AP® Biology
Investigation #9:
Genetics and Information Transfer:
Restriction Enzyme
(student guide)

Meets Revised College Board AP Biology Standards
safety precautions

lab specific safety

• Tris-Borate-EDTA is slightly toxic if ingested.

• Ward's QuickView DNA stain will stain clothing and skin.

• CAUTION: The power supply produces a high voltage that can 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**, including all cords, plugs, jacks, the electrophoresis chamber itself, and the power supply.

• Do not operate the electrophoresis apparatus in a damp or humid environment. Any condensed moisture may short out electrical components.

• 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.

general safety

• Know where all **emergency equipment** (safety shower, eyewash station, fire extinguisher, fire blanket, first aid kit etc.) is located.

• Remove all dangling jewelry and tie back long hair before you begin.

• **Read all instructions, SDSs and live care sheets** before starting the lab activities, and ask questions about safety and safe laboratory procedures. The SDSs and the most updated versions of live care sheets can be found at www.wardsci.com. Updated SDSs can also usually be found on each chemical manufacturer’s website.

• In student directed investigations, make sure that collecting safety information (like SDSs) is part of the experiment procedure.

• As general laboratory practice, it is recommended that you **wear proper protective equipment**, such as gloves, safety goggles, and a lab apron.

at the end of the lab:

• Follow your teacher’s instructions regarding clean-up and disposal of all chemicals and materials used in this lab.

• All laboratory bench tops should be wiped down with a 10% bleach solution or disinfectant to ensure cleanliness.

• Wash your hands thoroughly with soap and water before leaving the laboratory.
**Agarose**: a purified extract of seaweed that is a solid matrix used to separate molecules based on electrical charge and size.

**Gel electrophoresis**: the method used to separate proteins or strands of nucleotides according to charge using agarose.

**Mutation**: a change in the nucleotide sequence of an organism's genome.

**Polymerase chain reaction (PCR)**: the process used to amplify a piece of DNA to produce larger quantities. It is used in disease detection, tissue typing, forensic analysis, and research applications.

**Polymorphism**: found in many forms (lengths).

**Probe**: a piece of DNA or RNA with a specific sequence that is complementary to a sequence of choice to indicate the presence of that DNA or RNA.

**Restriction enzymes (endonucleases)**: proteins isolated from bacteria that cut nucleotides at specific sequences.

**RFLP**: a process using a restriction enzyme to digest a DNA sample at specific sites to create a DNA profile.

**Short tandem repeat (STR)**: short DNA sequences that are repeated numerous times within an individual's chromosomes.

**Southern Blot analysis**: a technique used following gel electrophoresis to identify fragments of DNA with specific complementary sequences to a previously-labelled probe.
Nucleic acid analysis has become a powerful tool in understanding organisms, their evolution, and their fundamental nature. Upon isolation of DNA from a cell source, that DNA can be examined using gel electrophoresis. **Gel electrophoresis** is a separation technology in which charged molecules are forced through a gel by an electrical current; activated electrodes at either end of the gel provide the driving force to move materials according to their charges. The frictional force of the gel acts as a “molecular sieve”, separating the material by size. The substrate used primarily in the separation of large macromolecules, such as DNA or RNA, is agarose. **Agarose** is a natural colloid extracted from seaweed that, when melted and re-solidified, forms a matrix of microscopic pores. The size of these pores depends on the concentration of agarose used to make the gel. Typically, the agarose concentration used in electrophoresis varies from 0.5% to 2.0%. The lower the concentration of agarose in a gel, the larger the pore size and the larger the nucleic acid fragments that can be separated. The length of a given DNA molecule can be determined by comparing the electrophoretic mobility on an agarose gel with that of a DNA marker sample containing fragments of known length.

**Figure S1**

A DNA sample can be digested by restriction enzymes which cut double stranded DNA at specific sequences between 4 and 10 kb long. These restriction enzymes were purified from bacterial strains and are usually named based on an abbreviation of their purification source. For example, EcoRI was purified from *Escherichia coli* and specifically cuts at the sequence 5’-GAATTC-3’. EcoRI is a restriction enzyme that leaves overhanging ends of sequence – it cuts between G and A on both strands. (See Figure S2, on the next page.) Other endonucleases may cut to leave “blunt” ends that don't have an overhang of single stranded nucleotides.

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Like so many of the techniques used in science, there is a natural role that science has exploited. Restriction enzymes can be regarded as a primary immune system for bacteria. They are meant to destroy viral DNA or RNA that enter the bacterial cell.

A simple bacterial genome is about 1000 times larger than a plasmid while the human genome is about $3 \times 10^9$ base pairs or $10^6$ times larger than a plasmid. Restriction enzyme digestion and agarose gel electrophoresis of genomic DNA similarly results in separation of the fragments according to size, however the individual bands are visible as a smear since there are so many bands of so many sizes.

Of the three billion nucleotides in human DNA, more than 99% are identical among individuals. The remaining 1% differs however, adds up to a significant amount of code variations among individuals, making each person's DNA profile as unique as a fingerprint. Due to the 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 approximately one site of variation, or polymorphism. The DNA polymorphisms change the length of the DNA fragments produced by digestion with restriction enzymes. The exact number and size of fragments produced by a specific restriction enzyme digest varies from person to person. The resulting fragments, called Restriction Fragment Length Polymorphisms (RFLPs), can be separated, and their size determined, by electrophoresis.

The difference 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 or tissue sample in a criminal investigation. Scientists have identified more than 3,000 RFLPs in the human genetic code, many of which are highly variable among individuals. It is this large number of variable, yet identifiable, factors that allows scientists to identify individuals by the number and size of their various RFLPs.

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Since human DNA has about $3 \times 10^9$ base pairs, digestion of the DNA with a restriction enzyme often produces tens or hundreds of thousands of DNA fragments. When run on an agarose gel, the sample will appear as a long smear with no resolution visible due to the extremely high number of fragments of all sizes. One way to identify an individual RFLP band is by **Southern Blot analysis**. This method transfers the DNA fragments in the agarose gel to a membrane; the fragments are denatured into single strands, and then hybridized with a smaller probe fragment of interest. The probe fragment adheres to the membrane where it can bind to complementary sequences. The probe is labeled in some way (by radioactive isotope or luminescent molecule that provides a visual tag for the hybridization probe), and then detected as a band of characteristic molecular weight on the membrane. Just as in restriction enzyme analysis, individual band size can be determined through comparison to a known size standard, and by measuring migration distance relative to migration distance of known fragments.

The main characteristic of a probe fragment of interest for DNA fingerprinting would be that it identifies the genomic fragments that vary greatly among individuals. Most of the DNA in a chromosome is not used for the genetic code; it is uncertain what, if any, use this 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. The regions contain short nucleotide sequences that repeat from 20 to 100 times (e.g., GTCAGTCAGTCAGTCA). The length of these short tandem repeat (STR) segments is highly variable in human populations, so the size of restriction fragments carrying the repeats will be variable. These sequences are excellent hybridization probes since they identify specific RFLPs that are known to vary in size among individuals.

Oftentimes only a small sample of DNA is available for analysis. Using the types of probes that are specific for targeted regions, those regions can be amplified for further analysis. The procedure is known as **polymerase chain reaction** (PCR). This concept was developed by Kary Mullis in 1983, and won him the Nobel Prize in 1993 because the technique is so powerful. PCR can be used to generate reliable DNA fingerprints from trace samples. In PCR the variable fragments are not formed by restriction digestion at specific endonuclease sequences; rather, the fragments are synthesized by amplifying the sequence between two specific hybridization sequences (which can be the same as the endonuclease sequences). A DNA fingerprint can be built by specifically amplifying regions that are highly variable among individuals.

A mutation is a change in the nucleotide sequence, which can lead to some disease states. A mutation can be identified more easily using these processes if the mutation is in the restriction sites. This could either shorten or lengthen some of the segments.

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The DNA fingerprinting technique is used with high frequency 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, tissue, or saliva. The DNA fingerprint matched to a suspect can be accurate to within one in 10 billion people, which is almost twice the total population in 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.

Further, DNA fingerprinting has many applications other than crime scene investigation. Since half of a person’s genome comes from each parent, DNA fingerprinting can be used to determine familial relationships. This technique has a much higher certainty than a blood test when used to determine the parents in a paternity suit.

DNA fingerprinting can be used to track hereditary diseases passed down through 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.
pre-lab questions

1. How would a scientist discover which company is responsible for a food contamination outbreak?

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2. What characteristics in a molecule are responsible for separation in a gel?

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modeling DNA and restriction digests (structured inquiry)

Materials needed per lab group:
- 60 red pop beads (phosphate)
- 60 white pop beads (deoxyribose sugar)
- 15 orange pop beads (thymine)
- 15 yellow pop beads (adenine)
- 15 blue pop beads (cytosine)
- 15 green pop beads (guanine)
- 30 plastic connectors (hydrogen bonds)
- 1 JanI restriction enzyme card
- 1 WardII restriction enzyme card
- 1 alkali card
- 1 probe card
- 1 suspect, victim, or crime scene DNA strip (assigned by your instructor)
- 1 self-sealing bag

scenario

Homicide investigators were called to the scene of a crime. They found bloodstains of two different blood types on the crime victim’s body. One of the stains was the blood of the victim. The investigators needed to determine the source of the other blood type found at the crime scene.

Four suspects were apprehended, each of them with the same blood type. You and your fellow lab technicians (classmates) have been chosen to compare the blood of the four suspects to the blood found at the crime scene. You will use several biotechnology procedures to accomplish your task. Be sure to follow the steps of each procedure precisely and pay attention to every detail. A mistake could result in the accusation of an innocent suspect or lead to the release of a violent criminal.

(continued on next page)
1. Assemble the simulated DNA molecule.
   a. Construct a DNA molecule using the DNA strip you were assigned as a blueprint. Use Figure S3, below, only as a guide. **Be sure to assemble the beads in the exact sequence indicated on your strip** to prevent incorrect results. (The color version of this figure shows yellow and orange beads, but depending on the DNA molecule you were assigned, you may be using blue and green beads.)

   **Remember:**
   - Thymine (orange) always pairs with Adenine (yellow).
   - Cytosine (blue) always pairs with Guanine (green).
   - The red and white beads form the sugar-phosphate backbone of the DNA molecule.
   - The clear connectors represent the hydrogen bond formed between bases.

   ![Figure S3](image)

   b. After your DNA molecule is assembled, **double-check the sequence of the model to the sequence on your DNA strip**. Remember, accuracy is absolutely essential.

   c. Place the DNA molecule on your desk or work area so that the 5’ TTT end is on the top left as shown below.

   5’
   TTT ........................................................................................
   AAA ........................................................................................
   3’

   From this point on, be sure to always keep the DNA molecule in this orientation. Never allow the chain to be turned upside down or rotated. The 5’ TTT should always be on the top left of the molecule. If your chain should accidentally be moved, refer to the DNA strip to place it back in the proper orientation. You can also use the overhang from the red pop bead to orient your DNA strands. The red bead is the 3’ end, and the white bead is the 5’ end.

   *(continued on next page)*
procedure: activity 1

2. Simulate restriction enzyme digest of the DNA molecule.

a. Examine the restriction enzyme cards (JanI and WardII). Restriction enzymes are able to recognize and cut DNA at a specific DNA sequence. Note the dotted line on the restriction enzyme cards. These lines indicate the manner in which the DNA will be cut.

b. Begin with the enzyme labeled JanI. Place the card on top of the left side of the DNA molecule, making sure the card is right side up. Move the card along the surface of the DNA molecule, from left to right, until the sequence on the card matches a sequence on the DNA molecule. When a match is found, stop and break the molecule apart where indicated by the dotted line on the card.

If the dotted line happens to lie on top of a bead, shift the molecule slightly until the dotted line lies between the beads.

c. Continue to move the restriction enzyme card along the molecule to the right, making sure to stop and break apart the molecule any time a sequence match is found. After you have completely digested the molecule with the enzyme, it is a good idea to double-check and make sure you have not missed any enzyme cut sites.

d. Repeat the procedure using the WardII enzyme card. Be sure to keep the newly generated DNA fragments in their proper orientation when making the cuts in the molecule.

e. Examine your DNA molecule. The enzymes have broken your original DNA molecule into fragments of various sizes. The fragments are called Restriction Fragment Length Polymorphisms, or RFLPs for short.

3. Simulate gel electrophoresis of the DNA fragments.

You now have several DNA fragments of varying sizes. In order to examine them further, you must first separate the fragments based on their size. To do this, you will now expose the DNA fragments to an “electric current”, using agarose gel electrophoresis.

Use the surface of your work area as an agarose gel. Evenly divide the length of your work area into eleven numbered sections, as seen on the right, with the largest number (22) at the top and the smallest number (2) at the bottom. Small pieces of numbered tape or a long strip of paper may be the easiest way to do this.

The top of your gel lane represents the negative (-) end of the electrophoresis chamber and the bottom end will represent the positive (+) end. Since DNA in an aqueous solution has a sugar-phosphate backbone that is highly negatively charged, it will migrate toward a positive charge. Using a small piece of tape, mark the top of the lane (-) and the bottom of the lane (+).

The gel used in electrophoresis contains millions of microscopic pores. When exposed to electricity, the DNA fragments move through these pores. Smaller DNA fragments are able to wind their way through the pores more easily, moving farther in a set amount of time than DNA fragments of a larger size. Starting with your smallest DNA fragment, place it at the top of your gel lane. Slide the fragment along the gel lane until the number of bases in the top strand of the DNA molecule matches the number labeled on your gel lane.

(continued on next page)
procedure: activity 1

For example, if the smallest DNA fragment has four bases on the top strand, slide the fragment down the gel lane until it reaches the spot marked four and leave it in that position. Continue to separate your DNA fragments in this manner. Work from smallest to largest fragment until they are all properly positioned along the length of your gel lane.

Steps 4 and 5 are optional. These steps model Southern Blot Techniques used in fingerprint analysis. If you choose not to do Steps 4 and 5, go directly to Step 6 of this procedure.

4. Optional Simulated Denaturing and Probing the DNA Fragments
   a. Since restriction enzyme digestion of actual human DNA may yield tens or hundreds of thousands of fragments, a probe is usually applied to RFLPs to select only certain DNA fragments for analysis. The probe is a small piece of DNA with a radioactive marker that will show up when exposed to X-ray film. In order to hybridize (attach) the probe to DNA fragments, they must first be separated into single stranded molecules. This is referred to as denaturing the DNA. Denature your DNA fragments by passing the alkali card over each fragment. The alkali card represents a chemical (such as sodium hydroxide) that will separate the DNA strands. Remove the bottom strand of each DNA fragment (including the hydrogen bonds) and set it aside.

   b. Construct two radioactive probes using six pink beads (radioactive phosphate), six white beads (deoxyribose), two orange beads (thymine), two green beads (guanine), and two blue beads (cytosine). Use Figure S4, as a guide when assembling your probes.

   c. Denature your DNA by separating the antiparallel strands of complementary nucleotides.
   d. Using a probe card, probe the single-stranded DNA fragments in your gel. Search the DNA fragments for a sequence complementary to the sequence found in the probes. When you encounter a sequence complementary (5' A C G 3') to the sequence of the probe (3' T G C 5'), use three hydrogen bonds to attach the probe to the DNA fragment containing the complementary sequence. Be sure to probe all of the DNA fragments in your gel.

   (continued on next page)
In an actual Southern blotting procedure, the DNA is transferred from the gel to a membrane, usually nylon or nitrocellulose, and then the membrane is exposed to the probes. The membrane is more durable and easier to handle than the flimsy gel. This is not necessary in this simulation so this step is omitted.

5. Optional Simulated Autoradiography
   
a. In order to examine the DNA fragments to which a probe has attached, a piece of X-ray film is laid on top of the membrane. The radioactive phosphate in the probe will cause the X-ray film to develop in that region, resulting in a dark band on the film. To simulate this, examine the size of the DNA fragments to which a probe has attached. In the gel lanes in the Analysis section of the lab, sketch dark bands for your suspect, or victim, or for the blood at the crime scene. If, for example, you have a DNA fragment ten bases long with a probe attached, sketch a dark band across the gel lane at the ten marking. If you have a fragment that is seventeen bases long, sketch a band halfway between the eighteen and sixteen on the gel lane.
   
b. After you have completed the autoradiograph, share your results with the rest of the teams in the class. Also, copy the results from the other suspects, the victim, and the blood at the crime scene, using the results obtained by the other groups.

6. Sketch your model.
   
Using this model, match the DNA fingerprint patterns of the suspects to the DNA fingerprint from the crime scene. Who left blood at the crime scene?
procedure: activity 2

agarose gel electrophoresis (guided inquiry)

When performing this lab activity, all data should be recorded in a lab notebook. You will need to construct your own data tables, where appropriate, in order to accurately capture the data from the investigation.

Materials needed per lab group:
- 1 (0.8%) agarose gel, on gel tray
- 200 mL 1X TBE running buffer
- 100 mL DNA stain
- 1 staining tray
- micropipets
- 1 metric ruler
- 1 calculator

Shared materials:
- DNA Samples
- Tube #1: DNA Marker Standard
- Tube #2: Crime Scene DNA Sample
- Tube #3: Suspect 1 DNA Sample
- Tube #4: Suspect 2 DNA Sample

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 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 whose blood was found at the crime scene.

1. Load a gel.

Use one of the following two methods to load 10 μL of each DNA sample into the corresponding gel well listed below.

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.

(continued on next page)
Method 1: Dry

a. Place the tray with the gel on the lab bench.

b. Use a micropipet to 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.

c. Place the tray with the loaded gel in the center of the electrophoresis chamber with the wells situated closest to the negative (black) electrode.

When filling the electrophoresis chamber with buffer, avoid pouring the solution directly onto the gel and be sure to pour it VERY SLOWLY. If the buffer is poured too quickly, it may wash away the DNA samples.

d. Add approximately 200 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. Continue to slowly add buffer until the level is approximately 2-3 mm above the top of the gel.

The current in the chamber is carried by platinum wires that extend along the bottom of the chamber. Be sure to fill your chamber until the buffer level is a couple of millimeters over the top of the gel. The buffer should not be touching the electrode junctions at the top of the chamber.

If the gel and casting tray begin to float, use a pipet to clear the air hole that is located on top of the casting tray.

(continued on next page)
**Method 2: Submarine**

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

b. Add approximately 200 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. Continue to slowly add buffer until the level is approximately 2-3 mm above the top of the gel.

   ![Note]

   The current in the chamber is carried by platinum wires that extend along the bottom of the chamber. Be sure to fill your chamber until the buffer level is a couple of millimeters over the top of the gel. The buffer should not be touching the electrode junctions at the top of the chamber.

   If the gel and casting tray begin to float, use a pipet to clear the air hole that is located on top of the casting tray.

c. Use a micropipet to 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.

   ![Caution]

   Do not overfill the chamber. Wipe off any spills.

2. **Run the gel.**

a. Making sure the cover is dry, place it onto the electrophoresis chamber. Wipe off any spills on the apparatus before proceeding to the next step.

b. Connect the power supply. Making sure that the patch cords and the female jacks on the chamber are completely dry, connect the red patch cord to the red terminal on the power supply. Connect the black patch cord to the black terminal on the power supply.

   ![Caution]

   Check the connections before proceeding to the next step.

c. Once the power supply is connected to the patch cords and is turned “on”, bubbles will form along the platinum electrodes.

d. Observe the migration of the tracking dye down the gel toward the red (positive) electrode. Disconnect the power supply when the tracking dye has reached the end of the gel.

(continued on next page)
procedure: activity 2

3. Visualize the bands.
   a. Lift the gel tray with the gel from the chamber, and gently push the gel off the gel tray into a staining tray.

Optional stopping point:

   If the lab period does not allow time to stain and destain the gel, measure the distance the loading dye has traveled (measure to the center of the diffuse band formed by the loading dye) on the gel, and record this measurement in Table 1.

   Be sure to take this measurement, as the loading dye will diffuse out of the gel within a couple of hours.

   Place the gel in a resealable bag, and add 1-2 mL of 1X TBE buffer.

   Store the gel refrigerated until the next lab period.

b. Wearing protective gloves, pour approximately 100 mL of warm dilute stain into the staining tray so the stain just covers the gel.

c. Let the gel stain for approximately 30-45 minutes.

d. Carefully decant the used stain. Make sure the gel remains flat and does not move up against the corner. Decant the stain directly to a sink drain and flush with water.

   The dilute DNA stain may be saved and reused several times.

   For best results, reheat the stain before using.

e. Add distilled or tap water to the staining tray. To accelerate destaining, gently rock the tray, and change the water frequently. Destain until bands are distinct, with little background color. This will take between 20 and 30 minutes, depending on the amount of agitation. Alternatively, change the water several times, or destain the gel overnight without changing the water.

f. View the gel against a light background, such as white paper, or on a light table. In the Analysis section, roughly sketch the bands you see on the blank gel.

g. Gels can be stored in self-sealing 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.

(continued on next page)
procedure: activity 2

4. Analyze your gel.

a. Measure the distance of the DNA marker standard bands, in millimeters, from the bottom of the sample well to the bottom of each DNA fragment on the marker. 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.

Record the measurements in Table 1 (on the following page).

b. 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 is expressed as a logarithmic scale.
Label the first series of lines 100 bp, 200 bp, 300 bp, etc.
Then label the second series of lines 1,000 bp, 2,000 bp, 3,000 bp, etc.
The third series would be 10,000 bp, 20,000 bp, etc.

c. Measure the distance traveled by each band in the lanes containing the two suspects and the crime scene DNA samples. Record the data in the appropriate sections of Table 1.

d. Calculate the base pair (bp) 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 the values in the table, in units of kilobases (kb).
# Table 1: Activity 2

<table>
<thead>
<tr>
<th>Band #</th>
<th>Distance migrated from well (mm)</th>
<th>Molecular weight (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Top</td>
<td></td>
<td>No measurement</td>
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<tr>
<td>Standard band 1 (first)</td>
<td></td>
<td>9.4</td>
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<tr>
<td>ST 2</td>
<td></td>
<td>6.6</td>
</tr>
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<td>ST 3</td>
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<td>ST 4</td>
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<td>2.3</td>
</tr>
<tr>
<td>ST 5</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Crime scene Band 1 (top)</td>
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<td></td>
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<td>CS 2</td>
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<td>CS 10</td>
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<td>Suspect 1 band 1 (top)</td>
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<td>S1 10</td>
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## Notes
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1. Based on the class results for activity 1, which suspect’s blood was found at the crime scene? Explain your answer.

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2. Compare the banding patterns formed on each lane of the gel in activity 2. 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?

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3. Which of the two suspects in activity 2 do you believe was at the crime scene? Explain your answer.

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_________________________________________________________________________________

4. Each of the following plays an important role in the process of DNA fingerprinting. Explain the function of each.

   **Restriction enzymes** –
_________________________________________________________________________________
_________________________________________________________________________________
_________________________________________________________________________________
_________________________________________________________________________________

   **Gel electrophoresis** –
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(continued on next page)
5. Suppose you had an enzyme that recognized a sequence of six nucleotides. What are the odds that this sequence would appear in a random chain of DNA?

6. Given the answer to the previous question, suppose you have a piece of human DNA that is three billion base pairs in length. How many fragments will be generated by digesting the DNA with the above enzyme?
7. EcoRI recognizes GAATTC, and HindIII recognizes AAGCTT. A student adds EcoRI to a genomic DNA sample. To another quantity of the same DNA, she adds HindIII. In a third tube she adds both enzymes. She runs a gel and the following occurs:

<table>
<thead>
<tr>
<th>HindIII</th>
<th>EcoRI</th>
<th>Double (EcoRI/HindIII)</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

After viewing the gel, the student draws the following map of the genomic DNA

![DNA map]

a. Explain why she placed the nucleotides as she did.
________________________________________________________________________________
________________________________________________________________________________
________________________________________________________________________________
________________________________________________________________________________

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

![DNA map with additional sequence]

b. Explain how she was able to add this sequence in this position based on the results of her gel.
________________________________________________________________________________
________________________________________________________________________________
________________________________________________________________________________
________________________________________________________________________________

(continued on next page)
8. You have used electrophoresis to perform DNA fingerprinting and provide evidence against a suspect involved in a criminal investigation. Research another use for electrophoresis and briefly describe the benefits of using this technology.

_____________________________________________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________

9. The application of DNA fingerprinting technology in forensic science as well as in medicine has raised many legal and ethical concerns. Discuss the following questions: Should data banks be established for DNA information? What are some benefits to society and individuals of DNA data banks? What are some of the risks to society and individuals of establishing a DNA databank?

_____________________________________________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________

10. 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?

_____________________________________________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________

11. 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?

_____________________________________________________________________________________
_____________________________________________________________________________________
_____________________________________________________________________________________

(continued on next page)
12. Below is a list of the components involved in agarose gel electrophoresis. Briefly describe the purpose of each component.

Agarose gel – ________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

TBE buffer – ______________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Electrophoresis chamber – ____________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Power supply – ______________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

DNA samples – ______________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

DNA stain – ______________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________
procedure: activity 3

genetics and information transfer
(design an experiment - open inquiry)

What questions occurred to you as you performed the DNA fingerprinting protocols? Now that you are familiar with DNA fingerprinting and agarose gel electrophoresis, design an experiment to investigate one of your questions. You may consider the following in your investigation:

How do the results of DNA fingerprinting change as different restriction enzymes are used?

How do changes in certain parameters of restriction digests or electrophoresis affect results of DNA fingerprinting?

Research and create a poster about another biotechnology technique. State its value to science and society.

Before starting your experiment, plan your investigation in your lab notebook. Have your teacher check over and initial your experiment design. Once your design is approved, investigate your hypothesis. Be sure to record all observations and data in your laboratory sheet or notebook.

Use the following steps when designing your experiment.

1. Define the question or testable hypothesis.
2. Describe the background information. Include previous experiments.
3. Describe the experiment design with controls, variables, and observations.
4. Describe the possible results and how they would be interpreted.
5. List the materials and methods to be used.
6. Note potential safety issues.

After the plan is approved by your teacher:

7. The step by step procedure should be documented in the lab notebook. This includes recording the calculations of concentrations, etc., as well as the weights and volumes used.
8. The results should be recorded (including drawings, photos, data print-outs).
9. The analysis of results should be recorded.
10. Draw conclusions based on how the results compared to the predictions.
11. Limitations of the conclusions should be discussed, including thoughts about improving the experiment design, statistical significance and uncontrolled variables.
12. Further study direction should be considered.