The popularity of crime scene dramas provides an opportunity for educators to engage students in science. Solving mock crimes helps students develop critical thinking skills and reinforces the importance of the scientific method. The expertise required in forensic science is extremely broad and includes mathematics and statistics, physics, chemistry, earth science, and biology (Funkhouser & Deslich, 2000). Numerous excellent reports for mock crime scene investigations integrated into general science units are available (Johnson, 1997; Hurley, 1995; Hein, 2003). Projects specific to biology include a forensic entomology simulation (Carloye, 2003) and several that outline methods for Restriction Fragment Length Polymorphism (RFLP) as the basis for genetic identification (Guilfoile & Plum, 1998; Pallandino & Cosentino, 2001; Reed, 2001). Since the O.J. Simpson trial, the techniques for DNA fingerprinting no longer rely on RFLP analysis, which requires a relatively large amount of non-degraded DNA for success. The standard developed by the FBI is now a PCR-based, fluorescently-labeled amplification of microsatellite markers, followed by capillary electrophoresis. Although this technique requires specialized instrumentation and expensive reagents, it is an excellent method to teach students Mendelian genetics and genome organization. We describe a method to introduce students to the state-of-the-art genetic profiling technique by forming partnerships with high school teachers, forensic science centers, and universities.

Many biology instructors include biotechnology principles and applications within the greater topic of molecular genetics. However, the sophistication of biotechnology education programs varies, as do the objectives, resources, and opportunities of instructors and institutions. Secondary biology courses introduce students to the concepts of restriction enzyme digestion, gel electrophoresis, DNA fingerprinting, and genetic engineering. The activities at this level are primarily designed for exposure and can take the form of either simulation-based dry labs or actual wet labs. Advanced Placement biology and college level biology courses emphasize gel electrophoresis, recombinant DNA technology, the gene libraries, PCR (polymerase chain reaction), determination of Alu insertions (Bloom et al., 1996b), and sometimes mitochondrial gene sequencing in expanded coverage of genetics and biotechnology. Students learn the science behind the concepts through the use of specialized kits designed for educators.

The latest method of DNA profiling relies on a form of microsatellite DNA called small tandem repeats (STRs). The technology is appropriate for biology students at the advanced secondary or introductory college level, and for teachers having prior experience with biotechnology. We have conducted a DNA profiling unit as a one-week summer mini-course, however the activities can be arranged to complement a genetics or biotechnology unit within a regular semester. Students genotype themselves first to learn the technique, then recover DNA from saliva left at a fictitious crime scene and from suspects for comparison. After a simple DNA isolation technique, they use PCR to amplify target DNA fragments, followed by capillary electrophoretic separation of the fragments and their identification with specific fluorescently-labeled probes. This lab is novel in that students work with the analytical reagents and instruments themselves made possible by partnerships with local college research labs, forensic science labs, biotechnology companies, and granting agencies. In New Mexico, we have established this lab through collaborative efforts between secondary schools, the New Mexico Institute of Mining and Technology in Socorro, and the Metropolitan Forensic Science Center in Albuquerque. The purpose of this article is to share our experiences in developing a forensic genotyping lab. We will review information on DNA profiling and the related biotechnology; explain how to obtain the necessary reagents and equipment; offer suggestions for establishing partnerships with local universities, crime labs, or biotechnology firms; and discuss appropriate forensic scenarios and course content. Furthermore, we will provide ideas for alternative activities, cover safety considerations and personal privacy issues.

DNA Profiling

The objective of DNA profiling is to determine the genotype of a person at several highly variable sites in the genome. Its value lies in the fact that it is based on genotype, not phenotype. A DNA profile, or genetic fingerprint, can be obtained from saliva left on a stamp, cigarette butt, or even on the mouthpiece of a telephone (Silverstein, 1996; Wickenheiser, 2002). Forensic analysts make a profile of tandem repeats of nucleotides found in small sections of DNA that are scattered across the chromosomes. Some regions of non-coding DNA sequences are highly polymorphic, so they vary from person to person in terms of the length of the repeated sequence and the number of times the sequence is repeated. STRs are an example of Variable Number Tandem Repeats (VNTR) and represent stretches of DNA containing tandemly-repeated nucleotide sequences in which the repeat unit is at least two bases but no more than seven in length. Many STRs are a four-base repeat unit, or tetranucleotide sequence, but some
are more complex (Table 1). STRs occur at various loci, or positions on a chromosome (Figure 1). A specific STR is characterized by the sequence of its repeat unit and the number of times that unit is reiterated. Located on Chromosome 5, the Locus D5S818 is an example of a tetrancleotide-repeat polymorphism that has at least 10 alleles as a result of variation in the number of the (AGAT) repeats. Alleles of D5S818 contain an STR having as few as seven repeats and as many as 16 repeats, thus the alleles can be resolved from one another based on size (Figure 2).

Inheritance of STRs follows basic Mendelian patterns. The individual shown in Figure 2 inherited a different allele from each parent and is therefore heterozygous. Thirty or more different alleles at some STR loci have been identified. This large number of different alleles means that each may be relatively rare in a population, with allele frequencies usually only 1% to 5% (0.01-0.05). Thus the probability of any two individuals, except identical twins, having exactly the same alleles at each STR is extremely low. For example, if four STR loci are screened and each allele at each locus occurred with a frequency of 1% (0.01) in a population, the odds of a chance match between any two random samples showing the same genotype would be one in ten million (0.01 x 0.01 x 0.01 x 0.01) (Lindahl & Johnson, 1995). Hence, forensic genotyping is highly discriminatory. Assuming no new mutations occur, an individual can be excluded as a suspect with absolute certainty on the basis of one allele mismatch. A positive identification is based on the unlikely probability that agreement in allele configuration is due to chance alone.

Biotechnology

This lab requires students to extract and isolate DNA, amplify target DNA fragments using PCR, then separate the fragments using capillary electrophoresis. The source of DNA can be from buccal cells that are naturally shed in saliva.
PCR

Polymerase chain reaction, or PCR, is based on the self-replicating properties of DNA and is used to produce multiple copies of a desired DNA fragment. PCR reactions are dependent upon cycling through several temperature steps that trigger the denaturing of the double helix, the annealing of primers to target DNA sequences, and an extension of new strands with deoxynucleotides. The result is a new identical copy of the desired DNA fragment as shown in Figure 3. The process is repeated until DNA sufficient for analysis has been produced. The enzyme DNA polymerase is required for the process. We use the thermostable AmpliTaq Gold™ DNA polymerase that is isolated from Thermus aquaticus bacteria that live in hot springs in Yellowstone National Park.

Capillary Electrophoresis

Separation of the DNA fragments for analysis occurs by capillary electrophoresis using the Applied Biosystem Inc (ABI, Foster City, CA) Prism® 310. As with gel-based separation technologies, fragments move through a matrix according to size, the smallest moving the fastest. In capillary electrophoresis, an acrylamide polymer acts as the matrix and functions as a sieve. DNA fragments separate as they move through the capillary, then pass a laser detector where alleles are detected and assessed based on size and quantity. STR analysis can be done on gel-based instruments, such as the Prism 377, but the forensic STR is optimized for capillary instruments such as the Prism 310.

Detection of the STR Fragments

Half of the primers used in PCR are labeled with a dye that fluoresces when it interacts with light from a laser. Some loci have alleles that overlap in size; they can be distinguished by using the locus-specific primers labeled with different color dyes. For example, the D21S11 green gene and D13S317 yellow gene overlap in their allelic size ranges at 189-243 base pairs and 206-234 base pairs, respectively, yet appear differently on the data printouts as green and black peaks (Figure 4). The black peaks on the printout represent the yellow genes but with better background contrast. The dye gives off a characteristic wavelength that corresponds to a specific color. This information is detected by the ABI PRISM® 310 and expressed as colored peaks.

Genotyping of the STR Fragments

An internal lane size standard is loaded with each sample to allow for automatic sizing of the alleles and to normalize differences in electrophoretic mobility between injections. The standard is represented by red peaks on the data printout. This provides an advantage over gel electrophoresis, as the size markers are run in every lane making sizing extremely accurate. Next, samples are loaded into the genetic analyzer, which are automatically injected, electrophoresed, and analyzed. Each student group prepares its personal DNA samples and the crime scene samples, and enters information for its samples into the database collection program. The GeneScan® software then automatically analyzes the data, which we import to Genotyper® software that automatically identifies the alleles. Students report enjoying this aspect of the genotyping process, as it allows for hands-on experience with the genetic analyzer and software. This confirms the importance of involving the students in the actual sample analysis as opposed to analysis by a remote facility. Although the software can generate a table of results, this task is done manually by the students using the form shown in Table 2. This reinforces basic genetic principles of heterozygosity and homozygosity.
Table 2. Gender and Genotype Form.

AmpFLSTR® Profiler STR Loci With Fluorescent Label.

<table>
<thead>
<tr>
<th>ID</th>
<th>AMEL Gender</th>
<th>D3S1358 Blue Gene</th>
<th>FGA Blue Gene</th>
<th>D8S117 Green Gene</th>
<th>D21S11 Blue Gene</th>
<th>D18S51 Green Gene</th>
<th>D5S818 Yellow Gene</th>
<th>D13S317 Green Gene</th>
<th>D7S820 Yellow Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sus</td>
<td>XX He</td>
<td>14/16</td>
<td>21/22</td>
<td>He/11/13</td>
<td>He/28/33.2</td>
<td>He/13/14</td>
<td>He/11/12</td>
<td>He/9/11</td>
<td>He/10/11</td>
</tr>
<tr>
<td>Vic</td>
<td>XY He?/18 OL?</td>
<td>21/23</td>
<td>He/8/14</td>
<td>He/28/31.3</td>
<td>He/13/16</td>
<td>Ho/11</td>
<td>Ho/11</td>
<td>Ho/13</td>
<td>Ho/12</td>
</tr>
</tbody>
</table>

1. Students assign unique 3-digit identifiers (ID) to each sample.
2. The genotype is either He for Heterozygote or Ho for Homozygote.
3. The alleles (number of repeats) are entered.

Equipment & Reagents

To extract DNA from buccal cells, sterile cotton swabs, a boiling water bath, and a micro-centrifuge are needed. The cell lysate obtained is crude and contains heavy metal ions that can interfere with PCR amplification by either inhibiting DNA polymerase or by acting as cofactors for nuclease that degrade DNA. Therefore, the buccal cell extract must be treated with Chelex® 100 (BioRad, Hercules, CA), a negatively-charged resin that binds the positive metal ions (Bloom et al., 1996b). The DNA is then amplified by PCR to insure a sufficient quantity for the analysis. A thermal cycler that automatically goes through a series of heating and cooling cycles is required. Mineral oil may be required as an overlay to prevent evaporation from the PCR tubes depending upon the type of thermal cycler used. The ABI PCR-based typing kit, AmpFLSTR (Amplification of Fluorescent STRs) ProfilerPlus™ (P/N 4303326) is one of several kits available for forensic genotyping. We have also used Cofiler™ and Profiler™ kits, and each works with equal effectiveness. The kit contains all of the reagents necessary for the amplification of 100 samples in a thermal cycler as well as the Allelic Ladder required for genotyping on a genetic analyzer. The Profiler Plus™ kit probes nine different STR loci and the gender indicator amelogenin (Table 1). Amelogenin is not a STR, but a gene displaying a 107 base X-specific band and a 113 base Y-specific band, thus it is used to identify the gender of an individual. The nine STRs examined in this lab are a subset of 13 STR markers recognized by the Combined DNA Index System (CODIS) database that enables crime labs to exchange and compare DNA profiles electronically, thereby linking crimes to each other and to convicted offenders. Once amplified, a red fluorescent marker used for the internal size standard (ABI GeneScan®500 ROX) is added and the DNA is denatured with heat and deionized formamide. The samples are then electrophoresed. The expense of the genetic analyzer and some of the reagents is prohibitive for high school budgets, so this requires the establishment of partnerships with forensic science laboratories and universities.

Partnerships

In New Mexico, secondary schools are fortunate to have a partnership with the New Mexico Institute of Mining and Technology in Socorro, where students experience the ABI® 310 Genetic Analyzer firsthand. Such programs are possible despite location or population size or limited budget. Our program evolved through collaboration between a secondary biology course within a public high school that is one of two in a city of 75,000, and the state science and technology college in a town of 9,000. We coordinated with the state’s largest crime lab, which is a one-and-a-half hour drive from each school, and with out-of-state biotech companies via phone/electronic communication. At present, an educational kit for forensic genotyping does not exist. Professional forensic genotyping kits are available but too costly for school budgets. However, we have obtained good results using expired reagents donated by the Metropolitan Forensic Science Center in Albuquerque. Also, we were given several complimentary kits from corporations after explaining our program. In addition, we have interviewed forensic science labs around the country and have discovered that many are willing to donate either expired reagents or a quantity sufficient to amplify a class set.

Amplified PCR products can be sent to a private lab for capillary electrophoresis. Some labs may run student samples for free or at a reduced cost if the polymer is provided. Ideally, if a partnership can be established, students can personally participate in the sample analysis. Local universities may have a Prism 310 Genetic Analyzer and some have forensic science programs that include genotyping in their curricula. To do STR analysis, the instrument must have Genescan® and Genotyper® software. Since this is an option at purchase, it is an important question when locating appropriate equipment. At New Mexico Tech, genotyping is taught as part of the undergraduate genetics course. The use of this equipment for a high school mini-course and for science fair projects is considered an important method of recruitment by the administration and can also fulfill National Science Foundation requirements for outreach to K-12. If collaboration with one of these labs is possible, no equipment is required other than what is necessary to perform PCR.

The Scenario

Any number of situations that require DNA genotyping may be fabricated here. However, based on results from student evaluations, a forensic DNA profiling case that involves DNA recovery from several “suspects,” a “victim,” and saliva left at a crime scene was found to be the most interesting. Students preferred a scenario in which suspect DNA was recovered directly from an artifact belonging to the victim. A case like this will capture students’ attention and provide them with a good example of how DNA evidence can link a crime to a suspect, or exonerate potential suspects. The crime does not have to involve sensitive issues that are inappropriate for high school or introductory college level settings. Crime scene artifacts and
suspect buccal cell samples are prepared using donated saliva and are created ahead of time and referenced for identity. We have recovered DNA from saliva left on soda cans, cigarette butts, sunflower seeds, chewing gum, an apple, and pistachio shells. Genotypes of the volunteers who donate saliva are kept on file and are used if reactions fail. Students are teamed and informed that they are field investigators responsible for writing or sketching a detailed description of the crime scene, and for collecting evidence likely to contain saliva, or to have been in close association with the victim. To collect and store the evidence, students wear gloves and use new plastic bags.

For the summer mini-course, we make use of a third floor lounge with a balcony. An outline of the victim is drawn in chalk on the sidewalk below, the lounge is in disarray, and evidence such as soda bottles, cigarette butts, and sunflower seeds are scattered around the crime scene. The crime scene involves three sections: the lounge, the balcony, and the sidewalk below. Other types of biological or non-biological evidence can be included as part of a comprehensive forensic science unit.

Course Content

The mini-course is run over five days. On the first day, students learn about DNA structure and function, then isolate and amplify their own DNA. They perform a PCR reaction and agarose-gel base separation that involves a single gene with two alleles, such as the Alu insertion polymorphism (Bloom et al., 1996b). This provides them with practice pipetting small volumes. The next day, they discuss the results and perform the STR analysis using their DNA. The results are available on the third day, and this is when the crime scene is investigated. Students perform the same STR analysis on the crime scene samples. These are loaded onto the instrument on the fourth day, and while they are running, the students tour the Metropolitan Crime Lab. The results are available when they return, and the students solve the crime and present their findings to the class on the last day. This activity can be completed in three days by omitting the agarose-gel base PCR: two days consisting of regular lab periods set up for DNA extraction and amplification, and one day spent as a field trip to a local lab for capillary electrophoresis. Each student team is responsible for the preparation of specific crime scene samples. We follow the procedure for DNA extraction from oral swabs and saliva stains in the ProfilerPlus™ Users Manual with the following modifications:

- Instead of using Proteinase K, samples are boiled for 10 minutes in a boiling water bath, followed by a one minute ice bath.
- It is not necessary to quantitate the DNA. We have found that a simple 1 to 4 dilution of the student and suspect DNA prior to amplification is generally sufficient.
- Do not dilute the crime scene DNA.

Results & Discussion

The Genotyper® data is usually straightforward and easy to read, and after an initial explanation, students report being able to interpret the profiles without assistance. DNA profiles obtained from a suspect and victim are shown in Figures 9 and 10. Students begin analysis of the DNA profiles by recording the gender and genotype of each individual sample in a chart (Table 2), then compare and contrast suspect and crime scene profiles.

In our example, the DNA profile of a certain suspect matches the profile of the saliva stain recovered from a certain crime scene artifact. This places the suspect at the scene or around the time the crime took place. Even though the probability of a random match between two samples using AmpF STR® ProfilerPlus™ kit is 1 in 82 billion, a suspect cannot be convicted based on this evidence alone. DNA profiling constitutes only part of a body of evidence that is built against a suspected offender. Exclusion of suspects by DNA evidence is actually simpler.

Safety Considerations

Sampling of saliva is noninvasive and is acceptable for college level courses, though regulations may vary in secondary institutions. Students are required to wear gloves throughout the procedure to reduce the possibility of biological or chemical...
cross contamination. The samples that are prepared for electrophoresis contain formamide. Though animal studies have shown formamide to be a teratogen, student exposure can be minimized if the 0.5 mL sample tubes are preloaded with formamide prior to student contact. The tubes should be stored in appropriate size racks, and double-wrapped with plastic to avoid contamination during storage or in transit to the analytical lab. It is advisable to inform students of the nature of the chemical on the chance that a participating student may be pregnant. The samples and any volumes of undiluted formamide should be consigned as “hazardous waste” and disposed of accordingly.

**Personal Privacy Issues**

We encourage students to genotype themselves as part of the exercise for two reasons. First, it personalizes the learning experience and engages their interest. Second, it adds authenticity to the process by mimicking the state and federal forensic analytical lab policy of genotyping the field investigator and keeping the profiles on file in the event of accidental background contamination. The alleles from the nine STR loci examined in this lab are not known to be associated with any genetic disease or human phenotype. Variation among individuals in these regions is not an indicator of health or genetic fitness. The gender indicator amelogenin can be eliminated from the printouts if there is concern about revealing a genotype that is inconsistent with phenotype, as in the rare case of androgen insensitivity syndrome (XY female). We explain to the students that the results of this lab are for teaching purposes only, and will not be used for diagnostic or identification purposes. The student’s name is not linked to his or her genotype and confidentiality is maintained by the use of anonymous personal identifiers that are known only by the individual student. However, the alleles are inherited in a Mendelian fashion and information about family relationships could be revealed, thus genotyping relatives in the same class should be avoided. All underage students and their parents should sign an Informed Consent Release form.

**Alternatives**

Many San Francisco Bay Area, Massachusetts, and other area teachers participate in the mitochondrial gene sequencing lab that is fully supported by Applied Biosystems in collaboration with BABEC (Bay Area Biotechnology Educational Consortium) and Cold Springs Harbor Lab. This program uses...
similar technology and is certainly worth investigating by those interested in incorporating biotechnology lab into their curriculum. Applied Biosystems presently provides analytical and reagent support for a forensic genotyping lab in the Bay Area only. However, in progress is a proposal to expand the program to include other regions. A kit for an agarose-gel based VNTR experiment is available (Bloom et al., 1996a). Otherwise, students can be exposed to the science of forensic genotyping through simulations (Kreuzer & Massey, 1996). Some DNA testing laboratories are willing to provide anonymous samples for nondiagnostic educational purposes and provide educators with the appropriate data to complete published exercises (Wray et al., 2001). The National Institute of Standards and Technology produces an excellent set of training slides on STR typing. The Cold Springs Harbor DNA Learning Center provides related bioinformatics activities through its Allele Server program. The Cornell Institute for Biology Teachers produces a collection of DNA profiling lab exercises and simulations that can be purchased from West Hill Biological. Also, we are willing to provide copies of our Forensic STR Genotyping Simulation activity upon request.

Conclusion

This activity requires that students interface with trained professionals and state-of-the-art technology. Our observations have been that this interaction works to engage student interest and to stimulate thinking. Following the exercise, the students are provided an opportunity to reflect upon their experience through a tour of a forensic science lab where they meet forensic scientists and STR analysts. We have formally assessed the value of the lab and field trip as a learning experience in the form of a questionnaire. In general, students feel that the forensic genotyping activities helped to increase their awareness of DNA science and its applications, and they rank the lab very high in terms of being a worthwhile experience. More importantly, they gain experience and knowledge of the genetics and biotechnology behind the popular field of forensic DNA science.

Acknowledgments

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