

The Case of the Druid Dracula



A Directed “Clicker” Case Study on DNA Fingerprinting

By Peggy Brickman

This article describes how case studies have been successfully implemented in an introductory biology course of 300+ students using available technologies ranging from WebCT, used to assign students to permanent small groups (as well as assign groups to regions of a large lecture hall), to hand-held response systems (aka “clickers”), which students use to collaboratively solve cases during class.

I use case studies in my one-semester, three-credit hour introductory biology course with typical class sizes of 300+ students. Taken primarily by freshmen and sophomores to fulfill a general education requirement, the course consists of three 50-minute weekly meetings in a lecture classroom with no recitation section, although 65% of students are enrolled in an optional one-credit hour lab section.

Using case studies in large-enrollment courses can be challenging. I have found that it can be done successfully with a little creative techni-

cal support. Before describing the technology I use, I’d like to discuss the kind of case study that I have found works best in this setting.

I recommend that the case study be short and self-contained, controllable, and gradable. For my purposes, I use cases that focus on the acquisition by students of important course-related concepts and facts and provide an opportunity for them to practice interpreting data and drawing inferences from observations. As *directed* cases, the focus of the case studies I use is more on the dissemination of facts and principles and less on the analysis of all the possible options and solutions to a problem. I have found that the best format for achieving my goals

is the Interrupted Case Format, where students receive the information in parts, or sections. Each section builds on the information and data presented in the previous section and is punctuated by questions that drive students’ learning. I use multiple-choice questions that have specific, correct answers. Students answer the multiple-choice questions in class using hand-held response systems, or “clickers,” as they are more commonly known (more on those in a moment).

Students work on the cases during the 50-minute lecture period in permanent small groups, which I institute the very first day of class. These groups, which consist of six students to a group, are randomly assigned using the Group Generation tool in WebCT, the online course management system adopted by the University of Georgia. Each group has an assigned seating location in the “stadium-style” lecture hall (see Figure 1), and each

Peggy Brickman (brickman@uga.edu) is an assistant professor in the Department of Plant Biology at the University of Georgia in Athens, Georgia.

group keeps handouts, grades, exams, and an attendance sheet in folders that they pick up in class each day. In their groups, students work daily on in-class activities that account for 15% of class time. Individual test and quiz scores determine 80% of each student's grade. An additional 20% of their grade is determined by group tests as well as mid- and end-of-semester peer evaluations.

Clickers in the classroom

Clicker technology has allowed me to successfully manage and encourage the type of classroom discussion and feedback critical for case study success in my classroom of 300+ students. I have turned to clickers because quality instruction depends on regularly assessing student comprehension and generating student discussion. The re-description by the student during discussion or questioning is a powerful way to promote learning that is unfortunately inhibited in very large classes.

Clickers are wireless transmitters used by students to instantly, accurately, and anonymously answer questions posed by the instructor. In my classroom, students use them to collaboratively solve cases during class.

Each group is assigned a clicker that a member of the group picks up at the beginning of class and turns back in when class is over. Using the device, student groups tackle the multiple-choice "clicker questions" associated with a case. I then call on groups to provide an explanation to the rest of the class of their problem-solving strategies, rather than just giving them the correct answer. This allows me to encourage the re-description that promotes learning as well as uncover sources of confusion and misconception.

The Case of the Druid Dracula

"The Case of the Druid Dracula," reprinted in Figure 2, is based on a lurid crime featured on the BBC program *Crimewatch UK* in December 2001 (Forensic Science Services Case Files) that was solved thanks to forensic DNA analysis. Students learn how the structure of DNA and the mechanism used by cells to duplicate DNA were critical to the forensic analysis. They then determine the statistical validity of the forensic data in the same way a prosecutor would prepare the case for a courtroom.

The case consists of three parts, with questions that can be completed within a single 50-minute lecture

session. In terms of the timing, there are approximately 15 to 20 minutes of student discussion punctuated by three "mini-lectures," each lasting about 10 minutes.

The case requires some preexisting knowledge on the part of students. My students receive a brief introduction to the biochemistry of DNA in a previous lecture and in their pre-class textbook reading (Krogh 2005). Students also need to understand the chromosomal differences between men and women in the total human karyotype.

Learning objectives

Upon completion of the case, students will:

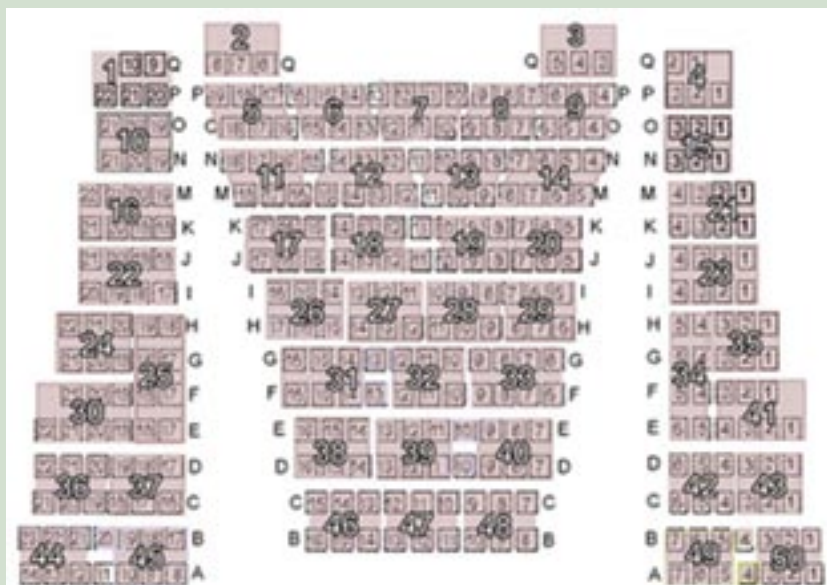
- ♦ Understand the similarities and differences in the DNA of humans and how those differences can be exploited for forensic identification.
- ♦ Understand the structure of DNA and how hydrogen bonds between the nucleotide bases dictate the complementary nature of the double helix. Students will be able to predict the nucleotide sequence of one strand of DNA in a double helix if given its complementary strand.
- ♦ Describe the technique of polymerase chain reaction (PCR) and relate it to the normal cellular process of DNA replication. Students will be able to predict the sequence of PCR primers that would amplify just one short stretch of DNA out of an entire genome.
- ♦ Understand how short tandem repeats (STRs) within human chromosomes can be used to generate fingerprints and how to interpret these fingerprints to match the DNA to a specific person.
- ♦ Use statistical prevalence of STRs to determine the probability that someone else at random in the population could have DNA that matched a sample found at a crime scene.

Case management

I hand out Parts I, II, and III of the case on opposite sides of a single sheet of paper at the beginning of class (with Part

FIGURE 1

Seating chart showing organization and location of student groups.



I on one side and Parts II and III on the other). I ask students to read the case, then describe to them the purpose of the case and ask them to answer two pre-assessments, using their clickers, on the structure of DNA from their pre-class reading, shown in Figure 3. If student responses to the pair of questions are below 90% correct, I will begin a quick review of the structure of DNA.

Part I—DNA structure and PCR

Part I of the case describes the murder of Mabel Leyshon and the evidence collected at the crime scene. I begin this part of the discussion and analysis with a short lecture describing the amelogenin gene. Found on the X chromosome, the amelogenin gene (AMELX) encodes a protein that is critical for the formation of the enamel on teeth. Individuals with deletions in this gene can have problems forming the normal thickness of enamel on their teeth, and problems in the mineralization of the enamel, so that their enamel may remain softer than normal (OMIM). A duplicate version of the amelogenin gene can be found on the Y chromosome in primates (AMELY), but there are significant differences between these two amelogenins. One of those differences is found in intron 1 of AMELX, which is missing six nucleotides found in AMELY. PCR can be performed using primers flanking this region to amplify DNA sequences from both the AMELX and AMELY. PCR products generated from AMELX will be 106 base pairs in length; PCR products from AMELY will be 112 base pairs in length.

After covering this material, I ask students to answer clicker questions 1 and 2 for Part I. I follow successful responses to these questions with a brief lecture on DNA replication and describe how the knowledge was exploited to create the technique of duplicating DNA in polymerase chain reaction (PCR). Before I go over the steps of PCR, I ask them to answer clicker question 3 to assess their understanding. After assessing their comprehension of normal replication, I lecture on the steps of PCR using animations on the internet (Dolan DNA Learning Center)

FIGURE 2

“The Case of the Druid Dracula”

Part I: DNA structure and PCR

In the northernmost corner of the Isle of Anglesey in Wales, in a village called Llanfairpwll, the wind-swept beaches and ancient Druid ruins provided a surreal backdrop for the murder of 90-year-old Mabel Leyshon. Her murder was not only brutal—her heart had been hacked out—but also creepy. It appeared as if the killer had collected Mabel’s blood in a small kitchen saucepan that had lip marks on the rim indicating the contents had been tasted. The murder showed other signs of the occult: a candlestick and a pair of crossed pokers had been arranged near the body. Further investigation indicated that this was no supernatural villain at work. The murderer had worn tennis shoes, which left distinctive footprints under the glass door that had been shattered with a piece of broken garden slate in order to gain entrance to the victim’s home. Inside the house, a windowsill had bloodstains on it. With luck, the evidence recovery unit hoped to use it to find the killer.

Questions

DNA differences can be used to identify people. For example, there is a gene found on both the X and Y chromosomes called amelogenin, but the version of the gene found on the X and Y chromosomes differs in length, so it can be used to tell if a blood stain, such as the one found on the windowsill inside Mabel Leyshon’s house, was left by a man or woman. This stretch of nucleotides shows one strand of the DNA double helix for the amelogenin gene. What is the sequence of the other, complementary strand?

5’CCCTGGGCTCTGTAAAGAATAGTGTGTTGATTCTTTATCCCAGATGTTTCTAAGTG3’

- 3’ACTGTTAGATTTCCCTTTTTAGGTCTAGGTCGTCGGCCTTATTTCCGAGGAATAA5’
- 3’GGGACCCGAGACATTTCTTATCACACA AACTAAGAAATAGGGTTTACAAAGATTCAC5’
- 5’GGGACCCGAGACATTTCTTATCACACA AACTAAGAAATAGGGTTTACAAAGATTCAC3’
- 3’CCCTGGGCTCTGTAAAGAATAGTGTGTTGATTCTTTATCCCAGATGTTTCTAAGTG5’
- 5’CCCTGGGCTCTGTAAAGAATAGTGTGTTGATTCTTTATCCCAGATGTTTCTAAGTG3’

To assist the investigators with the crime, you will need to perform polymerase chain reaction (PCR) to create copies of this gene so the sizes can be compared to determine if the blood was from a man or woman. During PCR it will be necessary to break the hydrogen bonds of the base pairs. Where are those hydrogen bonds normally found?

- Between two nitrogen-containing bases in a single strand of DNA.
- Between the phosphate and sugar of the same nucleotide.
- Between the sugar of one nucleotide and the phosphate of a different nucleotide.
- Between one nitrogen-containing base on a single strand of DNA and another nitrogen-containing base on the complementary strand of DNA.
- Between one phosphate on a single strand of DNA and a sugar on the complementary strand of DNA.

PCR creates copies of DNA using the exact same mechanism used by your cells to copy their own DNA (replication). Place the steps listed below in the order in which they occur during replication:

- Two strands, one new and one original template, wind together to form the double helix.
- Short stretch of primer (~20 nucleotides exactly complementary to the gene that is going to be copied) is made.
- Separation of the double helix from two parental DNA strands.
- Use of parental DNA as a template so that nucleotides are covalently bonded together to form a new chain that is complementary to the bases on the original template.
 - A, B, C, D
 - B, C, A, D
 - D, B, C, A
 - C, B, D, A

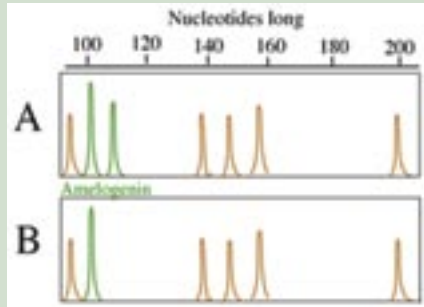
PCR can be used to selectively duplicate one single gene out of thousands because the only primers available to start replication are the one unique pair that is complementary to the regions on both sides of the gene. Which of these primers (one for each strand) could you use to copy just the stretch (highlighted in gray) of the amelogenin gene that differs between the X and Y chromosomes?

3’GGGACCCGAGACATTTCTTATCACACA AACTAAGAAATAGGGTCTACAAAGAGTTCCAGGACTT-TACAGTTCTACCAGCTTCCCAGTTAAGCTCTGAT5’

5’CCCTGGGCTCTGTAAAGAATAGTGTGTTGATTCTTTATCCCAGATGTTTCTCAAGTGGTCTGAAAT-GTCAAGGATGGTGGTCAAGGGTCAAATTCGAGACTA3’

- 3’GGGACCCGAGACATTTCTTATCAC5’ and 5’CCCTGGGCTCTGTAAAGAATAGTG3’
- 5’CCCTGGGCTCTGTAAAGAATAGTG3’ and 5’GTCGAAGGGTCAAATTCGAGACTA3’
- 5’CCCTGGGCTCTGTAAAGAATAGTG3’ and 3’CAGCTTCCCAGTTAAGCTCTGAT5’

Part II: The report



Question

Which of the above (A or B) represents the profile of a man?

Part III: More analysis

The crime was featured on BBC's *Crimewatch* program in December 2001 and North Wales Police received over 200 calls. Following up reports of a teenager who had attacked a German student, the police went to the home of Matthew Hardman (suspect 1), who gave police a cheek swab. During their visit, officers found a pair of Levi shoes. Forensic Science Service (FSS) scientists matched Hardman's shoes to the footwear marks found at the murder scene. Profiling, however, suggested a much older offender, so another suspect was also asked to give a cheek swab (suspect 2). Because both suspects were men, the officers needed to test for other genetic differences. They focused on STRs (short tandem repeats), stretches of DNA that exist in all people, but in different numbers of repeats. The allele ladder below shows all varieties in a population.

Questions

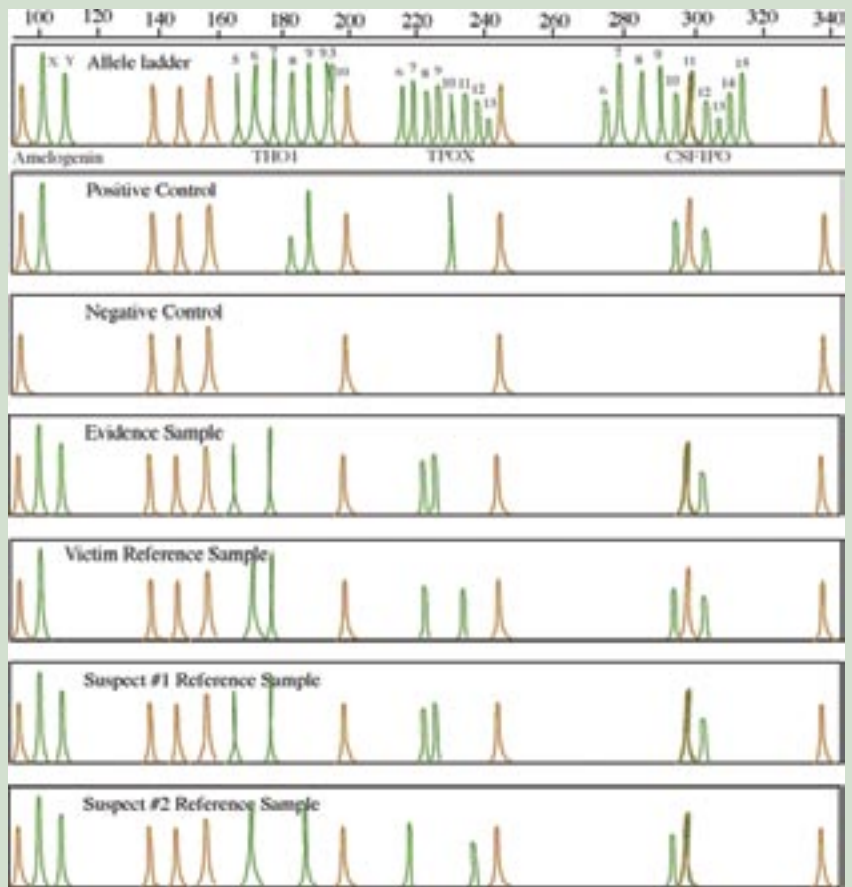
Did suspect 1 or 2 commit the crime?

- a. suspect #1
- b. suspect #2

There are only a few different numbers of repeats that are seen in our population—only five different TPOX STRs for example. By testing thousands of DNA samples, researchers know the distribution of these different STRs in the general population; those allele frequencies are shown in the table below. Using these frequencies, one can determine the probability that someone else at random would have the same matching pattern. For example, what is the likelihood that someone else at random would have the same pattern of Matthew Hardman (a 5- and 7-repeat for the THO1 STR)?

THO1	TPOX	CSF1PO
5: 1/200	8: 1/2	9: 1/40
6: 1/4	9: 1/8	10: 1/5
7: 1/6	10: 1/18	11: 1/3
8: 1/7	11: 1/5	12: 1/3
9: 1/6	12: 1/20	13: 1/10
9.3: 1/3		14: 1/50
10: 1/100		

- a. 1/200
- b. 1/206
- c. 1/1,200
- d. 1/2,600
- e. 1/20,060



What is the probability that someone else at random would have that same pattern of THO1 5 & 7, TPOX 8 & 9, and CSF1PO 11 & 12?

- a. 1/1,600
- b. 1/7,200
- c. 1/17,600
- d. 1/172,800
- e. 1/1,555,200

FIGURE 3
Pre-assessment “clicker” questions.

DNA is composed of nucleotides that are joined together by covalent bonds between which two portions of each nucleotide?

- between deoxyribose and a phosphate group
- between two deoxyribose groups
- between the nitrogen-containing rings
- between a phosphate group and the nitrogen-containing ring
- between the phosphate groups of both nucleotides

In the hydrogen bonds between nitrogen-containing bases ____.

- A always pairs with C
- A always pairs with G
- C always pairs with T
- G always pairs with T
- G always pairs with C

or the PowerPoint presentations that can be downloaded from the National Institute for Science and Technology’s website (Kline, Redman, and Butler 2001). To determine if they understand the idea of the primers, I ask them to answer clicker question 4. After five minutes of discussion, I call on groups at random to provide answers to the question. I ask them to explain why they chose the answer they did and try to probe for any confusion. If I discover major confusion, I will try to fill in any knowledge gaps. I then lecture on the way that the two different copies of the amelogenin gene (on the X versus the

Y) would generate different-sized PCR products (see Figure 4).

After explaining this to students, I describe how the technique of gel electrophoresis can be used to separate pieces of DNA like these two by size, again making use of the excellent animations available online (Dolan DNA Learning Center).

Obviously, this information only tells you the gender of the suspect who left the sample. It doesn’t help with much else: that’s where DNA fingerprinting comes in.

Background on DNA fingerprinting techniques used in Parts II and III

DNA fingerprinting has been used since the 1980s when Alex Jeffreys took advantage of regions of the human genome that have repetitive sequences that vary in number. These short stretches of DNA can be found in all humans, and consist of the same sequence of nucleotides repeated a different number of times in tandem (hence one of the names, short tandem repeats, or STRs). For example, a person could have five copies of one of these STRs, the THO1 sequence (TCAT), found on chromosome 11 on one homologue (a 5-repeat) and six repeats on the other homologue (6-repeat). Another person might have a 7-repeat and a 9-repeat, and therein lies the difference that can be used to match with a DNA evidence sample.

The procedure used to identify the number of repeats can vary. Most textbooks describe a technique called restriction fragment length polymorphism (RFLP) analysis. In this technique, researchers cleave chromosomal DNA with a restriction enzyme that recognizes restriction sites outside the repeat region so that the chromosome with the 6-repeat would yield a slightly longer fragment than the chromosome with the 5-repeat. Since smaller DNA fragments move farther into a gel during gel electrophoresis (toward the positively charged electrode), a 5-repeat will appear as a band lower in the gel than a 6-repeat. In this way, you can create a lineup of fragments with these repeats, with the largest number of repeats at the top of the gel, the one with the smallest number of repeats at the bottom, and the rest lined up in between. In reality, it is difficult to view fragments of DNA in a gel if the DNA concentration is low. So researchers transfer the DNA fragments from the gel to a membrane (Southern blotting), probe the blot with a labeled piece of DNA complementary to the nucleotide sequence of the repeat, and expose the blot to X-ray film to detect hybridization.

The likelihood that anyone else at random has the same repeat pattern was determined by sampling DNA from thousands of individuals. For example, they have discovered that only about 1/200 of all chromosomes have the

FIGURE 4
The sequence from the intron 1 of the AMELY gene.

The sequence reads:

3’G GGACCCGAGA CATTCTTAT CACCCACCTA AGAAGTAGGG TTTATTTCAC CAAAGAGTTC ACCAGGGTTA
5’C CCTGGGCTCT GTAAAGAATA GTGGGTGGAT TCTTCATCCC AAATAAAGTG GTTCTCAAG TGGTCCCAAT

3’TTACAGTTC CTACCATCAG CTCCAGTT TAAGCTCTGA T-5’
5’AAATGTCAAG GATGGTAGTC GAAGGTCAA ATTCGAGACT A-3’

The sequence from the intron 1 of the AMELX gene lacks 6 nucleotides compared to AMELY and reads:

3’G GGACCCGAGA CATTCTTAT CACACAATA AGAAATAGGG TCT----AC -AAAGAGTTC ACCAGGACTA
5’C CCTGGGCTCT GTAAAGAATA GTGTGTTGAT TCTTTATCCC AGA----TG -TTTCTCAAG TGGTCTGTAT

3’TTACAGTTC CTACACCAG CTCCAGTT TAAGCTCTGA T5’
5’AAATGTCAAG GATGGTGGTC GAAGGTCAA ATTCGAGACT A3’

The actual primers used for both regions generate a 106 bp fragment from AMELX and 112 bp fragment from AMELY.

5’-CCCTGGGCTCTGTAAAGAATAGTG-3’

3’-CAGCTTCCAGTTAAGCTCTGAT-5’ (backward 5’-TAGTCTCGAATTTGACCCTTCGAC-3’)

5-repeat, but 1 out of 4 chromosomes has a 6-repeat (Brenner). So, the overall chance that someone else at random in the population has both of those repeats can be determined by multiplying the two probabilities together: $1/200 \times 1/4 = 1/800$. This is much better than the odds that someone at random would have the same blood type as that left at a crime scene, for example, if 38%

of the population has type O positive blood. The beauty of using these STRs is that they occur in different regions of the human genome and the sequence of the repeats differs at these locations. So, researchers could subsequently probe this first blot for one of these different STRs (for example, TPOX found on chromosome 2) and determine that the DNA sample had an 8-repeat (1/2 probability) and a 9-repeat (1/8 probability). The probability that someone at random would have this pattern is $1/2 \times 1/8 = 1/16$. But the probability that someone at random would have a 5-, 6-repeat at THO1 *and* an 8-, 9-repeat at TPOX is the product of these two probabilities, or $1/16 \times 1/800 = 1/12,800$. Analysis with more STRs decreases the likelihood that someone else in the population could by random chance match the profile, thus increasing the validity of the data for use as evidence linking one suspect to the crime.

As the demand for analyzing DNA evidence grew in the last decade, it became imperative to find a cheaper and quicker way to generate these DNA fingerprints. The FBI began using specific PCR primers designed to amplify just those stretches of DNA with tandem repeats (Kline, Redman, and Butler 2001). This takes hours instead of days, and the PCR primers are labeled with different-colored dyes, removing the need for probing to discover the fragments. Moreover, a mix of PCR primers can be added to simultaneously amplify stretches, for example THO1, TPOX, AMEL, and CSF1PO STRs can all be amplified using the AmpFISTR Green 1 kit from Applied Biosystems. The green fragments created can be subjected to a very rapid type of gel electrophoresis using a thin capillary to separate by size, and then detected using a laser tuned to the green color of the dye used for the primers. Of course, controls must be run to determine the sizes of the fragments, and these are usually just a mixture of DNA samples of known size that can be mixed in with the fragments. This mixture of DNA fragments is labeled with another color-indicating dye, in this case an orange color, but for ease of reading I have placed them on the same

readout as the green peaks instead of in a separate window.

Part II—The report

Students next read Part II of the case and answer clicker question 1 in this section to assess their understanding of the process of gel electrophoresis.

Part III—More analysis

I then ask students to read Part III and answer clicker question 1 in that section. Almost every student group routinely gets this question correct but they are still unsure during questioning what the different peaks represent. So, I then lecture briefly on the presence and location of useful short tandem repeats of DNA in the chromosomes of humans and how those STRs can each be amplified using kits to generate different-sized PCR products that can be separated by gel electrophoresis (what they are seeing in the graphic from Part III). I ask students to consider clicker question 2. About a quarter of the groups routinely answer this question incorrectly. I will ask one of the groups that got it correct to explain to the class the logic of this probability and then assess students' comprehension by asking clicker question 3.

An answer key that provides answers to the questions posed in this case study is available on the website of the National Center for Case Study Teaching in Science—see www.sciencecases.org/druid_dracula/druid_dracula_notes.asp (Brickman 2006).

Case wrap-up

I wrap up the case with a description of what really happened. During the arrest of Matthew Hardman, a knife was found in his coat pocket, but there was no visible blood on it. DNA testing of the damaged knife handle, however, revealed two sources of DNA, one matching Hardman and a partial profile matching the victim. In the meantime, further forensics work on the partial profile from the windowsill improved the discrimination to 1 in 5 million and finally to 1 in 73 million. The picture was complete when police searched Hardman's home and found magazines

as well as evidence the computer had been used to access internet sites featuring vampires and how to become one. Matthew Hardman was found guilty of the murder of Mabel Leyshon at Mold Crown Court on August 2, 2002, and sentenced to life imprisonment.

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Note: The diagrams in the case are the same that would be generated by using ABI PRISM 310 Genetic Analyzer after using the AmpFISTR Green multiplex PCR kit from Applied Biosystems.