Whodunit? A Murder Mystery for Teaching Biology

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Abstract: From Sherlock Holmes to modern-days sleuths, the tremendous explosion of interest in the science of forensics is all around us. Turn on the television and you will see popular shows like *CSI: Crime Scene Investigation*, *Cold Case*, and *Crossing Jordan* all bolstering interest in forensics. In this exercise students gain hands-on experience in a breadth of fields, including latent fingerprints analysis, ABO/Rh typing, urine analysis, paper chromatography, as well as analysis of hair and fiber evidence. Students also execute molecular techniques such as setting up a restriction enzyme digest, preparing agarose gels, electrophoresis, and staining to visualize 'DNA profiles.'

Introduction

Although the science of genetics goes back to the time of Gregor Mendel, an understanding of the chemical basis of heredity was not fully achieved until the latter half of the 20th century. Major efforts to completely sequence the entire human genome were initiated in the 1990s, and have now been completed. Noncoding DNA comprises 95-97% of the genome, with only 3-5% coding for functional proteins (Varsha, 2006; Wong *et al*., 2000). These noncoding regions, often referred to as 'junk DNA', can differ widely between individuals.

When DNA is digested with restriction enzymes they cut the DNA into different fragments that can be separated and visualized using gel electrophoresis. The size and number of fragments produced by a restriction enzyme digestion varies between individuals and represents a 'DNA profile.' Just as each individual has a different physical fingerprint, they also produce a unique 'DNA fingerprint' (Jeffreys *et al*., 1985). The power of DNA-typing has fueled science to new heights. Today, 'DNA fingerprinting' is routinely used in the diagnosis of inherited disorders (Pagon, 2004), establishment of paternity (Bernal, 2004), to predict the origin of different ethnic groups (Bamshad *et al*., 2004), and in personal identification (Butler, 2006).

Although the science of molecular genetics is advancing at a rapid pace, and its applications are emerging into every facet of life, few people fully understand the technology behind it. Young people's perceptions about science are derived mostly from television and movies. The popularity of forensic-related television shows illustrates the public's growing interest in science. However, the accuracy portrayed often leads to unrealistic expectations and significant misunderstandings of the science behind the techniques (Bergslien, 2006).

The tremendous explosion and interest in forensic science represents a pedagogical opportunity for science educators. The National Science Teachers Association recommends incorporation of forensics into courses as a tool for getting students intrigued (Colgan, 2002). This laboratory exercise incorporates a variety of techniques and takes a multi-faceted approach in solving a mock crime scenario while giving students hands-on experiences in both current molecular techniques and data-analysis. This exercise was designed to introduce students to a multitude of molecular, biochemical, and microscopic techniques using a mock crime-scene, involving faculty participation in a murder scenario. Students execute molecular techniques such as setting up a restriction enzyme digest, preparing an agarose gel, gel loading, gel electrophoresis, and gel staining to visualize the 'DNA profile.' Students also analyze hair samples, perform fingerprint analysis, ABO blood-typing, and test urine for the presence of toxins.

This approach utilizes both active-learning strategies and inquiry-based laboratories as recommended by the National Research Council (2003). These strategies teach not only the process of science, but also basic laboratory methodologies (Handelsman *et al*., 2004). As a result, students develop critical-thinking and problem-solving skills while at the same time they gain knowledge through cooperative-learning. Evidence suggests that students who are engaged in this type of active learning exhibit improved knowledge retention (Handelsman *et al*., 2004; National Science Foundation, 1996). This is important because this exercise was designed as a precursor to a five-week cloning project that requires a high level of laboratory competence, and good critical-thinking and problem-solving skills.

Technical mistakes in the lab are often made due to students not having a full understanding of the theories behind the methodology. Accuracy in a laboratory setting is of the utmost importance for developing competent scientists. In the past, we have observed that either students lacked confidence about their laboratory abilities, were negligent about procedures, or were too hasty. Our goal was to introduce students to the theories and techniques behind the science, and to enhance their technical competency.

This exercise was used at the beginning of the semester in a junior-level *Molecular Biology* course and, with modification, has also been used in two different non-majors courses: *Introduction to Life Sciences* and *Modern Genetics*. This exercise might also be appropriate for the laboratory portion of undergraduate courses in *Cell Biology*, *Biochemistry*, *Biotechnology*, *Recombinant DNA Techniques*, or *Genetics*. It could even be tailored to a *Criminal Justice* course. Due to recent advances in DNA technology the laboratory procedures are routine, safe, and relatively inexpensive. The laboratory exercise can be completed in one 3-hour lab session with minimal molecular-biology equipment.

Student Outline

Background

The power of DNA-typing has fueled forensic science to new heights. Today, 'DNA fingerprinting' is routinely used in the diagnosis of inherited disorders, establishment of paternity, to predict the origin of different ethnic groups, and in personal identification. But perhaps, the most publicized use of DNA-typing is in criminal cases. Comparing the 'DNA profile' of a suspect with the evidence left at a crime scene may help determine guilt or innocence. The first successful criminal investigation using DNA- typing occurred in 1986 when Colin Pitchfork was convicted in England for the rape and homicide of two girls. Since then, huge national DNA databases have been created by law enforcement agencies. 'DNA fingerprinting' often involves *polymerase chain reaction* (PCR) amplification (to analyze minute quantities of DNA) or *restriction fragment length polymorphism* (RFLP) analysis (if large amounts of DNA are recovered). In recent years, news stories have reported that miniscule amounts of DNA (even if the DNA is many years old) have been used to identify individuals in a multitude of ways such as to exonerate innocent people from incrimination, identify unknown corpses, or to settle paternity cases.

Each person has similarities and difference in their DNA sequences. In humans, there are thousands of DNA segments that can be selected and used for fingerprinting analysis. Depending on demographic factors such as ethnicity or geographic isolation, some segments will show more variation than others. The degree of variation will affect the statistical odds of more than one individual having the same sequence. If 90% of a given population has the same frequency in its DNA fingerprinting pattern for a certain DNA segment, then very little information will be attained. But if the frequency of a DNA pattern in a population for a particular segment is extremely low, then this segment can serve as a powerful tool to discriminate between individuals in that population.

The objective of today's lab is to utilize both physical evidence and DNA evidence to solve a crime. You can analyze hairs left at the crime scene, determine ABO blood-type, test for the presence of toxins in the victim's urine, separate ink using paper chromatography, analyze fiber evidence, and examine fingerprints to identify potential suspects. After examining all of the evidence, you should be able to identify the 'murderer.'

DNA Fingerprinting

Today, we will use 'DNA fingerprinting' to solve a crime. We will use restriction enzymes to cleave DNA samples obtained from our suspects and then compare banding patterns using agarose gel electrophoresis. **Restriction enzymes** act like molecular scissors, making cuts at specific sequences of base pairs that it recognizes. A restriction enzyme sits on a DNA molecule and slides along the helix until it recognizes specific sequences of base pairs that signal the enzyme to stop sliding. The enzyme then cuts both strands of the DNA molecule at that site – called a **restriction site**. If a specific restriction site occurs in more than one location on a DNA molecule, a restriction enzyme will make a cut at each of those sites, resulting in multiple fragments. The length of each fragment will depend upon the location of restriction sites on the DNA molecule. The fragments can then be separated according to size by agarose gel electrophoresis and visualized by staining.

I. Restriction Digestion of DNA Samples

1. Obtain the six DNA samples (Crime Scene; Suspect 1 = Dr. ________; Suspect 2 = Dr.________;
   Suspect 3 = Dr. ___________; Suspect 4 = Dr. _____________; Suspect 5 = Dr. _____________).
2. Label seven microcentrifuge tubes and add 10 µl of DNA sample into the corresponding tube.
3. Pipet 10 µl of the enzyme mix "ENZ" to each tube and mix gently mix the contents.
4. Briefly centrifuge the tubes to force the liquid into the bottom of the tube.
5. Incubate the samples at 37°C for 50-60 minutes. (Start the crime scene analysis).
6. Add 5 µl of loading dye into each tube and gently mix the tube with your finger.

II. Agarose Gel Electrophoresis

1. Prepare a 1% agarose gel (0.4 grams of agarose and 40 ml of 1x TAE electrophoresis buffer).
2. Carefully boil the agarose until melted. Swirl the flask every 30 seconds to suspend any undissolved agarose. Continue until all the agarose is dissolved. Cool to 55°C before pouring.
3. Seal the ends of the gel tray securely with strips of masking tape. Press the tape firmly to the edges of the gel tray to form a fluid-tight seal.
4. Pour cooled agarose into tray and place the comb into the appropriate slot of the gel tray.
5. Allow the gel to solidify at room temperature for ~30 minutes.
6. Remove the comb from the solidified gel and remove the tape from the edges of the gel tray.
7. Place the agarose gel in the electrophoresis apparatus and fill the chamber with 0.25x TAE.
8. Load the indicated volume of each sample into your wells of the gel in the following order:

Lane 1	DNA Size Marker, 10 µl
Lane 2	Crime Scene, 20 µl
Lane 3	Suspect 1, 20 µl
Lane 4	Suspect 2, 20 µl
Lane 5	Suspect 3, 20 µl
Lane 6	Suspect 4, 20 µl
Lane 7	Suspect 5, 20 µl

9. Carefully place the lid on the electrophoresis chamber. Plug the electrodes into the power supply. Run at 200V for 20 minutes and continue with the crime scene analysis.

III. Visualization of DNA fragments

1. When the electrophoresis run is complete, turn off the power, unplug the wires from the power supply and remove the top of the chamber. Slide the gel into the staining tray.
2. Add enough 100x Fast Blast stain to cover your gel. Stain the gel for 2 minutes and gently agitate.
3. Rinse with warm (40-55°C) tap water for approximately 10 seconds.
4. Destain by washing twice in warm tap water for 5 minutes each with gently shaking.
5. Record results in the results section below.

Crime Scene Analysis

Forensics is a science, and science involves observations and experiments to make predictions. Below are examples of various types of data that forensic scientists can collect and test.

I. Hair Analysis: Microscopic analysis of hairs found at the crime scene.

Many types of physical evidence are encountered during the course of a criminal investigation. One of the most common is hair evidence. The identification and comparison of human hairs can help demonstrate physical contact between suspect and victim, giving the investigator valuable information about potential leads. Hair alone does not provide a positive identification, but can serve to narrow down the suspect pool. Human hairs can be classified by racial origin, length, color, or physical structure. Hair grows out the *hair follicle*; the root is embedded in the follicle and shaft extends above the skin's surface. If the hair has been pulled out, it should contain the follicle and shaft. The shaft has three forensically relevant layers: the cuticle, cortex, and the medulla (Figure 1). The cuticle is the outer covering that is formed when cells die and become keratinized. Within the cuticle lies the cortex which contains colored pigments. The distribution, color, and shape of pigments granules can be helpful in identifying a person (Figure 2). The features of the cortex can be examined microscopically to examine the internal features. The medulla varies in thickness, opacity, and continuity (Figure 3). Thus, the shape and texture of hair may also help to link a suspect to the scene of the crime. Other characteristics to keep in mind when examining hair evidence are length, color, condition of the tip, condition of the root, and the width of the medulla.

Figure 1: Diagram of hair.

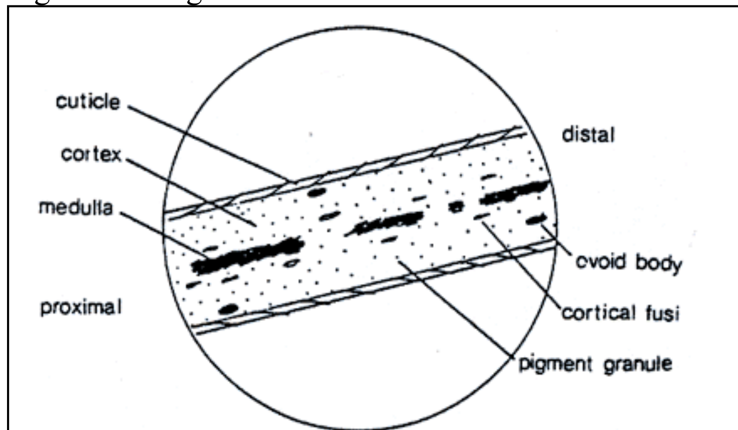
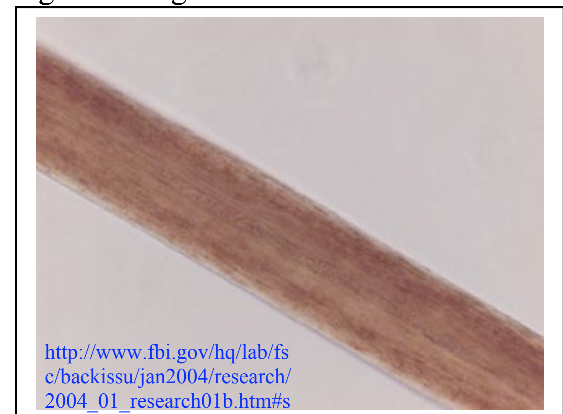
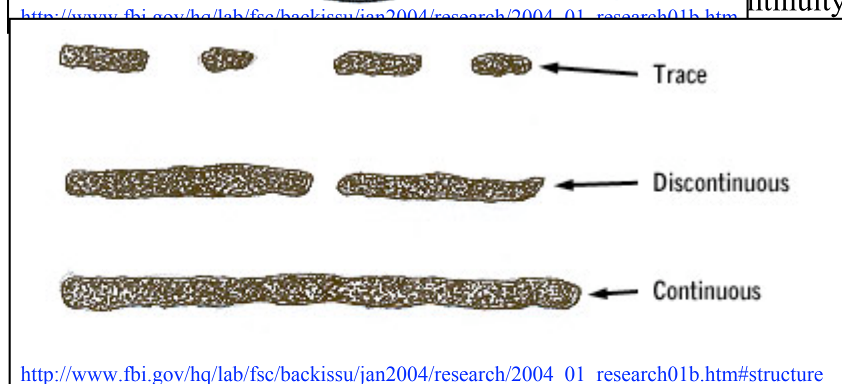


Figure 2: Pigment distribution in hair.



ntinuity.



Hair Preparation: Make a scale cast of the hair to examine scale impressions, shape and texture.

1. Carefully study the hair and record characteristics from each hair sample.
2. Prepare scale casts of each hair sample:
    a. Place a drop of latex near one end of a clean slide.
    b. Tilt a second slide over the first at a 30° angle so that the ends farthest from the drop of latex are touching.

    c. Slowly pull the tilted slide over the first until it touches the drop of latex.

    d. Allow the latex to run along the edge of the tilted slide.
    e. With a smooth motion, push the tilted slide back along the first slide to spread the latex into a thin film.

    f. Immediately place several strands of hair on the film of latex.
    g. Let the slide sit undisturbed for 10-15 minutes to allow the latex to harden.
    h. Once the latex is hard, use forceps to remove excess hair from the slide.
    i. Label the slide and examine it under a compound microscope.

II. Fiber Analysis: Microscopic analysis of fibers found at the crime scene.

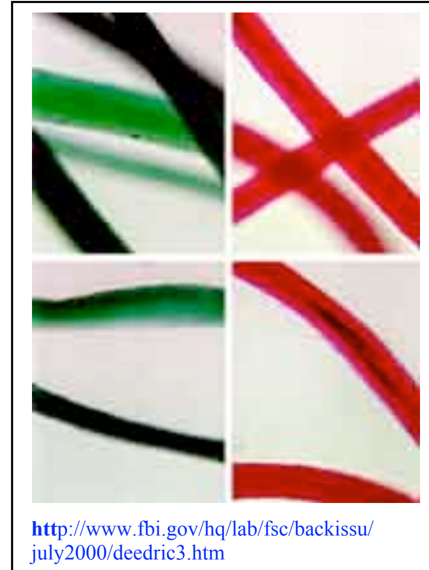
Fibers are small units of material and may occur naturally (from plants and animal sources) or synthetically (man-made). Examples of natural fibers include cotton (Figure 4), wool (Figure 5), linen, cashmere, and mohair; whereas examples of synthetic fibers include nylon (Figure 6), acrylics, polyester, and rayon. Fiber evidence may be transferred from clothing, carpet, furniture, bedding, car-seats, wigs, drapery, etc. These fibers can be transferred during commission of a crime from

suspect to victim or vice versa. Fibers are gathered from the victim and the crime scene with a vacuum, tape or tweezers. The value placed on fiber evidence depends on factors such as: the type, color, length, and number of fibers found, the location of the fibers in relations to the victim and the crime scene, and the length of time between the contact and collection of evidence.

Figure 4. Cotton fibers.

http://www.fbi.gov/hq/lab/fsc/backissu/july2000/deedric3.htm

Figure 5. Wool fibers.



http://www.fbi.gov/hq/lab/fsc/backissu/july2000/deedric3.htm

Figure 6. Nylon fibers.

http://www.mshp.dps.mo.gov/MSHPWeb/PatrolDivisions/CLD/TraceEvidence/Fibers.html

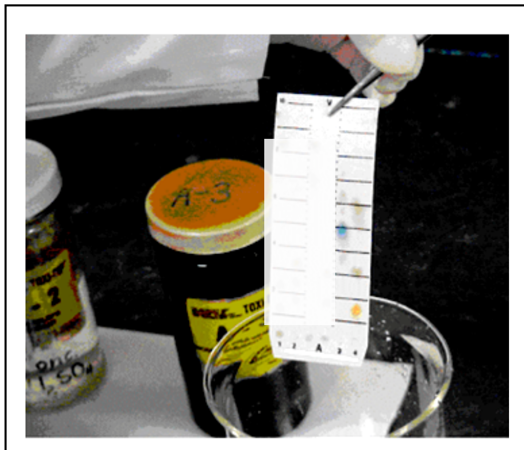
Fiber Preparation: Identify the source of fibers left at the crime scene.
1. Examine prepared slides of known fiber types under the microscope.
2. Record characteristics of each fiber type.
3. Prepare a wet mount of the fiber found at the crime scene:
    a. Place the fiber on a microscope slide.
    b. Add one drop of water.
    c. Cover the fiber and water with a cover slip.
4. Examine the fiber under a microscope.
5. Record any characteristics that you notice.

6. Determine if the unknown fibers are from a naturally-occurring source, or if they are man-made.
7. Determine who the fibers came from.

III. Toxicology Analysis: Presumptive test for toxins.

Toxicology is the study of poisons. A poison is any chemical that produces a harmful effect on a living organism. Blood, urine, and tissue samples can all be tested for the presence of poisons. Toxicologists have the responsibility of detecting and identifying the type of drug or poison and the amount present. Because there are tens of thousands of harmful substances, toxicologists routinely use 'presumptive testing' to narrow down the possibilities so that more specific tests can be conducted (Figure 7). A negative result requires no further testing. A positive result, as indicated by a pink color change (Figure 8) requires further confirmatory tests.

Figure 7: A presumptive test can be used to tentatively identify substances of interest.



http://www.mshp.dps.mo.gov/MSHPWeb/PatrolDivisions/CLD/Toxicology/Toxicology.htm

Figure 8: A positive presumptive test indicates the presence of a poison

http://www.mshp.dps.mo.gov/MSHPWeb/PatrolDivisions/CLD/Toxicology/Toxicology.htm

Presumptive test: Examine the likelihood that the victim was poisoned.

1. Conduct a presumptive test on the bottle of 'home-made brew' and on the recovered urine sample from the victim.
2. Dip the reaction paper into a sample of 'home-made brew' and observe the test strip for any change in color.
3. A pink color change indicates the presence of a poison.
4. Repeat the toxicological test on the recovered urine sample from the victim.
5. Record your results.

IV. Blood Analysis: Detection of blood.

Serology is the study and analysis of serums (blood, semen, saliva, etc.). Typically, the most studied serum is blood. Approximately 10 pints of blood circulates through our bodies. Therefore, it is not surprising that blood evidence is most often found at the scene of a crime, especially in crimes involving violence. Despite how thoroughly a crime is cleaned up, traces of blood can often be detected and tested further. Traces of blood can often be found between floorboards and inside drains. Even blood that has been washed away can be detected by chemical means. Tiny particles of blood remain attached to surfaces and can be detected using a light-producing chemical reaction, even after many years.

The oxygen-carrying protein in blood is known as hemoglobin. The central core of hemoglobin contains iron. In the presence of luminol and a solution of hydrogen peroxide, iron acts as a catalyst that results in electrons being excited. As the electrons fall back to their ground state, a photon of visible light is produced and is seen as a 'glowing' blue light. This 'glowing' phenomenon is known as chemiluminescence.

Any chemiluminescence produced only indicates to investigators that blood might be present. Other substances, such as bleach can also produce 'blue glowing' light. Thus, further tests are needed to verify the presence of blood. The presence of luminol may interfere with other tests on a piece of evidence, and thus needs to be used carefully.

Detection of blood: Examine the murder weapons for the presence of blood.

1. Spray the ends of the femur and hammer with the luminescent blood detection solution.
2. Darken the room as much as possible.
3. If blood is present, a 'blue glow' will appear.
4. Record your results.

V. Blood Analysis: Presumptive Blood Test.

Any darkish substance found at a crime scene must first be determined to be blood. Presumptive blood tests are used for screening and will differentiate between blood and other substances. The Kastle-Meyer Test uses phenolphthalein and hydrogen peroxide which turns pink in the presence of blood. However, this test reacts with both human and animal blood, so further testing to distinguish between the two is required.

Presumptive test for blood: Determine if blood is present on the potential murder weapons.

1. Using a cotton swab, place 1 drop of dH_2O to moisten it.
2. Lightly rub the swab over the surface where blood is suspected.
3. Apply 1 drop of alcohol onto the swab.
4. Apply 1 drop of Phenolphthalein solution onto the swab.
5. Pause for a few seconds and examine your swab.
6. Apply 1 drop of hydrogen peroxide onto the swab.
7. Record your results.

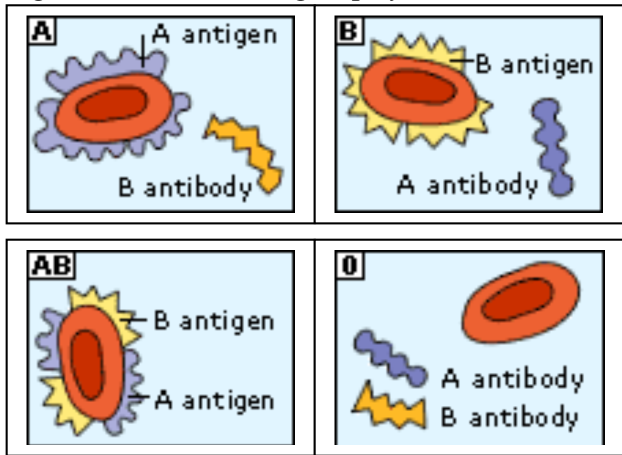
VI. Blood Analysis: Identification of ABO blood type.

Blood-typing is perhaps one of the most important pieces of evidence in the world of criminal justice. Forensically, differences in human blood are due to either the presence or absence of proteins called antigens and antibodies. The antigens are located on the surface of red blood cells and antibodies are in the blood plasma (liquid portion). Individuals have different combinations of antigens and antibodies which are both genetically determined. The ABO system, discovered by Dr. Karl Landsteiner in 1930, is used to classify human blood groups. Blood Group A contains A antigens on the surface of red blood cells and B antibodies in the plasma. Blood Group B contains B antigens on the surface of red blood cells and A antibodies in the plasma. Blood Group AB contains both A and B antigens on the surface of red blood cells and neither A or B antibodies in the plasma. Blood Group O contains neither A or B antigens on the surface of red blood cells and both A and B antibodies in the plasma (Figure 9).

The ABO system is especially important during blood transfusions. Antibodies attach to specific antigens. Anti-A antibodies will stick to A antigens and cause the red blood cells to agglutinate or clump together. Anti-B antibodies will have the same effect on B antigens. This clumping is dangerous because it blocks capillaries, resulting in tissue damage and eventual death. Type-O blood is considered the 'Universal Donor' because it lacks antigens on the surface of its red blood cells. Individuals with Type-O blood cannot receive blood from anyone with another blood type, but they can donate blood to any blood type. Type-AB is considered the 'Universal Acceptor' since it has no antibodies. Individuals with Type-AB can receive blood from anyone with another blood type.

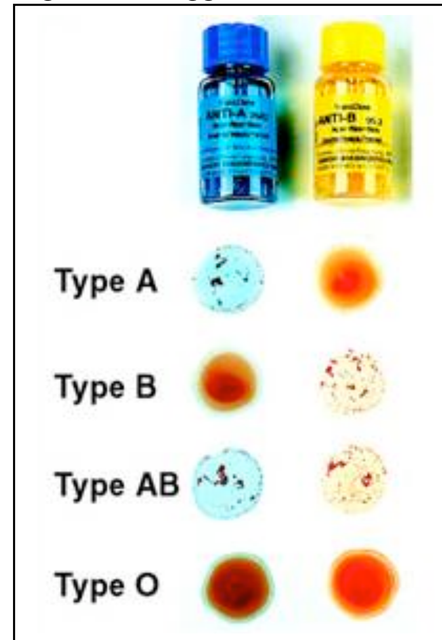
Blood left at the scene of a crime can be typed by observing the formation of clumps when the same type of antibody meets the same type of antigen. Blood typing is performed using antiserum (blood that contains specific antibodies). Anti-A serum contains anti-A antibodies and anti-B serum contains anti-B antibodies. To perform a blood typing test, anti-A and anti-B sera are separately mixed with a drop of blood and observed for agglutination or clumping (Figure 10).

Figure 9: ABO blood group system.



http://nobelprize.org/medicine/educational/landsteiner/readmore.htm

Figure 10: Agglutination reactions.



http://science.tjc.edu/Course/BIOLOGY/jbet/Lecture%203%20%20Blood%20(cont)_files/slide0013_image016.jpg

Blood-typing: Determine blood-type from crime scene.

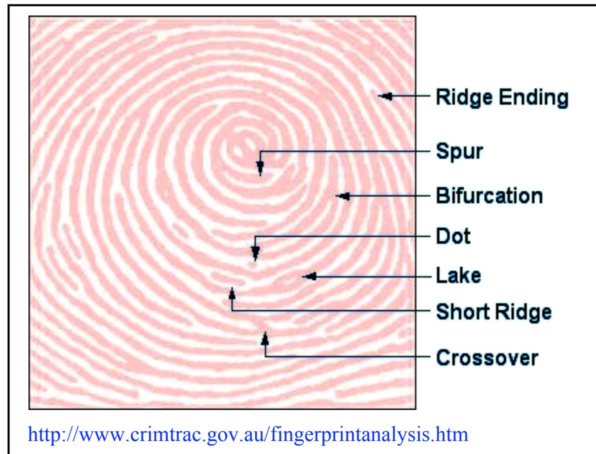
1. Place 5 drops of blood recovered from the crime scene into wells "A" and "B" on the blood-typing tray.
2. Add three drops of anti-A serum into well "A" and three drops of anti-B serum into well "B."
3. Mix the blood with the serum in each well with a separate stirring stick for 10 seconds.
4. Carefully examine each well to determine the blood-type of the recovered blood.
5. Repeat the procedure with blood recovered from the possible murder weapons.
6. Compare your results with the suspect's identification cards and record your results.

VII. Fingerprint Analysis: Microscopic analysis of latent fingerprints.

Fingerprints are one of the most commonly used identifiers. The contact surfaces of your hands and feet have a series of raised spiral and concentric patterns called "friction ridges." "Friction ridges" provide a gripping surface and are the only part of the skin that lack hairs. They are formed at birth, remain unchanged over a lifetime, and display a number of unique characteristics. Figure 11 details several principle ridge characteristics: Ridge Endings (abrupt ridge ending), Bifurcation (single ridge that divides into two ridges), Lake or Enclosure (single ridge that bifurcates then reunites), Short Ridge (ridge that commences, travels a short distance, then ends), Dot (independent ridge with equal length and width), Spur (bifurcation with a short ridge that branches off longer ridge), and Crossover (ridge that runs between parallel ridges).

Additionally, fingerprint ridges may be grouped into three general patterns. The Arch Pattern has ridge lines that start from one side of the fingertip, rises at the center, and exits on the other side of the fingertip (Figure 12). The Loop Pattern consists of ridge lines that start and end on the same side of the fingertip (Figure 13). The Whorl Patterns consists of ridge lines that are circles and do not begin or end on either side of the fingertip (Figure 14).

Figure 11: Examples of ridge characteristics.

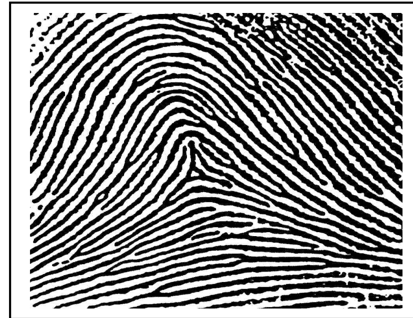


http://www.crimtrac.gov.au/fingerprintanalysis.htm

Figure 12: Arch Patterns.

Plain arch shows ridges lying above one another.

Tented arch consisting of one upthrusting ridge.



http://www.ridgesandfurrows.homestead.com/fingerprint_patterns.html

Figure 13: Loop Patterns.

Radial loop starts at the thumb side.

Ulnar loop starts at the little finger side.

http://www.pbs.org/wgbh/aso/resources/campcurr/patterns.htm

Figure 14: Whorl Patterns.

Plain whorl with recurving ridges.

Twinned whorl with two loop formations.

Central pocket whorl showing obstruction of flow.

Lateral pocket whorl showing two loop formations.

Accidental whorls with two or more patterns.

http://www.ridgesandfurrows.homestead.com/fingerprint_patterns.html

Lifting latent fingerprints:

1. Dip the brush into the container of graphite powder.
2. Shake off excess powder by tapping the handle on the container.
3. Lightly brush the powder over the object of interest.
4. Use a magnifying glass to locate fingerprints on the object.

5. When a fingerprint is found, place a piece of lifting-tape over the print. Never rub the tape, this will
   ruin the print.
6. Carefully lift the tape and place it on the back of a fingerprint lifting card.
7. Examine the print under a microscope.
8. Characteristics to keep in mind: the type of ridge patterns (arches, loops, and whorls) and ridgeline characteristics.

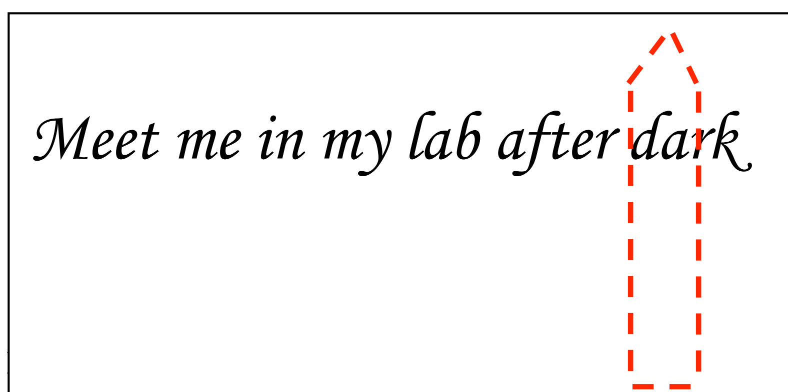
VIII. Ink Chromatography: Paper chromatography to separate inks.

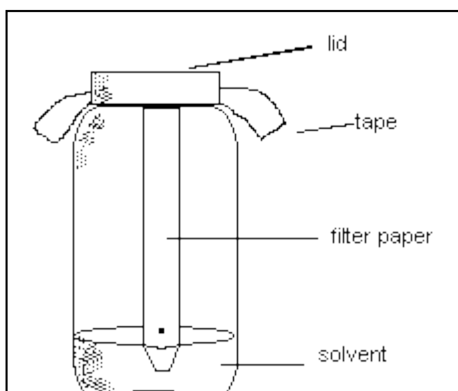
Chromatography is a technique used for the separation and identification of a mixture of components based on their solubility. When an ink sample is placed on chromatography paper and placed into a solvent solution, the solvent will rise up the paper via capillary action carrying dissolved molecules with it. As a result, chromatography can be used to provide a 'chemical fingerprint' of colored pigments, such as dyes, inks, and ingredients used in flavorings. The inks used in pens are composed of a mixture of different colors, with each brand of pen being composed of a slightly different mixture and quantity of dyes. After chromatography, the Relative Mobility Factor (R_f) is characteristic of a particular substance and can be determined for each spot on the chromatogram. The R_f value is a ratio of the distance a substance (i.e. ink) travels to the distance the solvent travels, and is calculated as follows:

$$R_f = \frac{\text{Distance ink colorant traveled}}{\text{Distance solvent traveled}}$$

Using the note found at the crime scene and pens collected from the suspect's offices, perform paper chromatography to identify who wrote the note. Choose a letter from the note found on the victim at the crime scene and cut out the letter as showed in the example below (Figure 15).

Figure 15. Example of how to cut out letters from the note.





Ink Chromatography: Determine which pen was used to write the note.

1. Spot ink samples from pens collected from the suspects onto a strip of chromatography paper.
2. Wrap each strip of paper around skewers to form a support.
3. Place the strips of paper into the vial containing solvent (ethanol), so that the tip of you paper just touches the solvent (Figure 16).
4. Allow the ink to separate, then examine the banding patterns and determine which suspects pen was used to write the note.

Case Notes:

As you are examining the physical evidence, record your findings and keep in mind the following:

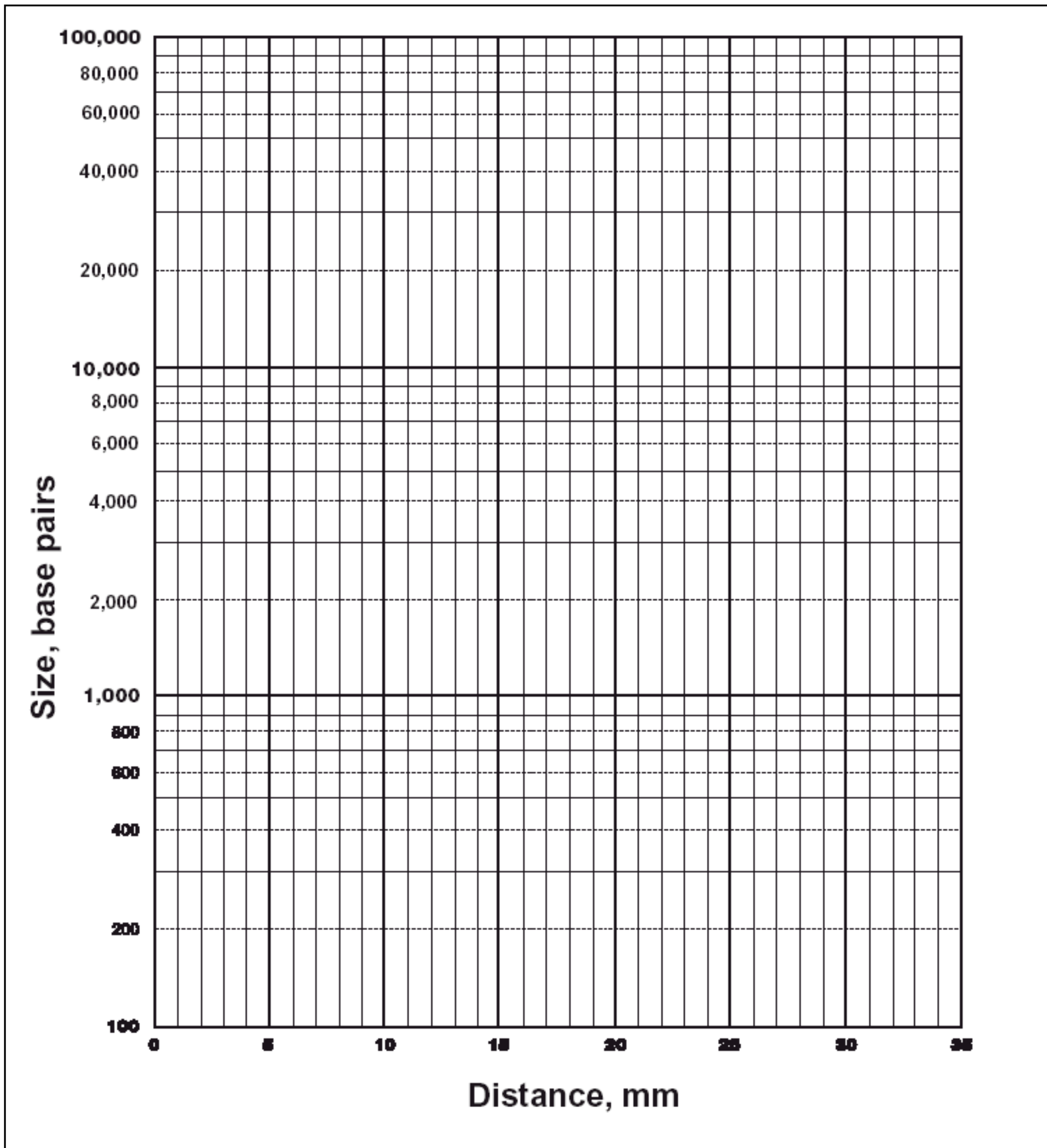
Whose hair was found at the crime scene?
Was a toxin present in the victim's urine sample?
Whose blood was found at the crime scene?
What was the murder weapon?
Whose fingerprints were found on the murder weapon?
Were the fibers at the crime scene natural or synthetic?
Who left fibers at the crime scene?
Whose pen was used to write the note found on the victim?
Based on the current evidence, who do you suspect murdered our victim?

Additional Detective Notes:

Fill in the data from your gel electrophoresis results. Measure the distance (in mm) that each the DNA fragments 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 below. Make a standard curve using the distance your molecular weight markers traveled vs. their size. Use this standard curve to predict the sizes from digested DNA samples.

	Lambda/*Hind*III size marker		Crime Scene		Suspect 1		Suspect 2		Suspect 3		Suspect 4		Suspect 5	
Band	Distance (mm)	Actual size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)	Distance (mm)	Approx. size (bp)
1		23,130												
2		9,416												
3		6,557												
4		4,361												
5		2,322												
6		2,027												

Use the data in the table to construct a standard curve showing the relationship between the actual size of each Lambda/*Hind* III marker and how far it moved down the gel. Connect the dots and use this curve to estimate the sizes of the restriction fragments from the crime scene and suspects (fill in the approximate size of your fragments in the table above).



Compare the fragment sizes of the suspect’s DNA to that found at the crime scene. Who do you think murdered our victim?

Why is it important to look at both the physical evidence as well as DNA evidence when trying to solve a crime?

Materials

For a lab section of 16 students (4 students per group; 4 groups per lab section):

Equipment

P20 micopipettors (2-20 μl):	4
37°C water bath or heating block:	1
Gel electrophoresis apparatus:	4 individual boxes with 8 well combs
Electrophoresis power supply:	2
Dissecting microscope:	4
Magnifying glasses:	4
Compound microscope:	4
Microcentrifuge:	1
Microwave:	1
Light box:	1
Computer and projector:	1

Supplies

Plasmid DNA:	4 sets
P20 Pipette tips (2-20 μl):	4 boxes
Fingerprinting tape:	1 roll
Lifting powder:	1
Fingerprint lifting brushes:	4
pH paper:	1 package
Evidence labs:	1 package
Microscope slides:	1 package
Microscope cover slips:	1 package
Crime Scene tape:	1 roll
Fiber slides:	1 set
Evidence bags:	various sizes
Evidence labels:	1 package

Artificial urine:	1 bottle
Simulated blood-typing kit :	1
Simulated 'home-made' brew':	1 microcentrifuge tube
Femur:	1
Hammer:	1
Spray bottle:	1
Luminol:	1
Phenolphthalein detection:	1
250-ml Erlenmeyer flask:	4
Gloves:	1 box of each size
1-liter Chromatography jars:	4
Chromatography paper:	4 sheets
Various water-soluble pens:	1 set
Identification cards:	1 package
Fingerprints from suspects & victim:	1 set
Hair samples from suspects & victim:	1 set
Containers for holding ice:	4

Safety precautions and disposal: Overall, the reagents used in this exercise are safe. Students need to be reminded that ethanol is flammable. Instructors should consult the directions provided by the manufacturers regarding safety and disposal of reagents.

Notes for the Instructor

The beauty of this lab is that is can be repeated numerous semesters with a different crime scenario staged each semester. No single source of evidence has to be the deciding factor. DNA evidence could be used to implicate suspects, but could also be used to exonerate a prime suspect. The first time this lab was implemented, we told the students that the 'crime scene' DNA was isolated from the victim's fingernails. But it could just as easily been from blood evidence left at the scene, or a cigarette butt, piece of chewed gum, etc. This gives the instructor the opportunity to tailor the exercise to their students or college campus. The crime does not even have to involve a murder, but rather scenarios such as dog-napping, disappearance of a mascot, vandalism, environmental dumping, etc. The instructor can made this exercise as difficult or as easy is they want. Furthermore, the entire scenario or details within (such as the perpetrator) can be changed year to year. The sky's the limit…let your creativity shine!

Alternatively, the instructor can give the student more freedom in formulating their overall strategy as well as details like: positive and negative controls, the order in which gels are loaded, whether to use a compound vs. dissecting microscope vs. a magnifying glass. The instructor also has the option of omitting the standard curve in the gel electrophoresis portion.

Time required:

This lab has been completed in one three-hour laboratory session or during two consecutive weeks (two-hour laboratory sessions each). If time is limited, groups of students could be assigned

to specific stations with the data being shared amongst groups. Alternatively, multiple stations could be set-up for blood-typing, hair analyses, fiber analyses, etc.

Crime scene setup:

Since this laboratory exercise involved faculty participation, we gathered hair and fiber samples from each participant and placed them into plastic bags with evidence labels (Wards Cat. 15W5072; $12.75 US per 100). We also took mug-shots and fingerprints (using an inkpad) and placed them onto an identification card. Furthermore, on their identification cards we assigned a blood-type that would be used for later blood-typing comparisons. The 'victim' volunteered to be covered in fake blood and we set-up a mock crime scene and took photos. Photos from the crime scene and mug-shots of each suspect were put into a PowerPoint and presented to the students. Throughout the lab session students could refer to the PowerPoint slides for fiber identification and for additional clues.

An alternative version of this laboratory exercise has involved students portraying both the victim and suspects. Students were eager to participate (and with the help of work-study students) we staged a murder scene with the victim ahead of time. Each suspect had their mug-shot taken along with fingerprints well before the laboratory session.

To enhance the realism of the experience, the crime scene was restaged and cordoned off with crime-scene tape. Crime scene tape was purchased from Wards (Cat. 15W5060) for $14.50 US. An outline of the body was also drawn on the floor.

DNA fingerprinting:

The Forensic DNA Fingerprinting Kit (Cat. 166-0007) was purchased from Bio-Rad Laboratories for $95.00 US. This kit includes all the necessary DNA samples, restriction enzymes, and instructions for gel preparation and gel electrophoresis. Refills are available separately and the company gives an educational discount.

Hair analysis:

For this laboratory exercise, suspects, and victims donated hair samples. However, hair samples are available for purchase from Carolina Biological (Cat. WF-69-9871) for $49.95 US.

Fiber analysis:

Fiber types slide sets (used for comparison) were purchased from Wards for $24.95 US (Cat. 95W0065). Fibers were collected with transparent tape from our victim and from suspect's clothing. These fibers were placed onto labeled microscope slides. An alternative is to collect individual fiber samples and place them into plastic bags with evidence labels. Students can then make their own mounts and examine the fiber evidence.

Toxicology analysis:

Simulated urine was purchased from Flinn Scientific (Cat. FB1444) at $17.00 US per liter. Urine was placed into a 1.5 ml microcentrifuge tube and HCl was added to lower the pH enough to be detected with pH paper. The simulated urine was placed into an evidence bag. Simulated 'home-made brew' was created by mixing distilled water with food coloring to create a brown liquid. The pH of this can be adjusted as desired. The home-made brew was placed into a 1.5 ml

microcentrifuge and placed into an evidence bag. Alternatively, a simulated drug testing kit using the Semiquantitative Enzyme Immunoassay technique can be purchased from Wards (Cat. 36W5481) for $41.50 US.

Blood analysis:

Chemiluminescence blood detection:

Simulated blood was wiped on the potential murder weapons (hammer and femur) and wiped on the floor near the crime scene. A chemiluminescent solution was purchased from Flinn Scientific (Cat. AP6292) for $16.95 US. This kit also includes simulated blood.

Presumptive blood test:

The phenolphthalein presumptive test was purchased from Wards (Cat. 36W6134) for $17.95 US. This kit reacts with the simulated blood used in the chemiluminescent kit.

Blood-typing:

ABO blood-typing was done with simulated blood using a kit purchased from Carolina Biological (Cat. WF-70-0101) for $27.50 US. Refills are available for $12.00 US.

Fingerprint analysis:

Fingerprint brushes were purchased from Ward's (Cat. 15W5061) for $7.25 US each. Fingerprint powder was purchased from Wards (Cat. 37W2345) for $6.99 US. Ward's also sells a magnetic fingerprint kit that is more effective, but the kit is more expensive. Dustless fingerprinting kits are also available. Fingerprint lifting tape was purchased from Wards (Cat. 15W5063) for $6.25 US a roll. Identification cards (package of 100) for fingerprinting and blood-typing were purchased from Wards (Cat. 15W5062) for $21.75 US.

Ink Chromatography:

Various pens were used for ink chromatography. Beakers and saran wrap or test tubes with corks can be used as alternatives to chromatography chambers. Wards sells a kit (Cat. 36W6237).

Additional lab ideas:

Many companies market a variety of forensic science kits that could be easily incorporated into this exercise. Other sources of evidence that can be analyzed are:

- Handwriting
- Footprints
- Blood-splatter
- Human vs. animal bones
- Environmental forensics
- Tire-treads
- Forensic entomology
- Ballistics evidence
- Blood alcohol determination

Student feedback:

Of the nine exercises completed throughout the semester, no other lab exercise was rated higher by students for learning new laboratory skills, with three-fourths reporting it as their favorite.

This exercise succeeded in fostering curiosity and excitement about molecular and biochemical techniques that might be remembered for years.

Acknowledgments

Thanks to Bio-Rad Laboratories, Biotechnology Explorer Program for their support and advice. We appreciate the participation of the Biology faculty at Bellarmine University: Drs. Thomas Bennett, Mary Huff, David Porta, William Tietjen, and Steven Wilt. Special thanks to Dr. Joanne Dobbins for a great portrayal of the victim, and for not getting upset about the "blood" that took days to come out of her hair. We extent our gratitude to all of the students in our classes for their helpful suggestions on improving this laboratory exercise.

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Supplemental Resources:

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Joann M. Lau earned a Ph.D. from the University of Illinois Urbana-Champaign and a B.A. from Bellarmine University. While at UIUC, she was a USDA Agriculture Genome Sciences and Public Policy Fellow, she had also received the Colgate-Palmolive Graduate Fellowship, and the Eugene S. Boerner Graduate Fellowship. She currently teaches Drugs and the Human Body, Modern Genetics, Introduction of Life Sciences, Principles of Biology labs, Cell Biology lab, and Molecular Biology labs at Bellarmine University. Her research currently involves studying the evolution of triple repeat diseases in non-human primates, the effects of Reishi mushrooms on lung cancer cell proliferation, and the expression of allergenic-related genes in ragweed.

Dave L. Robinson received his B.S. and M.S. in plant science from the University of Arizona, and his Ph.D. in plant physiology from the University of Minnesota. Now an Associate Professor of Biology at Bellarmine University in Louisville, KY he has taught Principles of Biology, Plant Diversity, Molecular Biology, Environmental Science, and Genetics, as well as seminar courses in ethnobotany. He has served as Biology Department Chair as well as principal investigator on a 3-year grant from NIH-NCRR. His research interests are in the physiology of weedy plants like ragweed, dandelion, and white snakeroot.

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Appendix

Example of DNA gel electrophoresis results.