Case Teaching Notes

for

"The Case of the Druid Dracula"

by

Peggy Brickman
Department of Plant Biology
University of Georgia

Introduction / Background

In this directed case study, students read about a lurid crime featured on the BBC program *Crimewatch* in December 2001 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. Since this real-life scenario is one that many students may encounter as prospective jurors, the case provides them with some important background knowledge to help them make more informed decisions.

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

The case requires some existing knowledge from the students: my students have had a brief introduction to the biochemistry of DNA in a previous lecture and in their pre-class assigned reading, which includes the chapter on "DNA Structure and Replication" (Chapter 13 in *Biology: A Guide to the Natural World* by David Krogh, Prentice Hall 2005). Students also need to understand the chromosomal differences between men and women in the total human karyotype.

Objectives

As a directed case, the focus is more on the dissemination of facts and principles and less on analyzing all of the possible options and solutions to a problem. 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.

Classroom Management

The case takes about an hour to complete. It consists of approximately 15 to 20 minutes of student discussion punctuated by three "mini-lectures," each lasting about 10 minutes, such that the case can be completed within a single 50-minute lecture session. Students are assigned to work on the case in groups of six that are instituted the first

day of class. I have used this case successfully in a class with 50 groups. Organizing and interacting with permanent student groups on that scale can be challenging. I accomplish it with the aid of several technology tools.

WebCT

I form permanent student groups with the aid of the group generation tool in WebCT. Each group has an assigned seating location in the lecture hall (see Figure 1, below) and each group keeps handouts and an attendance sheet in folders they are responsible for bringing to class each day.

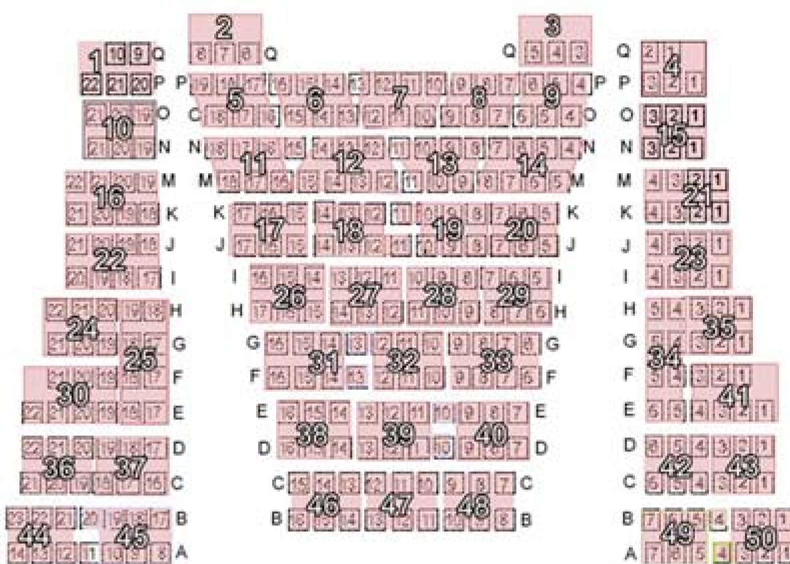

Figure 1—Seating Chart Showing Organization and Location of Student Groups

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, as they are commonly called, are wireless transmitters used by students to instantly, accurately, and anonymously answer questions posed by the instructor. Students work in groups to tackle the multiple-choice "clicker questions" associated with the 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 teaching technique allows me to encourage the re-description that promotes learning as well as uncover sources of confusion and misconception.

Case Management

Overview

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 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 the following two pre-assessment clicker questions on the structure of DNA from their pre-class reading:

1. DNA is composed of nucleotides that are joined together by covalent bonds between which two portions of each nucleotide?
    a. between deoxyribose and a phosphate group
    b. between two deoxyribose groups
    c. between the nitrogen-containing rings
    d. between a phosphate group and the nitrogen-containing ring
    e. between the phosphate groups of both nucleotides
2. In the hydrogen bonds between nitrogen-containing bases ____.
    a. A always pairs with C
    b. A always pairs with G
    c. C always pairs with T
    d. G always pairs with T
    e. G always pairs with C

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"

I begin 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/or problems in the mineralization of the enamel, so that their enamel may remain softer than normal. 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 6 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 on the handout. 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 showing animations on the Internet from the Dolan DNA Learning Center (see References) or using PowerPoint presentations that can be downloaded from the National Institute for Science & Technology's website (see Kline, Redman, and Butler 2001). To determine if they understand the idea of the primers, I ask them to answer clicker question 4. After 5 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, then 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.

The sequence from the intron 1 of the AMELY gene reads:

3′G GGACCCGAGA CATTTCTTAT CACCCACCTA AGAAGTAGGG TTTATTTCAC CAAAGAGTTC ACCAGGGTTA
5′C CCTGGGCTCT GTAAAGAATA GTGGGTGGAT TCTtcatCCC AAATAAAGTG GTTTCTCAAG TGGTCCCAAT

3′TTTACAGTTC CTACCATCAG CTTCCCAGTT TAAGCTCTGA T-5′
5′AAATGTCAAG GATGGTAGTC GAAGGGTCAA ATTCGAGACT A-3′

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

3′G GGACCCGAGA CATTTCTTAT CACACAACTA AGAAATAGGG TCT-----AC -AAAGAGTTC ACCAGGACTA
5′C CCTGGGCTCT GTAAAGAATA GTGTGTTGAT TCTTTATCCC AGA-----TG -TTTCTCAAG TGGTCCTGAT

3′TTTACAGTTC CTACCACCAG CTTCCCAGTT TAAGCTCTGA T5′
5′AAATGTCAAG GATGGTGGTC GAAGGGTCAA 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′-CAGCTTCCCAGTTTAAGCTCTGAT-5′ (backwards 5′-TAGTCTCGAATTTGACCCTTCGAC-3′)

After explaining this to the 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 from the Dolan DNA Learning Center (see References).

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, STRs). For example, a person could have 5 copies of one of these STRs, the THO1 sequence (TCAT), found on chromosome 11 on one homologue (a 5-repeat) and 6 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 positive-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 line-up 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, with 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 5-repeat, but 1 out of 4 chromosomes has a 6-repeat. 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 × 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, say if 38% of the population has type O positive blood, but it is far from ideal. 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 × 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 × 1/800 = 1/12,800. Thus, 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 use 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. 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 labeled mixture of DNA fragments are 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 the 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 which can be separated by gel electrophoresis (what they are seeing in the graphic from Part III). I then ask them 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.

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 one in five million and finally to one 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.

Answer Key

Answers to the questions posed in the case study are provided in a separate answer key to the case. Those answers are password-protected. To access the answers for this case, go to the key. You will be prompted for a username and password. If you have not yet registered with us, you can see whether you are eligible for an account by reviewing our password policy and then apply online or write to answerkey@sciencecases.org.

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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 AmpFlSTR® Green multiplex PCR kit from Applied Biosystems.

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