A's) within the recognition site, resulting in a staggered cut.

A staggered cut exposes single-stranded regions of the molecule which are known as "sticky ends". These are especially useful in making recombinant-DNA molecules. DNA restriction fragments produced by the same restriction enzyme can be spliced together. The sticky ends produced as a result of the staggered cut by the restriction enzyme allow complementary regions in the sticky ends of another molecule to recognize one another and pair up.

Another class of restriction enzymes cuts cleanly through the DNA molecule by cleaving both complementary strands of DNA at the same nucleotide position within the recognition sequence. These enzymes produce a blunt-end cut. For example, the restriction endonuclease *Bal* I will cut a double strand of DNA in the following way,

Electrophoresis

Molecular-level concepts in biology are often difficult to grasp. When trying to visualize the invisible world of the molecule, students are too often confronted by abstract theory. Yet one product of biotechnology's advances - gel electrophoresis - actually allows students to visualize and separate DNA, proteins, and other gene products such as polypeptides and nucleotide sequences.

Gel electrophoresis is a separation technology that uses gel, a substrate (like gelatin), electricity ("electro"), and movement. "Phoresis", from the Greek verb *phoros*, means "to carry across". Gel electrophoresis, then, refers to the technique in which molecules are forced across a gel by an electrical current; activated electrodes at either end of the gel provide the driving force.

With gel electrophoresis, nucleic acids, both DNA and RNA, can be separated on the basis of size and charge to identify structural forms of plasmid DNA, as well as determine the size of DNA fragments. These determinations are critical to the analysis of the structure and function of genes. Furthermore, the size of unknown DNA fragments can be determined by constructing a standard curve using the migration distances and sizes of a known DNA marker.

Band patterns of separated DNA on a gel visually resemble a bar code - that familiar pattern used to identify consumer products. Each band is a unique "signature" revealing a recorded and identifiable DNA fragment. Scientists and clinicians regularly use scanning instruments to glean vital information from electrophoresis samples. This information is critical in a range of applications: pinpointing cancer types, identifying diseased tissue, characterizing genetic dysfunctions, assessing coronary risk, even reading and compiling nucleotide sequences to map genomes of many life-forms, including our own.

DID YOU KNOW?
Arne Tiselius, a Swedish biochemist, won the Nobel Prize for chemistry in 1940 for his work with electrophoresis.

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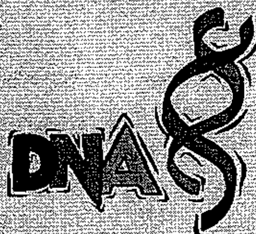
Separation of large (macro) molecules depends on two forces: charge and mass. When a biological sample is mixed in a buffer solution and applied to a gel, these two forces act in concert. The electrical current from one electrode repels the molecules while the other electrode attracts, and the frictional force of the gel material acts as a "molecular sieve", separating the molecules by size and charge. Negatively charged molecules will migrate toward the positive pole, while positively charged molecules will migrate toward the negative pole. The net negative charge of their phosphate backbones results in the DNA fragments having a negative charge and thus will always migrate toward the positive pole. The material is roughly analogous to that of a thoroughly wetted sponge, except that in this case, the "pores" are submicroscopic. During electrophoresis, macromolecules are forced to migrate through the "pores", always from the closest electrode and toward the farther electrode when electrical current is applied. After staining, the separated macromolecules in each lane can be seen; they appear as a series of bands spread from one end of the gel to the other.



DID YOU KNOW?
There are two types of electrophoresis: moving boundary electrophoresis uses liquid media and zone electrophoresis which uses solid or semi-solid media

There are two basic types of materials used to make gels: agarose and polyacrylamide. Agarose is a natural colloid extracted from seaweed. Agarose gels have a very large "pore" size and are used primarily to separate very large molecules such as DNA with a molecular mass greater than 2,000 kdal. kdal is the abbreviation for kilodalton, or 1,000 daltons. A dalton is a unit of molecular weight very nearly equivalent to the mass of a hydrogen atom, or 1.000 on the atomic mass scale. When agarose is heated to about 90°C it melts, but re-solidifies when cooled below 45°C. During the solidification process, agarose forms a matrix of microscopic pores. The size of these pores depends on the concentration of agarose used. Typically the concentration varies from 0.5% to 2.0%. The lower the concentration, the larger the pore size of the gel and the larger the nucleic acid fragments that can be separated. During electrophoresis, DNA molecules wind through the pores in the gel. As the pore size decreases (by increasing agarose concentration), it is harder for longer DNA fragments to travel properly. Smaller DNA fragments can thread their way through the pores more easily, migrating faster. Polyacrylamide is a material similar to that found in soft contact lenses; it is primarily used to separate proteins.

DNA Fingerprinting

Of the three billion nucleotides in human DNA, more than 99% are identical among all individuals. The remaining 1% that is different, however, adds up to a significant amount of code variations between individuals, making each person's DNA profile as unique as a fingerprint. Due to the very large number of possible variations, no two people (with the exception of identical twins) have the same DNA sequence.

For every 1,000 nucleotides inherited, there is one site of variation, or polymorphism. These DNA polymorphisms change the length of the DNA fragments produced by the digestion of restriction enzymes. The exact number and size of fragments produced by a specific restriction enzyme digestion varies from person to person. The resulting fragments, called Restriction Fragment Length Polymorphisms (RFLPs), can be separated and their size determined by electrophoresis.

Most of the DNA in a chromosome is not used to code for genes. It is uncertain what, if any, use this "unused" DNA may have. Because these regions are not essential to an organism's development, it is more likely that changes will be found in these nonessential regions. These regions contain nucleotide sequences (e.g., GTCAGTCAGTCAGTCA) that repeat from 20 to 100 times. These restriction enzymes that flank these repeating sequences cut the DNA strand creating RFLPs.

The differences in the fragments can be quantified to create a "DNA fingerprint". Distinct RFLP patterns can be used to trace the inheritance of chromosomal regions with genetic disorders or to identify the origin of a blood sample in a criminal investigation. Scientists have identified more than 3,000 RFLPs in the human genome, many of which are highly variable among individuals. It is this large number of variable yet identifiable factors that allow scientists to identify individuals by the number and size of their various RFLPs.

This technique is being used more and more frequently in legal matters. Using DNA fingerprinting, the identity of a person who has committed a violent crime can be determined from minute quantities of DNA left at the scene of the crime in the form of blood, semen, hair, or saliva. The DNA fingerprint matched to a suspect can be accurate to within one in 10 billion people, which is about twice the total population of the world. Certain limitations in the technique prevent two samples from being identified as a "perfect match", yet it is possible to measure the statistical probability of two samples coming from the same individual based on the number of known RFLPs that exist in a given population.

DNA fingerprinting has many other applications. Since half of a person's genome comes from each parent, DNA fingerprinting can be used to determine familial relationships. It has a much higher certainty than a blood test when used to determine fatherhood in a paternity suit. DNA fingerprinting can be used to track hereditary diseases passed down family lines and can be used to find the closest possible matches for organ transplants. It can also be used to ascertain the level of inbreeding of endangered animals, aiding in the development of breeding programs to increase animals' genetic health and diversity.

DID YOU KNOW?

In 1989, Alec Jeffreys, a professor at Leicester University, coined the term DNA fingerprinting. He was the first person to use DNA in court cases involving paternity, immigration, and murder.

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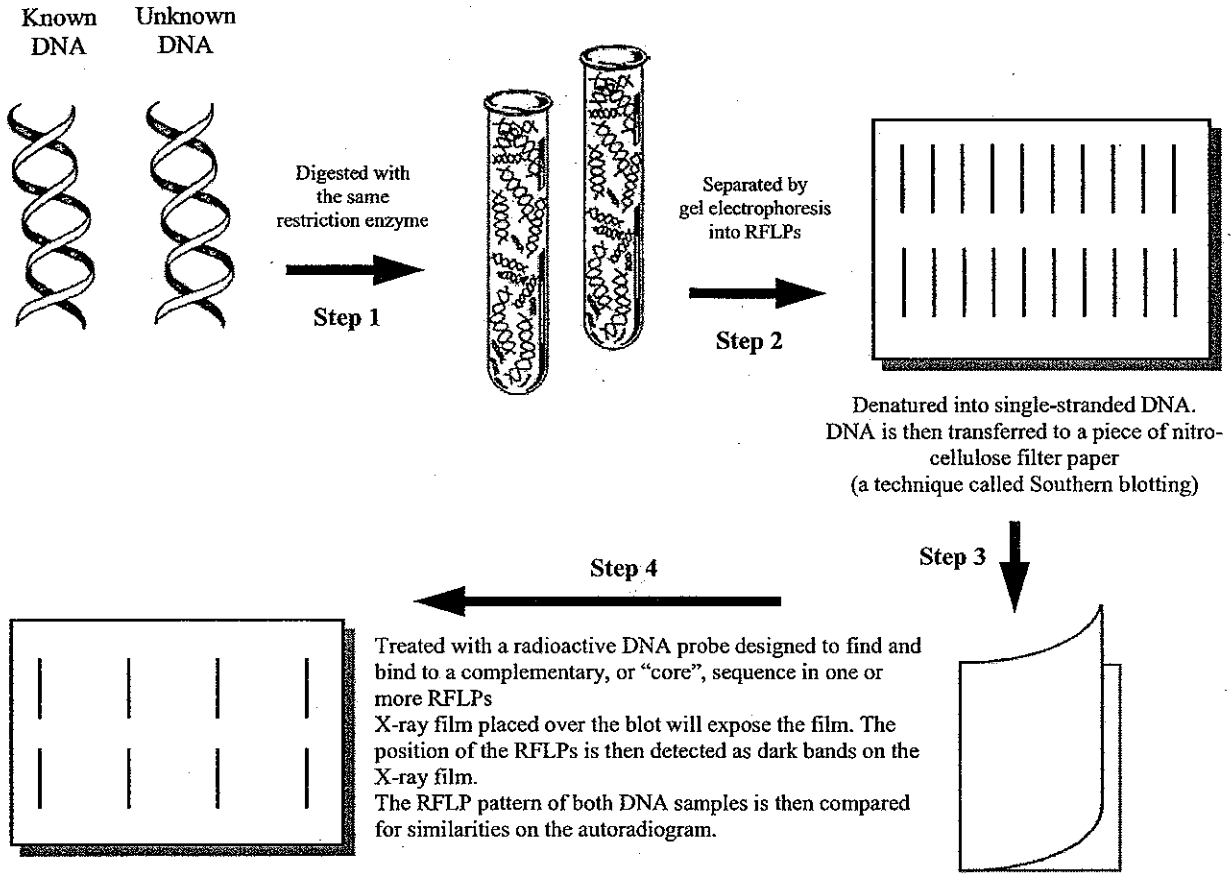


DNA Fragment Length Determination

Under a given set of electrophoretic conditions such as pH, voltage, time, gel type, concentration, etc., the electrophoretic mobility of a DNA fragment molecule is standard. The length of a given DNA fragment can be determined by comparing its electrophoretic mobility on an agarose gel with that of a DNA marker sample of known length. The smaller the DNA fragment, the faster it will move down the gel during electrophoresis.

Using a technique called Southern blotting, the separated fragments are transferred to nitrocellulose paper, labeled with a radioactive probe, and developed against X-ray film. The probe, which is coded to bind to specific RFLPs being tested, will develop the film. The greater the concentration of DNA in that particular band, the darker the band will be. The resulting image, called an autoradiogram, shows a series of dark and light bands. This pattern is the DNA fingerprint of the tested individual. Comparing the distances between the bands in different samples determines the similarities between the samples.

PRE-LAB PREPARATION

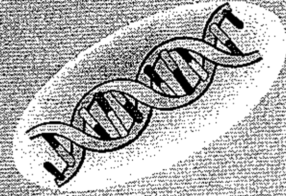
Agarose Gel Electrophoresis

To conserve class time, the teacher can to do the following in advance:

- *Prepare 1X TBE Running Buffer*
- *Melt agarose and cast the gels*
- *Prepare DNA stain*

The procedure for each step listed above appears in the procedure.

The procedure used in this lab activity will result in a gel showing the results of a Southern Blot DNA Fingerprint.



DID YOU KNOW?

Real human DNA is billions of base pairs long. Cutting human DNA with restriction enzymes results in millions of fragments on a gel, which is why the Southern blotting procedure is performed.

The DNA samples used in this investigation are actually viral DNA, which is much smaller and gives distinct patterns on agarose gels, eliminating the need for Southern blotting.

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MATERIALS

MATERIALS NEEDED PER GROUP

1 0.8% Agarose gel, on gel tray
TBE running buffer 1X, 350 ml
DNA stain
Staining tray
Micropipets
Metric ruler
Calculator
Semi-log graph paper
Goggles
Aprons
Gloves

SHARED MATERIALS

DNA Samples:
DNA marker standard
Crime scene DNA
Suspect 1 DNA sample
Suspect 2 DNA sample
Waterbath or microwave
lab markers
UV transilluminator (88 W 9900)
UV goggles (88 W 9900)
Biohazard bag (88 W 9900)

DID YOU KNOW?
Homes without security systems are approximately 3 times more likely to be burglarized than homes with security systems.

SCENARIO

Investigators were called to the scene of a burglary where it appeared that, as the burglar rushed to leave, he ran into a glass door cutting his arm and tearing his shirt. The investigators removed small pieces of bloodstained fabric from the door to be tested. The blood sample was determined to be type A. Two suspects were apprehended; unfortunately, both had type A blood. Investigators have now resorted to DNA fingerprinting to determine which of the two suspects is the burglar.

PROCEDURE

Prepare 1X TBE Running Buffer

1. Add 280 mL of distilled water to 70 ml of 5X TBE concentrate to obtain 350 mL of 1X buffer.

Any remaining 1X TBE or 5X TBE buffer may be stored at room temperature in a clean container. The diluted buffer will provide best results if used within 18 months. If the concentrated buffer contains a white precipitate it should be discarded.

Melt Agarose

The bottle of prepared agarose provides enough material to produce eight 25 mL *gels.*

Always handle agarose bottle with heat-protective gloves.

1. Melt the agarose using a boiling waterbath or a microwave.

   Waterbath: Loosen the cap on the agarose bottle before placing it in the boiling waterbath. Water temperature should be 100°C. Boil until the agarose is completely liquefied. Remove the bottle from the water bath and swirl occasionally.

   Microwave: Loosen the cap on the agarose bottle before microwaving. Heat in one-minute intervals on low to medium power until the agarose is melted.

If, when heating in a microwave, the agarose starts to boil before melting completely, stop the microwave, swirl the agarose bottle, and continue.

Melted agarose may be stored in a 70°C water bath until ready to pour gels. Unused agarose may be stored in a screw-top bottle and remelted.

DID YOU KNOW?

The first time DNA was used as evidence in a trial was in 1985.

The first time DNA evidence actually sent someone to jail was in 1988.

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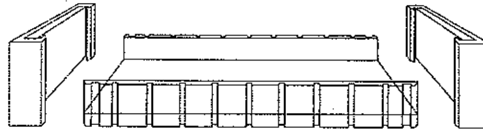
Aliquot the DNA (Optional)

1. You may wish to provide each group with their own DNA samples. If so, aliquot each of the samples into eight sets of the provided microcentrifuge tubes. Be sure to label the tubes appropriately.

Casting an Agarose Gel

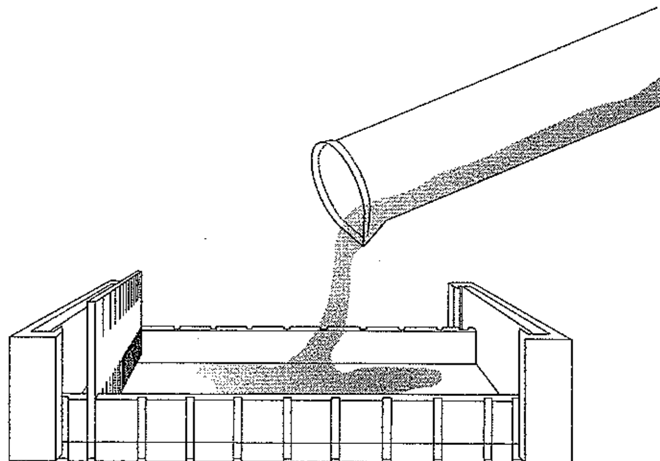
You may pour the gels in advance and store them refrigerated for up to one week prior to performing the electrophoresis portion of the lab. Place the gel, on the casting tray, in a resealable bag and add 1-2 ml 1X TBE buffer. Seal the bag and store refrigerated until needed. As a precaution, you may wish to pour one or two extra gels.

1. Place a gel casting tray on a flat surface and snap the end dams onto each end of the casting tray. The end dams seal the ends of the tray, eliminating the need to tape the ends.



2. Insert the gel comb, using the 8-well side, into the slots near the end of the tray.

3. Pour approximately 25 mL melted agarose into the tray, until it reaches a depth of about 3 mm.



4. Allow the gel to solidify for approximately 20-30 minutes. Do not disturb the gel tray or comb. When the agarose has solidified, it will turn opaque.

DID YOU KNOW?
Besides agarose, other types of semi-solid media used for electrophoresis are cellulose acetate and polyacrylamide gel.

5. After the gel has solidified, carefully remove the comb from the gel by lifting the comb straight up. Remove the end dams from the tray by carefully sliding them up.

Prepare Stain

Wear gloves, apron and eye protection when working with either of the following two stains.

WARD'S QUIKView DNA Stain Lab Activity (88 W 8400):

The WARD'S QUIKView DNA Stain may be prepared just prior to staining the gel. Warm DNA stain results in enhanced banding of the DNA fragments.

1. Add 5 mL WARD'S QUIKView DNA Stain concentrate to 95 mL warm (50° to 55°C) distilled or tap water to obtain 100 mL of dilute stain.

2. Dilute WARD'S QUIKView DNA Stain can be stored at room temperature indefinitely in a labeled screw-top bottle. Rewarm before use.

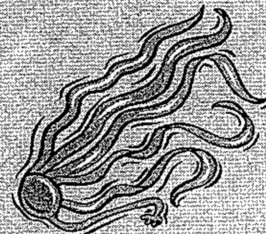
WARD'S Ethidium Bromide (EtBr) Stain Lab Activity (88 W 9900):

Ethidium Bromide is considered a possible mutagen and should be treated with great respect. It is __HIGHLY__ recommended that __ONLY__ the teacher perform procedures and handle items involving Ethidium Bromide. Check with your local authorities for proper disposal procedures.

1. Fill the labeled, brown glass bottle with 500 mL distilled water. Add the contents (50 µL) of the concentrated ethidium bromide tube into the water. Tighten the cap and swirl to mix.

2. Storage directions:

   Ethidium bromide is light sensitive and should be stored in the brown, screw-top bottle supplied with this activity. It can be stored indefinitely at room temperature.



DID YOU KNOW?
Agarose is a highly purified form of agar, which is a material extracted from seaweed.

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Loading and Running a Gel

1. Place the gel, on the gel tray, in the center of the electrophoresis chamber with the wells closer to the negative (black) electrode.

2. Add approximately 350 mL of 1X TBE running buffer to the chamber: **Slowly** pour buffer from a beaker into one side of the chamber until the buffer is level with the top of the gel. Add buffer to the other side of the chamber until the buffer is level with the top of the gel. Continue to **slowly** add buffer until the level is approximately 2-3 mm above the top of the gel.

If you are running two gels, place the first gel in the chamber and add buffer until the first gel is completely submerged, then load the gel. After the first gel is loaded, place the second gel with the gel tray into the electrophoresis chamber. Slowly add more buffer until it reaches a level that is 2-3 mm above the second gel and load the second gel. Do not overfill the chamber. Wipe off any spills.

3. Load 10 µL of each DNA sample into the corresponding lane. Do not pierce the bottom of the wells with the micropipet tip. Do not overload wells.

Lane #1: DNA marker standard
Lane #2: Crime scene DNA sample
Lane #3: Suspect 1 DNA sample
Lane #4: Suspect 2 DNA sample

The amount of DNA in the reaction tubes is extremely small. Demonstrate the correct procedures needed to transfer samples from these reaction tubes to the wells on the gel.

DID YOU KNOW?
New DNA technology has been used to clear the names of several people who were wrongly convicted of crimes. One man, falsely convicted of rape in 1969, was released from prison after 23 years when advances in biotechnology allowed minute amounts of his DNA to be analyzed, the result proving his innocence.

Preparation Notes

The power supply produces a high enough voltage to cause severe electrical shock if handled improperly. For safe operation, follow all directions and precautions.

- Examine all components of the electrophoresis apparatus prior to each use: all cords, plugs, jacks, the electrophoresis chamber itself, and the power supply.

- Do not operate electrophoresis apparatus in a damp or humid environment; any condensed moisture may short out electrical components. You may wish to designate one area of the laboratory specifically for electrophoresis equipment, where cells and power supplies are connected. Ensure that power cords and patch cords are free from moisture and that any wall outlet is properly wired; i.e., that correct polarity exists (use a circuit tester).

- Be sure that students are well acquainted with the correct procedure for making electrical connections. Students should be supervised at all times when performing this investigation.

- Do not come in personal contact with or allow metal or any conductive material to come in contact with the reservoir buffer or the electrophoretic cell while the power supply is on.

4. Make sure the cover, as well as the female jacks and the plugs, are dry, then slide the cover onto the electrophoresis chamber. Wipe off any spills on the apparatus before proceeding to the next step.

5. Make sure that the patch cords attached to the cover are completely dry, then connect the red patch cord to the red electrode terminal on the power supply. Connect the black patch cord to the black electrode terminal on the power supply.

Check the connections before allowing students to proceed to the next step.

6. Plug in the power supply and set it to the desired voltage.

It is recommended that you set the power supply between 75-125 volts. The system may be run at lower voltage settings but this will increase the running time of your agarose gels.

7. Turn on the power supply. The red power light will illuminate, and bubbles will form along the platinum electrodes.

DID YOU KNOW?
Gel electrophoresis is used in many fields of biology. Examples include genetic testing, paternity analysis, forensics, conservation biology, animal behavior, taxonomy, and evolutionary biology.

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8. Observe the migration of the loading dye down the gel toward the red electrode. Turn off the power when the loading dye has reached the end of the gel. Unplug the power supply.

The loading dye is a special dye added to the DNA samples prior to performing electrophoresis and serves two purposes. It is heavier than the electrophoresis buffer, causing the DNA samples to sink into the wells. It is also smaller than most of the DNA fragments in the samples so it runs to the end of the gel faster than the DNA, giving an indication of when to end the electrophoresis run.

9. Wait approximately 10 seconds and then disconnect the patch cords from the power supply. Remove the cover from the electrophoresis chamber.

10. Carefully remove the gel, on the casting tray, from the electrophoresis chamber.

Optional stopping point:

If the lab period does not allow time to stain and destain the gel, place the gel in a resealable bag and add 1-2 mL of 1X TBE buffer and refrigerate the gel until the next lab period.

DID YOU KNOW?

DNA testing was used to prove that Thomas Jefferson was in fact the biological father of at least one child of his slave, Sally Hemings.

Staining Gels

Using WARD'S QUIKView DNA Stain

Wear gloves, apron and eye protection when working with WARD'S QUIKView DNA Stain.

1. Gently slide the gel from the casting tray into the staining tray and pour approximately 100 mL of warm dilute stain into the staining tray so that it just covers the gel.
2. Cover the tray and let the gel stain for approximately 30-40 minutes. Make sure the gel remains flat and does not move up against the sides.
3. When finished staining, you may decant the stain directly to a sink drain and flush with water or, save the stain by decanting it into a sealable container. The dilute DNA stain may be saved and reused several times. For best results, re-warm the stain before reusing.
4. Flush the gel under streaming tap water. Then add distilled or tap water to the staining tray. It is best not to pour water directly onto the gel. To accelerate destaining, gently rock the tray. Destain until bands are distinct, with little background color. This will take between 30 and 60 minutes, depending on the amount of agitation and number of water changes. Change the water several times, or destain the gel, without changing the water, overnight.
5. View the gel against a light background, such as white paper, or on a light table. Gels can be stored in resealable plastic bags. For long-term storage, add several drops of dilute stain to the bag to prevent the DNA bands from fading. If fading does occur, the gel can be restained using the above procedure.
6. Accurately sketch the bands you see on the blank gel in the Analysis section. Be as exact as possible in sketching the bands in their actual positions.

All materials from the QUIKView DNA Stain Lab Activity can be disposed of in the trash.

DID YOU KNOW?

The unique banding patterns of each individual suspect's DNA can be compared to those contained within searchable national computer databases to determine whether the suspect has been implicated in any other crimes. Many criminals have been brought to justice for crimes they thought they had gotten away with due to this sharing of data.

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Using WARD'S Ethidium Bromide (EtBr) DNA Stain

*Ethidium Bromide is considered a possible mutagen and should be treated with great respect. It is **HIGHLY** recommended that **ONLY** the teacher perform procedures and handle items involving Ethidium Bromide.*

Wear gloves, apron and eye protection when working with EtBr.

1. After electrophoresis, gently slide the gel from the chamber into a disposable plastic staining tray.
2. Cover the gel with 1X EtBr solution.
3. Let stand for 10 minutes.
4. Decant the EtBr solution from the staining tray back into its original container. The EtBr stain may be saved and reused several times.
5. Flush the gel and staining tray with running tap water.
6. Cover the gel with distilled or tap water for 10 minutes.
7. Remove gel from tray and place on UV Transilluminator for examination.

Be certain that the UV Transilluminator has a UV-blocking shield and/or have everyone in the area wear UV-blocking safety glasses or face shields.

8. Accurately sketch the bands you see on the blank gel in the Analysis section.

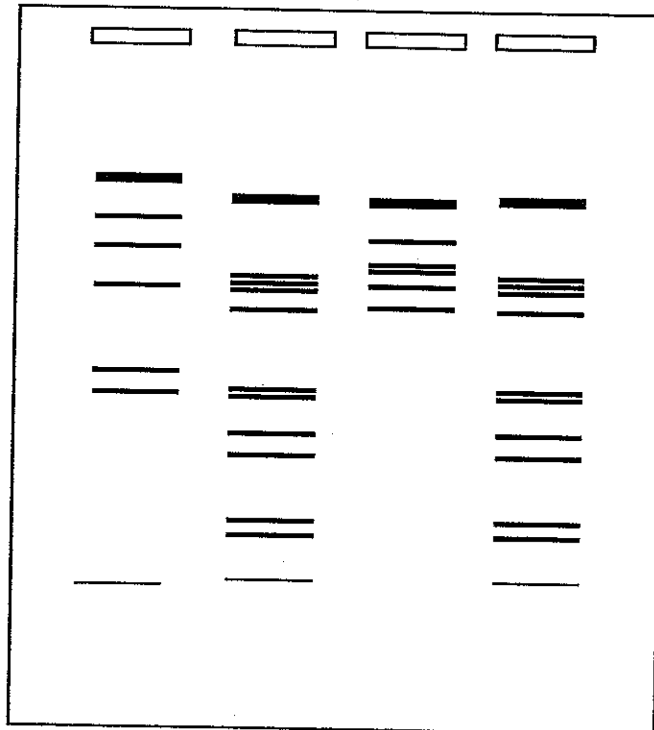
All gels, gloves, staining trays, etc. that contact EtBr should be placed in a biohazard bag and disposed of according to local regulations.

Wash hands thoroughly before leaving the lab.

DID YOU KNOW?

The fluorescent dye ethidium bromide intercalates between the base pairs of DNA. UV light at 260 nm is absorbed by the DNA and transmitted to the EtBr. UV light at 300 nm and 360 nm is absorbed by the dye itself. Ethidium Bromide fluoresces by emitting a wavelength of 590 nm, which is in the red-orange range of the visible spectrum.

ANALYSIS



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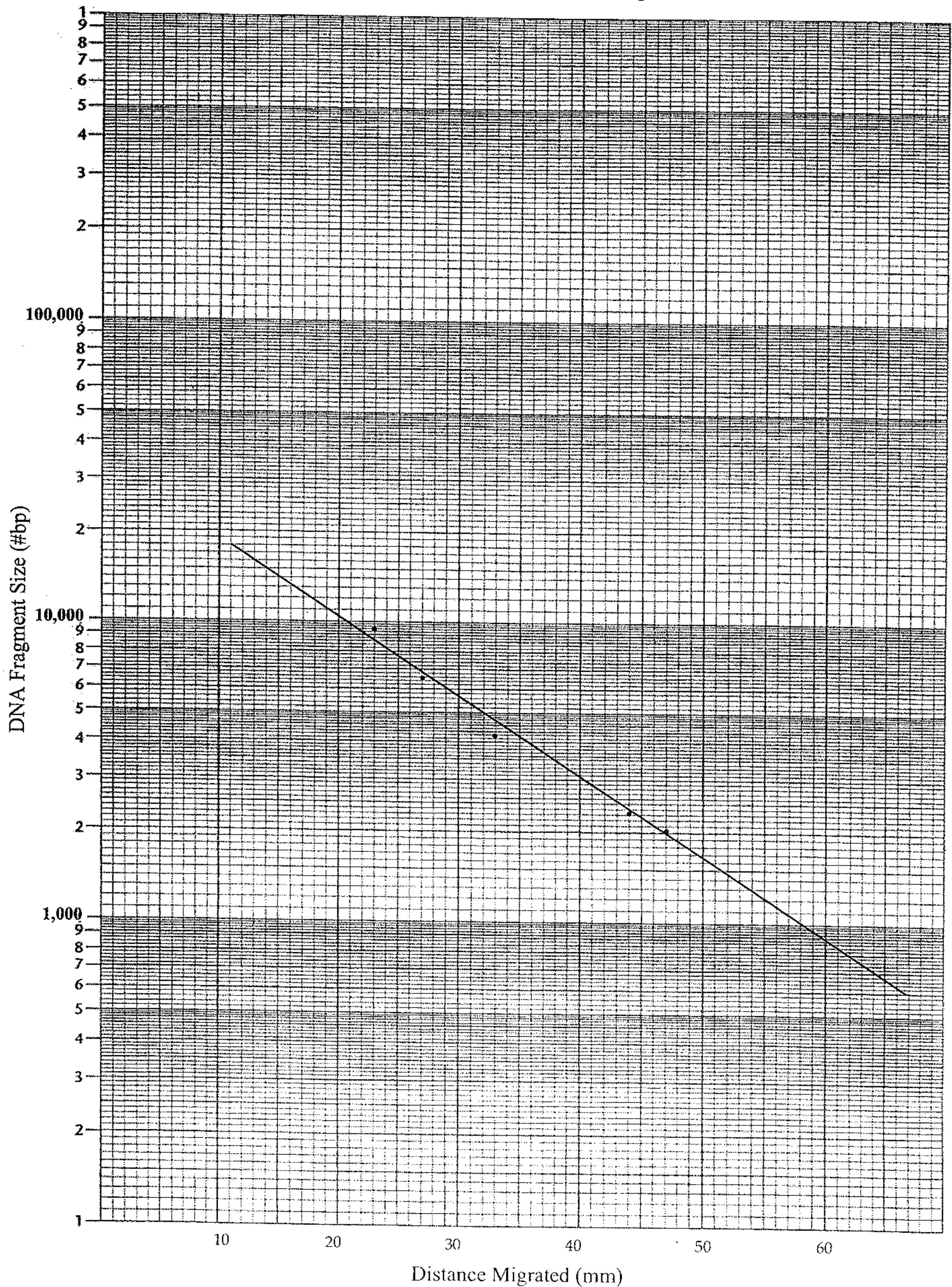

Determination of Unknown DNA Fragment Size
DNA Fragment Size (#bp)
100,000
10,000
1,000
1
9
8
7
6
5
4
3
2
10
20
30
40
50
60
Distance Migrated (mm)

Analyzing your gel

1. Measure the distance of the DNA bands, in millimeters, from the bottom of the sample well to the bottom of each DNA fragment for the DNA marker standard. Measuring to the bottom of each fragment band ensures consistency and accurate measurements. Do not measure the migration distance of the largest fragment nearest the well; it will not be on the standard curve and will skew results.

2. Record the measurements in Table 1 in the Analysis section.

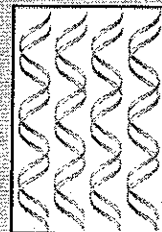
3. On semi-log graph paper, plot a standard curve for the DNA marker standard. Plot the migration distance in millimeters on the X-axis, against the molecular size in base pairs (bp) of each fragment. Draw the best-fit line to your points.

When plotting on semi-log graph paper, the fragment size on the Y-axis is expressed on a logarithmic scale. Label the first series of lines 100bp, 200bp, 300bp, etc. Then label the second series of lines 1000bp, 2000bp, 3000bp, etc. The third series would be 10,000bp, 20,000bp, etc.

4. Measure the distance that each band traveled for the lanes containing the two suspects and crime scene DNA. Record the data in the Tables 2, 3, and 4 in the Analysis section.

5. Calculate the base pair size of each of the fragments by moving along the X-axis until you have reached the distance traveled by the fragment. From that point, move upward until you intersect the line of best fit on the graph. Determine where that point is on the Y-axis and estimate the base pair value at that point. Enter this data in the Tables 2-4.

This interpolation technique is not exact. Students' results may vary from 10% to 20% from the actual values, but should be similar to the above data.



DID YOU KNOW?
Ethidium Bromide will stain both single- and double-stranded DNA; however, the fluorescence is poorer with single-stranded DNA.

Table 1
DNA Marker Standard

Fragment	Distance Migrated (mm)	Length (bp)
1 (top)	18	23109
2	23	9416
3	27	6557
4	33	4361
5	44	2328
6	47	2027
7	74	564
8	Not imaged	125

The first fragment is too large to migrate properly in agarose and will not fit within your line of best fit, and should be disregarded.

Table 2
Crime Scene DNA Sample

Fragment	Distance Migrated (mm)	Length (bp)
1 (top)	21	21226
2	29	5148
3	33	4973
4	34	4268
5	36	3530
6	47	2027
7	48	1904
8	52	1584
9	56	1375
10	65	947
11	67	831
12	74	564
13	Not imaged	125

Table 3
Suspect 1 DNA Sample

Fragment	Distance Migrated (mm)	Length (bp)
1 (top)	21	21226
2	26	7421
3	29	5804
4	30	5643
5	32	4878
6	36	3530
7	—	—
8	—	—

Table 4
Suspect 2 DNA Sample

Fragment	Distance Migrated (mm)	Length (bp)
1 (top)	21	21226
2	29	5148
3	33	4973
4	34	4268
5	36	3530
6	47	2027
7	48	1904
8	52	1584
9	56	1375
10	65	947
11	67	831
12	74	564
13	Not imaged	125

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ASSESSMENT

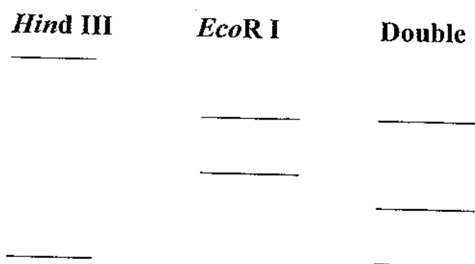
1. Compare the banding patterns formed on each lane of the gel. Do you think the three DNA samples tested are the same? Explain. How can you further verify whether or not any of the DNA samples tested are the same?

   Two of the three DNA samples tested are identical; they have the same banding pattern. To further verify whether or not any of the DNA samples tested are the same, the DNA fingerprinting protocol could be followed by a procedure called sequencing. DNA sequencing would allow the determination of the exact DNA sequence of the DNA fragments on the gel.

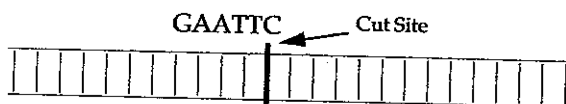
2. Which of the two suspects do you believe is the real burglar? Explain your answer.

   Suspect 2's DNA banding pattern matches the DNA pattern of the crime scene DNA sample. It can be concluded that, based on the similarities of these two samples, suspect 2 cannot be ruled out as having committed the crime.

3. *Eco*R I recognizes GAATTC, and *Hin*d III recognizes AAGCTT. A student adds *Eco*R I to a linear DNA sample. To another quantity of the same DNA, she adds *Hin*d III. In a third tube she adds both enzymes. She runs a gel and the following occurs:



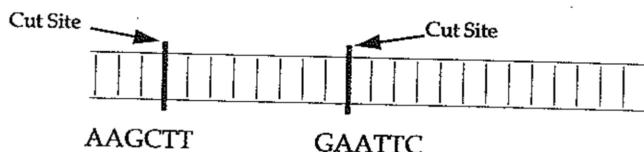
The student, after viewing the gel, draws the following map of the linear DNA



a. Explain why she placed the *Eco*R I restriction enzyme site as she did.

The *Eco*R I cut in one place which is why there are two bands. One fragment is slightly smaller than the other.

After a little more thought, she added another sequence to the DNA:



b. Explain how she was able to add the Hind III restriction enzyme site in this position based on the results of her gel.

The *Hin*d III by itself only cut once resulting in one very large band and one very short band. The *Eco*R I also cut once. In the double digest, the *Hin*d III cut the smaller of the *Eco*R I fragments.

5. Different restriction enzymes are isolated from different types of bacteria. What advantage do you think bacteria gain by having restriction enzymes?

   Restriction enzymes serve as a means of defense for a bacterial cell. Restriction enzymes can prevent DNA from other organisms, such as bacterial viruses or other bacterial species, from entering the cell and taking over vital cell processes.

6. In your lab, you ran your DNA samples on a 0.8% agarose gel. Would you get the same results if you ran your samples on a higher percentage agarose gel? Why or why not?

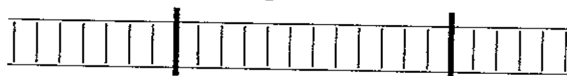
   Student answers will vary. Running the samples on a gel of only slightly higher percentage of agarose (0.9% or 1.0%) would probably not affect the results significantly. However, if the percentage were greatly increased, such as 2.0% or 2.5%, the larger fragments may not be able to move through the decreased pore size, resulting in less effective electrophoretic separation.

7. Predict what would happen if you place your gel in the electrophoresis chamber with the wells containing the DNA next to the red electrode instead of the black?

   The DNA would still be attracted to the positive electrode. The DNA samples would run quickly toward the top of the gel and out into the buffer.

8. If you have a restriction enzyme that cuts a piece of linear DNA at two recognition sites, how many DNA fragments would you see on a gel?

   Three. Two cut sites would result in three pieces of DNA

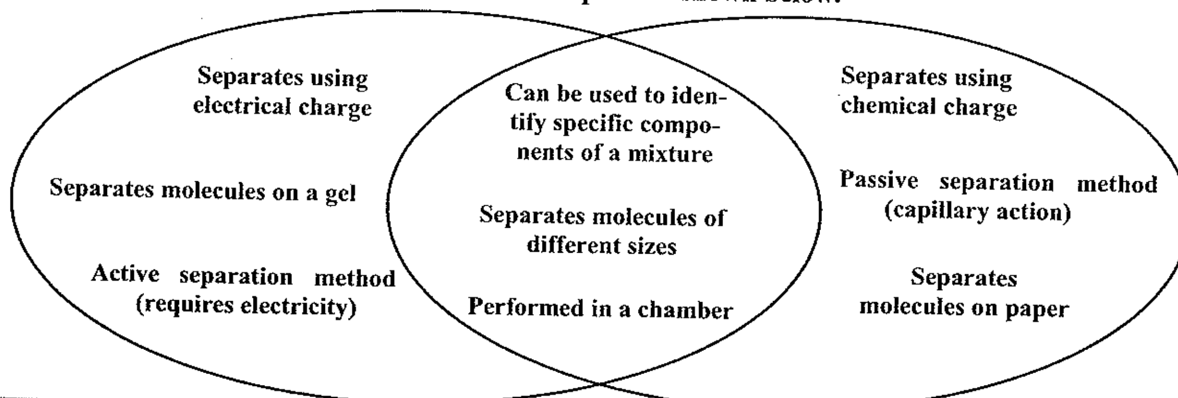


9. *Hind* III recognizes a sequence of six nucleotides (AAGCTT) as a cut site. What are the odds that this sequence will occur in a random chain of DNA?

   1 out of 4,096 (4 x 4 x 4 x 4 x 4 x 4). Since there are four bases (A, C, G, and T) possible and they must appear in a six base sequence the answer is 1 out of 4^6

10. Electrophoresis is one method of separating molecules. Paper chromatography is another method of separating molecules. Create a Venn diagram showing at least two similarities and two differences between these two methods.

    Accept any reasonable answers. Some examples are shown below.



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11. Below is a list of the components involved in agarose gel electrophoresis. Briefly describe the purpose of each component.

Agarose gel – **the substrate used to separate molecules. Contains microscopic pores that al low smaller molecules to move through faster.**

TBE buffer – **a liquid buffer that protects the DNA molecules and allows electricity to move through the chamber, driving the molecules across the gel.**

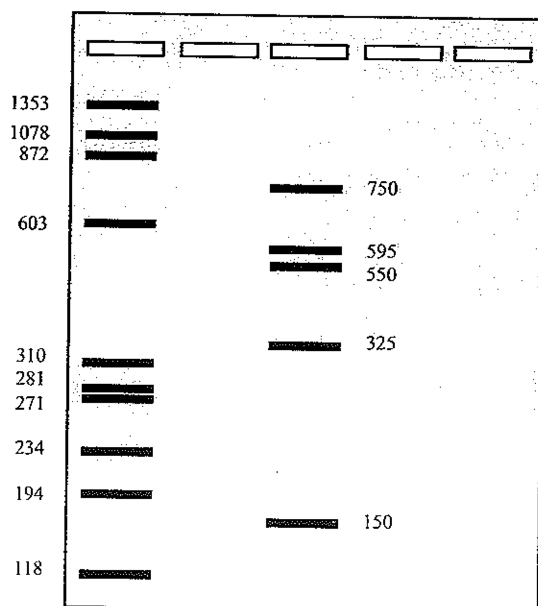
Electrophoresis chamber – **holds all of the components of the separation. Creates a closed sys tem in which to submerge a gel and apply electrical current.**

Power supply – **the unit generating the direct current electricity being applied to the chamber.**

DNA samples – **the substance to be separated. A mixture of differently-sized fragments that separate when electricity is applied.**

DNA stain – **used to stain the gel after electrophoresis. Since DNA cannot be seen with the na ked eye, DNA stain is necessary to visualize your DNA fragments.**

12. Below is a restriction map made from the weight marker ΦX174, a different marker than the one you used in your lab exercise. You ran a DNA sample containing fragments of the size 750 bp, 595 bp, 550 bp, 325 bp, and 150 bp. Draw in the gel below approximately where your DNA fragments would appear.



13. You have used electrophoresis to perform DNA fingerprinting and identify a probable perpetrator involved in a criminal investigation. Research another use for electrophoresis and briefly describe the benefits of using this technology.

    Answers will vary. Students may present topics such as paternity testing, screening for genetic disorders, protein identification, studying evolutionary relationships, genetic engineering, etc.

14. The application of DNA fingerprinting technology in forensic science as well as in medicine has raised many legal and ethical concerns. As a class, discuss the following issues: Should data banks be established for DNA information? What are some benefits of DNA data banks? How will these be controlled? Should the DNA taken from a suspect for identification be used to determine other genetic characteristics? Should newborn children have a DNA fingerprint created and kept in a DNA data bank?

    Answers will vary.

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