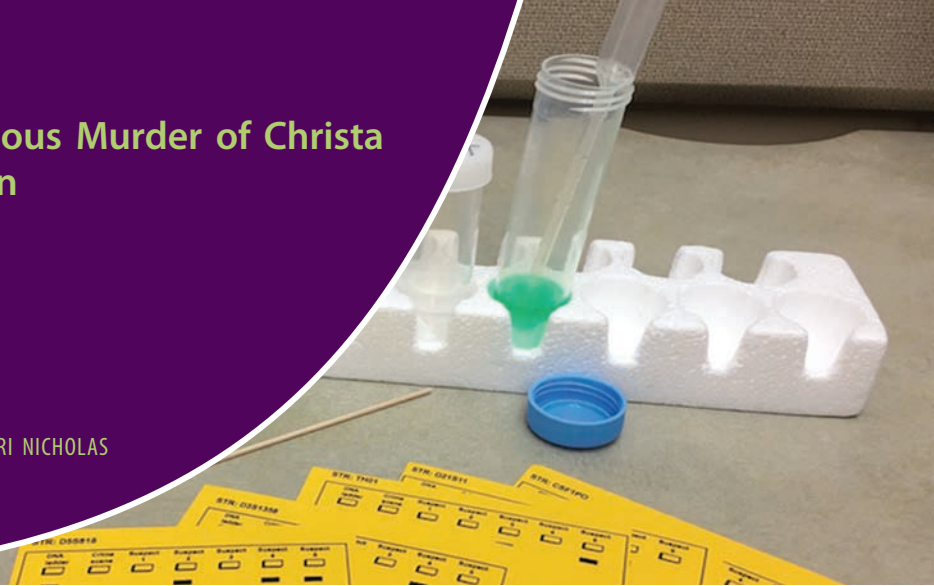


• KEVIN M. BONNEY, LORI NICHOLAS



ABSTRACT

This lesson presents an interrupted case study based on the true story of the 2002 murder of Christa Worthington in Massachusetts. The case was developed for use in an undergraduate non-majors life science course, but would also be appropriate for a high school biology course or a forensic science course. During this lesson, students examine a crime scene diagram and discuss evidence collection. Students then conduct a hands-on activity extracting DNA from wheat germ to simulate how DNA would be isolated from crime scene samples. Lastly the students will analyze simulated DNA profiles produced using STRs, polymerase chain reaction, and gel electrophoresis to help match a crime scene sample to one of five suspects. The pros and cons surrounding the use of a DNA dragnet are also discussed.

Key Words: biotechnology; DNA extraction; DNA profile; forensics; flipped classroom.

○ Introduction

This lesson is based on the true story of the murder of Christa Worthington in 2002 in Cape Cod, Massachusetts. No suspect was charged for this crime for over three years, during which time investigators used forensic DNA analysis techniques to identify potential suspects. The methods and motivations of investigators were questioned before, during, and after the trial due to allegations of racial bias and encroachment of civil rights. As a result of these issues, there have been three requests for a new trial since the original verdict. This case now serves as an example of DNA profiling and ethical issues related to criminal justice.

Students first examine a simulated picture of the crime scene and decide where and what kinds of evidence they would want to collect from the scene. Next, students are asked to determine which kinds of evidence collected from a

This lesson is based on the true story of the murder of Christa Worthington in 2002 in Cape Cod, Massachusetts.

crime scene could be used to construct a DNA profile. In the second part of the case, students conduct a hands-on activity extracting DNA from either wheat germ or human cheek cells to simulate how DNA would be isolated from crime scene samples. Lastly the students analyze simulated DNA profiles produced using short tandem repeats (STRs) and gel electrophoresis to identify a killer.

Students may want to know who was identified using crime scene DNA evidence and the outcome of the trial in the real-world case of Christa Worthington. The man arrested for the murder was Christopher McCowen, who was the trash collector for Worthington's neighborhood at the time. He did not submit a DNA sample as part of the dragnet but had actually given one a year earlier at the request of the police. He was subsequently convicted and sentenced to three consecutive life sentences in prison based mostly on the DNA evidence. Although he confessed to having sex with Worthington, he has always said it was consensual. His lawyers have asked for a new trial three times based on accusations of racial bias by members of the jury, questions of McCowen's competency, and issues with some of the DNA evidence. As of 2016, the Massachusetts courts have turned down all requests for a new trial, and McCowen remains in prison. More information about the details of the Worthington case are available by reading the NY Times article "Trash collector guilty in Cape Cod slaying" (Belluck, 2006) and watching the episode of the television show *48 Hours* included in the additional resources (Yager & Zied, 2007).

This lesson is an interrupted case study with three separate but related activities. It was developed for use in an undergraduate non-majors life science course, but is also appropriate for high school biology or a forensic science course. This case could be taught as part of a unit on genetics, biotechnology, or forensics. To facilitate use in the high school setting, the relevant Next Generation Science Standards (NGSS) are identified. Some prior knowledge or supplemental

instruction about polarity, chemical properties of DNA, the genetic code, meiosis, and gel electrophoresis will be necessary for completing this lesson. Instructors may find chapters in the free, online textbook *Concepts of Biology*, listed in additional resources, as a good source of information on the general biology topics (OpenStax, 2017). Information regarding DNA collection, DNA profile construction, and analysis can be found on the National Institute of Justice website “DNA Evidence Basics” (2012), also listed in the additional resources.

○ Overview

This lesson is organized into three parts: mock crime scene investigation, isolation of DNA from cell samples, and analysis of DNA profiles. Each part of the lesson can be completed in one 50-minute class period. In courses with 75-minute class periods, the first two parts can be completed within one class, and the third part can be completed in a second class period. Use of a laboratory may facilitate completion of the DNA extraction, but is not necessary.

After completing this case study, students will be able to:

- Identify potential pieces of evidence that may be recovered from a crime scene
- Identify crime scene evidence that can be used to construct DNA profiles
- Describe and demonstrate how DNA can be extracted from cells
- Discuss how gel electrophoresis can be used to separate pieces of DNA
- Describe the construction of a DNA profile using STRs
- Apply knowledge of STRs and gel electrophoresis to analyze representative data
- Identify the pros and cons associated with the use of DNA dragnets.

Connections to the Next Generation Science Standards

The Next Generation Science Standards (NGSS) are widely used in K-12 education to organize classroom instruction around core learning objectives. The goal of the NGSS is to provide a framework of science and engineering education that effectively prepares students for a range of higher education and careers. The lesson presented here was designed to promote achievement of several objectives from the NGSS aligned with the National Research Council’s *Framework for K-12 Science Education*. The *Framework* is divided into three dimensions: disciplinary core ideas, science practices, and crosscutting concepts. Table 1 identifies which aspects of these three dimensions are integrated into the activities described here.

○ Part I: The Scene of the Crime

Teacher Preparation

Students read the short narrative describing the discovery of Worthington’s body and the biographical and contextual information about the people involved. This should take five to ten minutes. Next they will look at the mock crime scene photo and answer the questions with their partners. Before moving on to Part II, it

is helpful to discuss the answers to these questions. This part of the activity should take about 15 to 20 minutes.

Student Activity

On a cold morning in January 2002, in the town of Truro, Massachusetts, Tim Arnold decided to return a borrowed flashlight to his former girlfriend and neighbor Christa Worthington. As he approached her kitchen door he noticed it was open. “That’s odd,” he thought as he entered the kitchen. To his horror he saw the motionless body of his friend lying in a pool of blood on the floor with her two-year-old daughter, Ava next to her. “Mommy fell down,” said Ava as Tim scooped her up and called 911.

Worthington, the 46-year-old Vassar graduate, moved to this rural town on Cape Cod, Massachusetts, a few years earlier, leaving behind a more glamorous life as a fashion writer in Manhattan. Her family had deep roots in Truro. Although she had left behind a hectic and stressful life in NYC, these past years on the Cape had still been a struggle for her. An only child, she had already lost her mother to cancer and was losing her father to a woman he was dating, Elizabeth Porter. Porter was nearly 50 years younger than her father, a heroin addict and a former prostitute who seemed to be using him for his money. There was some joy in her life though, too. When she arrived in Truro, she began an affair with a local fisherman named Tony Jackett, who was married with several children. Much to Worthington’s surprise, she became pregnant and eventually gave birth to Ava. In a small town it is hard to keep secrets, and soon Jackett’s wife Susan learned of the affair and the existence of Ava.

As part of the crime scene investigation team, you are assigned to the case of Christa Worthington. You should examine the crime scene diagram of her home (Figure 1) to determine what type of evidence to collect and then answer the questions.

Questions

1. Provide a hypothesis to explain who committed this crime. Provide evidence to support your reasoning.
2. Provide a hypothesis to explain the motive of the perpetrator of this crime. Provide evidence to support your reasoning.
3. Who would you want to speak to regarding Worthington’s homicide? What information would be helpful to learn?
4. Indicate on the crime scene diagram where you would look for evidence and what types of evidence you would collect. Briefly describe the information you hope to obtain from each piece of evidence.
5. What kinds of evidence can be used to make a DNA profile?

○ Part II: Isolating DNA from the Samples

Teacher Preparation

As a surrogate for analyzing actual crime scene evidence, almost any cell type can be used as a source of DNA. Isolating DNA from students’ own cells is perhaps the most engaging since students can visualize their own genetic material. However, some circumstances, such as lack of biohazard disposal or institutional approval, may prohibit use of this method. Wheat germ is preferred over other

Table 1. Connections between these activities and the Next Generation Science Standards.

Dimension	Standard	Examples
Disciplinary Core Ideas	LS3.B Variation of Traits "The variation and distribution of traits in a population depend on genetic and environmental factors. Genetic variation can result from mutations caused by environmental factors or errors in DNA replication, or from chromosomes swapping sections during meiosis."	Part III: Students examine genetic variation in terms of STR number.
Science Practices	Asking Questions and Defining Problems "Ask questions that arise from careful observation of phenomena, or unexpected results, to clarify and/or seek additional information."	Part I: Students synthesize hypotheses and propose questions during the crime investigation section. Part III: Students predict phenomena and solve problems using data related to STRs.
	Developing and Using Models "Develop and/or use a model (including mathematical and computational) to generate data to support explanations, predict phenomena, analyze systems, and/or solve problems."	Part III: Students analyze data about STRs and make scientific claims.
	Analyzing and Interpreting Data "Analyze data using tools, technologies, and/or models in order to make valid and reliable scientific claims or determine an optimal design solution."	Part II: Students apply scientific reasoning to support explanations about how the DNA extraction protocol works.
	Constructing Explanations and Designing Solutions "Apply scientific reasoning, theory, and/or models to link evidence to the claims to assess the extent to which the reasoning and data support the explanation or conclusion."	Part III: Students communicate scientific information about STRs graphically and textually, while also communicating orally during the activity.
	Obtaining, Evaluating, and Communicating Information "Communicate scientific and/or technical information or ideas in multiple formats (i.e., orally, graphically, textually, mathematically)."	Part III: Students communicate scientific information about STRs graphically and textually, while also communicating orally during the activity.
Crosscutting Concepts	Patterns "Observe patterns in systems at different scales and cite patterns as empirical evidence for causality in supporting their explanations of phenomena."	Part III: Students observe and cite patterns in STR variation to support explanations about the suspect and crime.

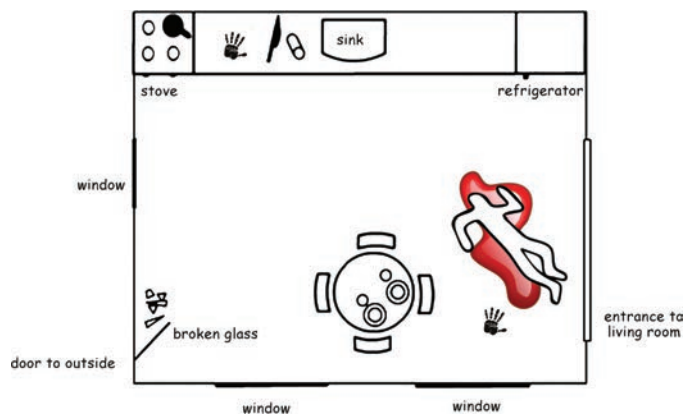


Figure 1. Crime scene diagram.

commonly used plant products, such as strawberries, because it can be cheaply purchased and easily stored from semester to semester, is easier and less messy to handle, and does not contain large amounts of pectin and other cellular components that may obscure results.

The DNA extraction protocol (Table 2) is similar regardless of the source of DNA. It may be helpful to assemble a DNA extraction "kit" for each student team in a reusable plastic bag that contains a teaspoon of wheat germ, wooden stir stick, and a plastic test tube. Then the water, detergent, and isopropyl alcohol can be made available as needed according to the DNA extraction procedure. The 0.9% NaCl solution can be made easily by adding 2 tbsp to one liter (quart) of water. Plastic test tubes can be purchased from many biological supply companies, along with racks to hold the tubes, if necessary. Groups of two to four students are best for

Table 2. Protocol for extraction DNA from wheat germ and human cheek cells.

	DNA extraction from wheat germ	DNA extraction from human cheek cells
Materials (per group)	<ul style="list-style-type: none"> • raw wheat germ (1 tsp) • detergent (1 mL) • water (25 mL) • isopropyl alcohol (10 mL) • 50 mL plastic test tube (1) • wooden stir stick (1) • teaspoon (1) • small plastic funnel (1) 	<ul style="list-style-type: none"> • detergent (5 mL) • water with 0.9% NaCl (10 mL) • isopropyl alcohol (10 mL) chilled on ice • drinking cup (1) • 50 mL plastic test tube (1) • teaspoon (1) • small plastic funnel (1)
Procedure	<ol style="list-style-type: none"> 1. Add one teaspoon of wheat germ to test tube using funnel. 2. Add 25 mL of water to test tube. 3. Gently stir wheat germ for 3 minutes. 4. Add 1 mL of detergent to test tube. 5. Gently stir wheat germ for 5 minutes. Be careful not to create bubbles or foam. 6. Tilt test tube at a slight angle. Slowly add 10 mL of isopropyl alcohol by pouring it down the side of the test tube. The alcohol should form a layer on top of the water. Be careful not to mix the alcohol with the water. 7. Cap test tube. Place test tube on desk or in holding rack for 10 minutes without disturbing. 8. The DNA should be floating in the layer of alcohol, on top of the water. Observe and record appearance. 	<ol style="list-style-type: none"> 1. Swish 10 mL of 0.9% NaCl water in mouth for 30 sec. 2. Spit water into drinking cup. 3. Add 5 mL detergent to test tube. 4. Pour water from cup into test tube using funnel. 5. Cap test tube. Gently roll test tube across desk for 3 mins. 6. Tilt test tube at a slight angle. Slowly add 10 mL of isopropyl alcohol by pouring it down the side of the test tube. The alcohol should form a layer on top of the water. Be careful not to mix the alcohol with the water. 7. Cap test tube. Place test tube on desk or in holding rack for 10 minutes without disturbing. 8. The DNA should be floating in the layer of alcohol, on top of the water. Observe and record appearance.

this activity. The activity takes 30 to 45 minutes, including time to answer the accompanying questions.

Students may require prior instruction about the structure of the cell membrane and the physical and chemical characteristics of macromolecules. It may be helpful to remind students that both the cell membrane and detergents are made of lipids, and that DNA and water are highly polar compared to alcohol and lipids. If additional instructional material is needed, instructors can consult Chapters 2.2, 2.3, and 3.4 of *Concepts of Biology* (Open Stax, 2017).

Student Activity

The biological samples collected from the Worthington crime scene have been taken to the lab for further analysis. The first step of making a DNA profile is to extract DNA from all of the crime scene samples. Today you will practice extracting DNA from either wheat germ or your own cheek cells. Using the appropriate protocol listed below, collect your sample and extract the DNA. While you are waiting for the DNA to precipitate (come out of solution) in step 7, work on answering the questions.

Questions

1. Where in the cell is DNA located?
2. Identify the purpose of the water, detergent, and alcohol in the DNA extraction procedure.

3. Sometimes meat tenderizer is added to the DNA extraction protocol. Meat tenderizer contains enzymes that break down proteins. Why would this be helpful during DNA extraction?
4. Describe the appearance of the DNA you extracted.
5. List several reasons why scientists would need to extract DNA from cells.

○ Part III: Constructing and Analyzing DNA Profiles

Teacher Preparation

During this part of the activity, students take 15–20 minutes to read about STRs, constructing and analyzing DNA profiles, and using a DNA dragnet in a criminal case. Students should already have an understanding of genes, alleles, and independent assortment of chromosomes before this activity. Instructors can read more about the use of DNA dragnets in the *Time* magazine article “The DNA dragnet” (Ripley et al., 2005), which is listed in the additional resources. Each student group will then examine 12 simulated DNA profiles (Figure 5, below) to determine which suspect’s DNA sample matches the sample from the crime scene. Students will construct a banding pattern for the 13th DNA profile while answering the questions. The DNA profiles were simplified by having DNA

ladders that represent the numbers of repeats rather than length of base pairs. Every DNA profile has more than one match to the crime scene, so that students need to examine several profiles before determining which suspect's DNA sample matches. The DNA profiles can be printed on a single sheet of paper and then cut into 12 separate profiles. Printing the profiles on card stock or laminating the paper will make them sturdy enough to be reused for several classes. This part of the activity should take students about 30 minutes, including answering questions.

Student Activity

DNA profiles for criminal and paternity cases can be produced using STRs, which are sequences of two to six nucleotide bases repeated a certain number of times in a row. For example, an STR called TPOX located on chromosome #2 has the sequence AATG. An individual might have three repeats on one copy of chromosome #2, which would look like this: AATGAATGAATG (STR Fact Sheet, 2007). Since STRs are not genes and as a result are not transcribed and translated into proteins, they have more variability from person to person. This is what makes them more appropriate to use for identification purposes than genes, since there are a limited number of alleles for a gene. TPOX, for example, has alleles that range from four to sixteen repeats (STR Fact Sheet, 2007). Just as everyone inherits a pair of alleles of a gene from their parents, a person also inherits a pair of alleles of STRs from their parents. For example, as seen in Figure 2, Kid 1 inherits five repeats of TPOX from their father and three repeats from their mother, whereas their sibling (Kid 2) inherits five repeats from their father and nine repeats from their mom.

Since 1997, the FBI has required that DNA profiles used in criminal cases be constructed using 13 core STR loci. The 13 STRs are spread out across the genome on different chromosomes. These 13 core STR loci were chosen so that construction of DNA profiles is standardized across the United States. The use of 13 STRs also ensures that it is extremely unlikely that two DNA profiles would match and not be from the same person (Butler, 2006). Effective January 1, 2017, the FBI require an additional seven core loci when constructing a DNA profile. Adding more loci will enhance accuracy in matching DNA profiles, increasing compatibility with international DNA databases and in decreasing statistically the chance that two non-related samples will match (Hares, 2015).

Construction of a DNA profile involves many steps. First the DNA must be extracted and purified from each sample collected

from the crime scene, the victim(s), and any suspects. Next these samples must be copied many times, or amplified, using polymerase chain reaction (PCR). This step is necessary because a single molecule of DNA is too small to visualize, but when there are many identical copies of the same DNA sample, the sample can be easily analyzed. PCR is a technique that allows sequences of DNA to be copied millions of times by essentially mimicking DNA replication in a controlled setting. The DNA to be copied is combined with a mixture of nucleotide bases, small sequences of DNA called primers, and a special version of DNA polymerase called *Taq* polymerase. This mixture is then placed in an instrument called a thermocycler, which as the name suggests cycles through various temperatures.

The first stage of PCR is called denaturation and involves heating the samples to about 95°C, which causes the hydrogen bonds between the base pairs to break, allowing the DNA strands to separate. The next step is called annealing. The samples are cooled to about 55°C, which allows the primers to bind to the single-stranded DNA. The primers are designed to match nucleotide sequences in the DNA that are found right next to the STR sequence on both strands. The last step is called extension. The mixture is heated to about 72°C to provide the optimal temperature for *Taq* polymerase to attach, which is the enzyme that adds free nucleotides to the nascent DNA strand. This heating-cooling-heating cycle is then repeated 30 to 40 times and results in millions of copies of the STR. See Figure 3 for the steps of PCR.

The process of PCR is repeated using different primers to amplify each of the 13 STRs. After the STRs for every DNA sample have been amplified, they are analyzed using a technique called gel electrophoresis. Gel electrophoresis uses electricity to separate DNA molecules based on their lengths. A positive electrode is connected to one end of the gel electrophoresis apparatus, and a negative electrode is connected to the other end. Because DNA is negatively charged, it will move away from the like-charged negative electrode and toward the oppositely charged positive electrode. Shorter pieces, which have fewer repeats, migrate to the bottom of the gel faster than longer pieces, which remain closer to the top. Each lane of an agarose gel is loaded with a different DNA sample that has been processed for a specific STR. The places on the gel where the STRs are located are called bands. In addition to crime scene related samples, the gel is also loaded with samples of DNA of known lengths, that when separated produce a set of reference points or a DNA ladder. The pattern of bands for each STR is the DNA profile. If the pattern matches for all 13 STRs between two

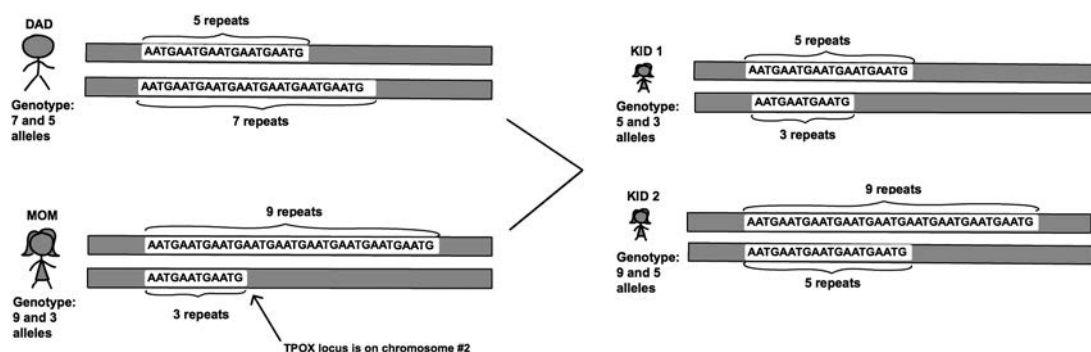


Figure 2. Inheritance of the STR TPOX.

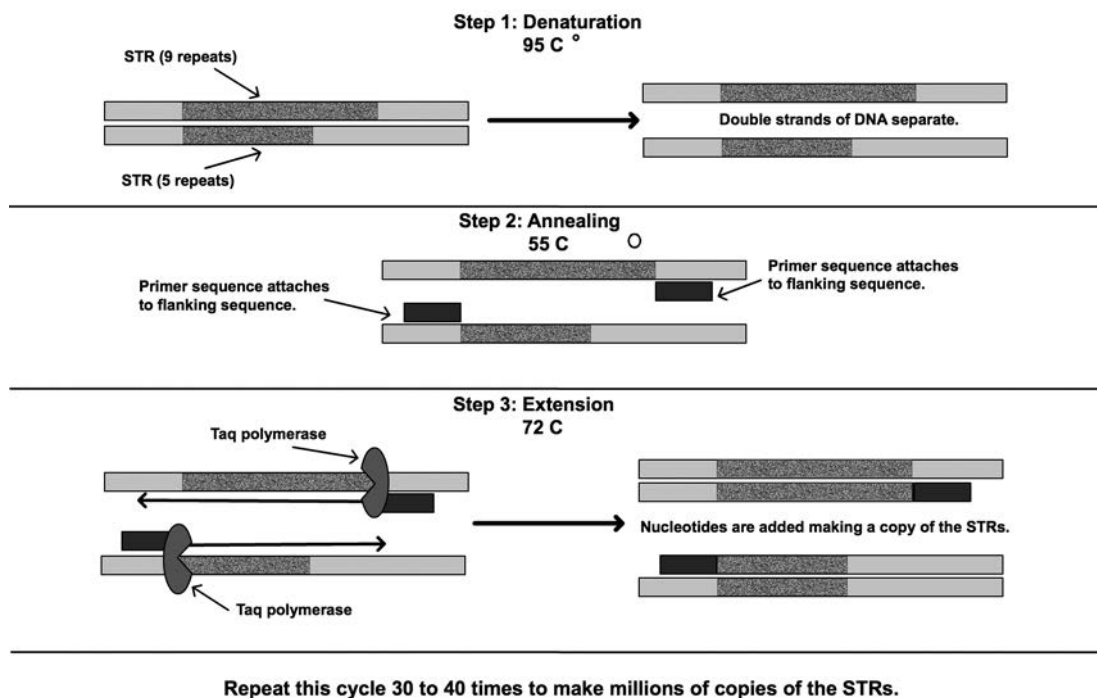


Figure 3. The steps of polymerase chain reaction.

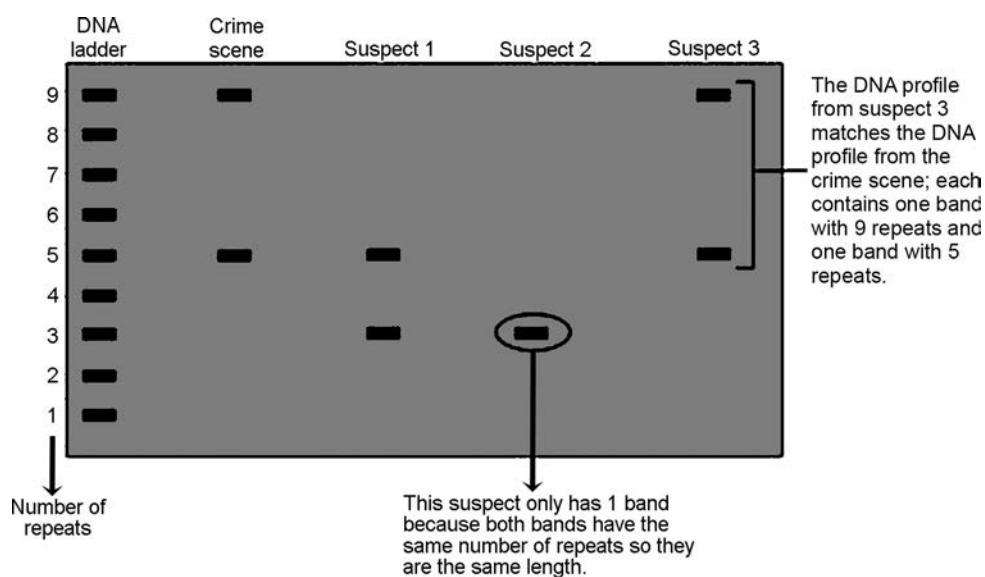


Figure 4. DNA profile for the STR TPOX.

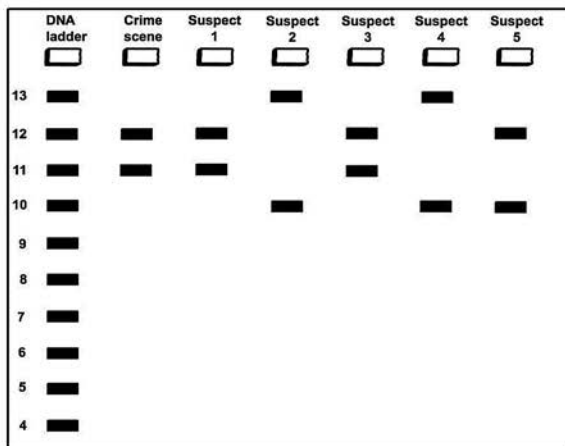
samples, it is statistically unlikely it could match more than one person. See Figure 4 for an example of a DNA profile.

After three years the police had not arrested anyone for Worthington’s murder. Family, friends, and neighbors were both nervous and angry about the lack of progress in the case. The police chief decided to try a technique called a DNA dragnet. A DNA dragnet involves collecting DNA samples from volunteers that may match characteristics of the perpetrator (Rothstein & Talbott, 2006). At various places in town the police asked Truro residents between the ages of 18 and 70 to volunteer to give a DNA sample. The license plate was recorded of people that refused.

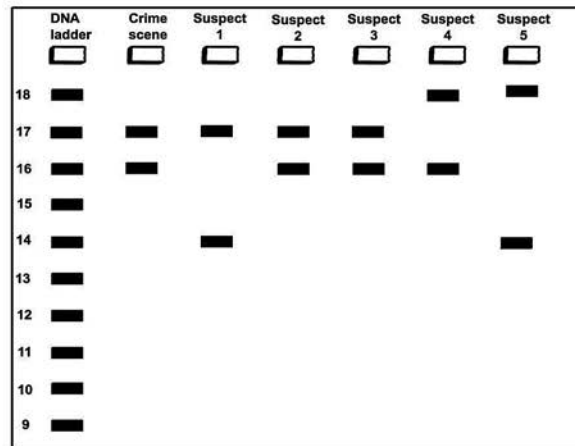
These samples were processed and compared to DNA profiles constructed from crime scene evidence.

Use the DNA profiles constructed from the following STRs: TPOX, CSF1PO, D3S1358, D8S1179, FGA, TH01, VWA, D5S818, D7S820, D13S317, D16S539, and D21S11 (Figure 5). One lane contains a DNA ladder for comparison, which for simplicity uses the number of repeats as the scale (like in Figure 4). The other lanes contain DNA collected from the crime scene and five suspects. Oftentimes a crime scene sample contains a mixture of victim’s DNA and a suspect’s DNA. For this simulation we have already removed the victim’s alleles, so the crime scene sample is only

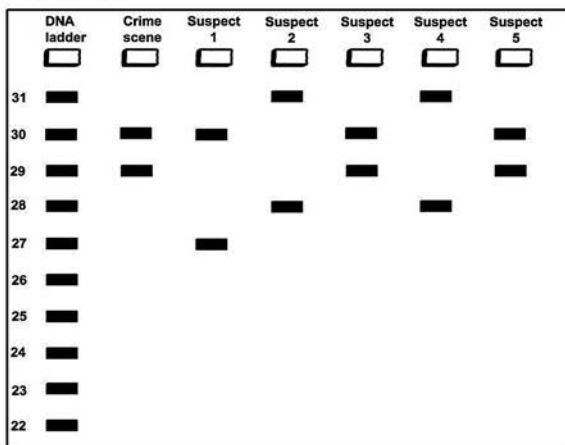
STR: D16S539



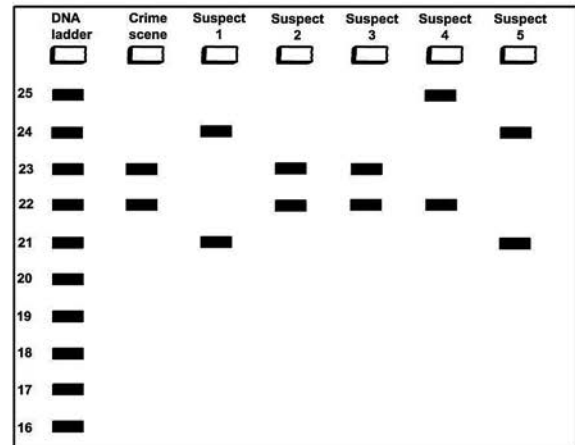
STR: VWA



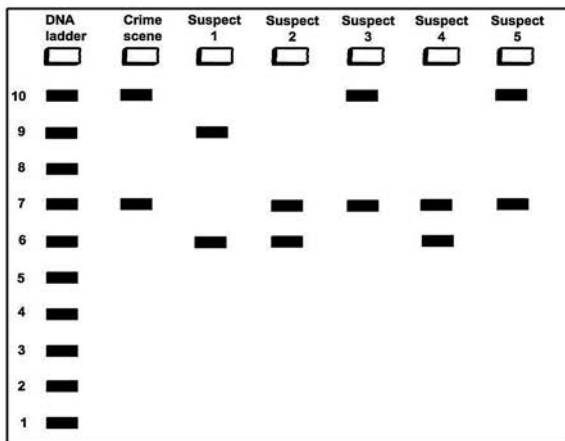
STR: D21S11



STR: FGA



STR: TH01



STR: TPOX

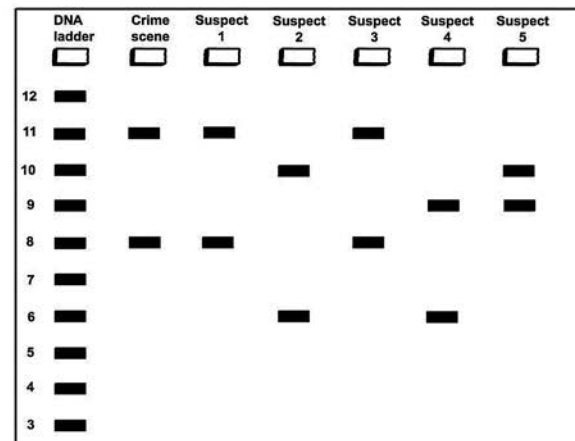


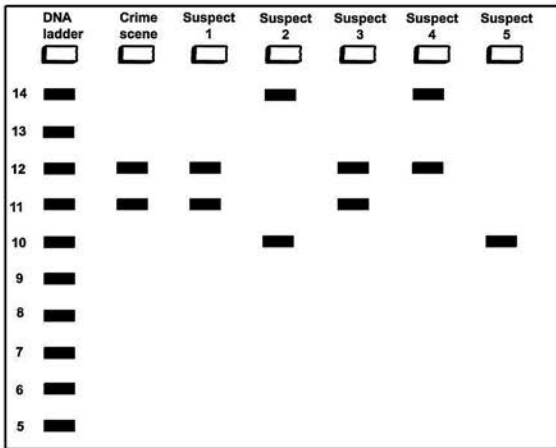
Figure 5A. STR results.

from whomever left the sample. Four suspects are people who refused to give DNA samples during the DNA dragnet and were later compelled by a warrant to give one. The other suspect is someone the police called in for questioning and voluntarily gave a sample. You should examine all the STRs to see if there is a match between the crime scene and any of the suspects. Once you have examined the 12 STRs you should answer the questions.

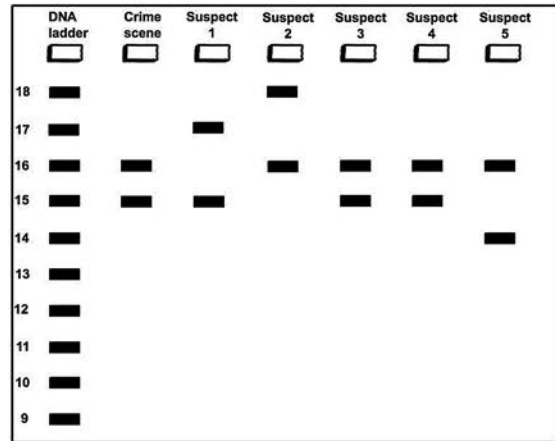
Questions

1. Based on the 12 DNA profiles, which suspect matches the crime scene DNA evidence? Explain why it is a match.
2. Suspects #2 and #4 are brothers. Provide evidence from the DNA profiles to support this claim.
3. Explain why suspect #5 only has one band for STR CSF1PO.

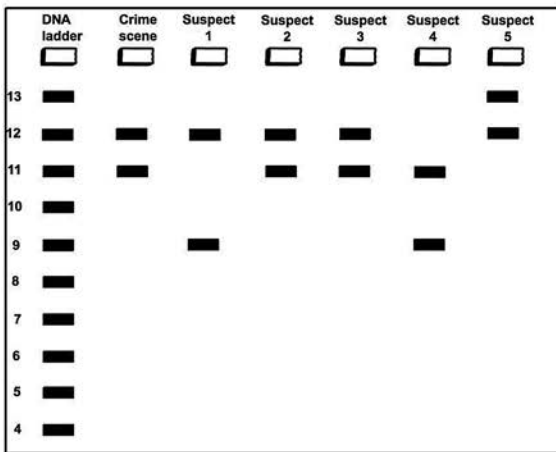
STR: CSF1PO



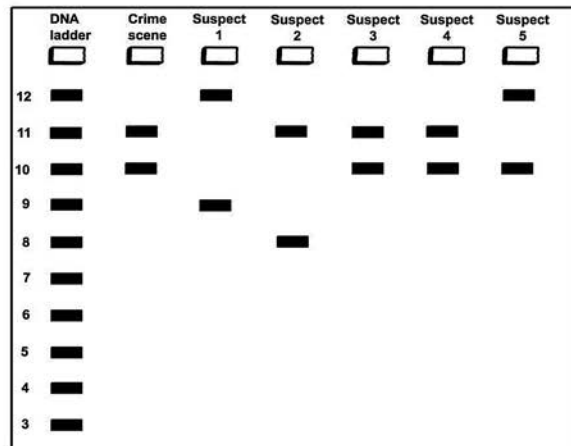
STR: D3S1358



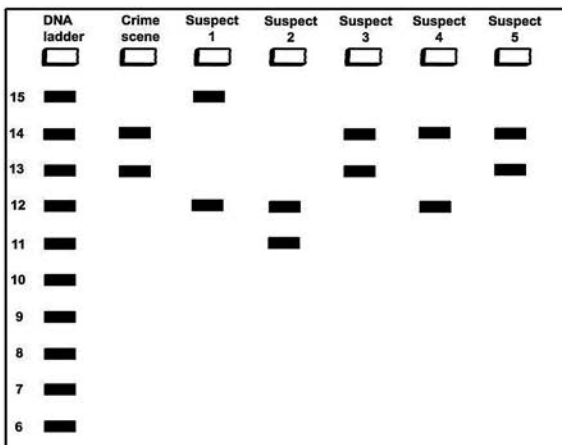
STR: D5S818



STR: D7S820



STR: D8S1179



STR: D13S317

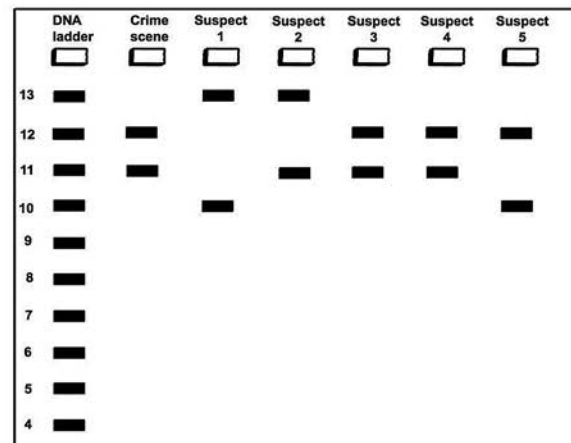


Figure 5B. STR results (continued).

- Below is a blank gel for the 13th STR D18S51 (Figure 6). Based on the data you have already examined, draw bands on the gel for the matching suspect.
- Does the presence of a suspect's DNA sample at the crime scene mean that person is guilty of the crime? Explain your answer.

- The killer's DNA was not collected from the dragnet but was from someone the police had questioned a year earlier. The sample was not tested for four years because Massachusetts, like most states, has a several year backlog of DNA samples to be processed. Knowing this, what do you think about using DNA dragnets to catch criminals?

STR: D18S51

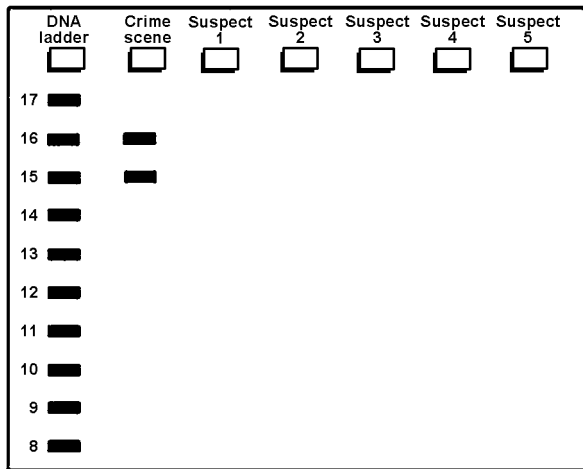


Figure 6. A blank gel for the 13th STR D18S51.

○ Conclusion

More information on the basics of constructing and using DNA profiles can be obtained from the additional resources provided. Note that these resources contain references to the sexual assault that was part of the real crime but was not mentioned in this learning activity. Also included in additional resources is an article about the use of DNA dragnets.

Instructors who do not have access to wet lab space and equipment can still teach their students basic concepts in biotechnology. This activity provides a hands-on set of activities related to forensic science and biotechnology, but without using expensive equipment like a thermocycler or gel electrophoresis. Through the use of a simulated crime scene, students can discuss the collection of DNA and other evidence. Students can then extract DNA from plant or human cells with minimal equipment and safe, minimally toxic chemicals. Lastly students can examine simulated DNA profiles to gain a deeper understanding of how they are constructed and analyzed in criminal cases without having to do PCR or gel electrophoresis.

Additional Resources

- Belluck, P. (2006, November 17). Trash collector guilty in Cape Cod slaying. *The New York Times*. Retrieved from <http://nyti.ms/2ejYAxJ>
- National Institutes of Justice. (2012). DNA evidence basics. Retrieved from <http://nij.gov/topics/forensics/evidence/dna/basics/Pages/welcome.aspx>
- OpenStax (2017, February 6). *Concepts of Biology*. Retrieved from <http://cnx.org/contents/b3c1e1d2-839c-42b0-a314-e119a8aafbdd@8.21>.
- Ripley, A., Bates, T., Hequet, M., & Laney, R. (2005, January 16). The DNA dragnet. *Time*, 165, 39–40. Retrieved from <http://content.time.com/time/magazine/article/0,9171,1018083,00.html>
- Yager, J., & Zied, M. (Prods.). (2007, September 20). "Murder on the Cape." *48 Hours*. *Cbsnews.com*. Retrieved from <http://www.cbsnews.com/news/murder-on-the-cape/>

References

- Butler, J. M. (2006). Genetics and genomics of core short tandem repeat loci used in human identity testing. *Journal of Forensic Science*, 51(2), 253–265. doi:10.1111/j.1556-4029.2006.00046.x
- Hares, D. R. (2015). Selection and implementation of expanded CODIS core loci in the United States. *Forensic Science International: Genetics*, 17, 33–34. doi:<http://dx.doi.org/10.1016/j.fsigen.2015.03.006>
- Rothstein, M. A., & Talbot, M. K. (2006). The expanding use of DNA in law enforcement: What role for privacy? *The Journal of Law, Medicine and Ethics*, 34(2), 153–164.
- STR Fact Sheet—TPOX. (2007). Retrieved from http://www.cstl.nist.gov/strbase/str_TPOX.htm

LORI NICHOLAS (lnicholas4@alamo.edu) is an Instructor in the Natural and Physical Sciences Department of Northwest Vista College, 3535 North Ellison Drive, San Antonio, TX 78251; KEVIN M. BONNEY (kevin.bonney@nyu.edu) is a Clinical Assistant Professor in Liberal Studies at New York University, 726 Broadway, New York, NY 10003.