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Laboratory Exercises

DNA Fingerprint Analysis of Three Short Tandem Repeat (STR) Loci for Biochemistry and Forensic Science Laboratory Courses[S]

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We have devised and implemented a DNA fingerprinting module for an upper division undergraduate laboratory based on the amplification and analysis of three of the 13 short tandem repeat loci that are required by the Federal Bureau of Investigation Combined DNA Index System (FBI CODIS) data base. Students first collect human epithelial (cheek) cells using sterile buccal swabs and then utilize commercially available kits and materials to extract genomic DNA. This is followed by the PCR amplification of three specific short tandem repeat loci (*i.e.* CSF1PO, TPOX, THO1). Polyacrylamide gel electrophoresis is used to resolve the allelic bands associated with the three short tandem repeat loci, and the results are statistically analyzed in the context of human population genetics. In addition, DNA was collected from a family, and the children's allele sets were compared with those of the parents to help illustrate paternal and maternal relatedness. This module enables students to use the materials and methods employed by actual law enforcement agencies and therefore can be used for laboratory exercises in traditional biochemistry curricula as well as for the growing field of forensic science and education.

Keywords: DNA fingerprinting, forensic science, genomic DNA, PCR, short tandem repeat (STR) loci, polyacrylamide gel electrophoresis (PAGE), silver staining.

DNA fingerprinting was introduced to forensic science in the 1980s and has since proved to be one of the most accurate methods of distinguishing the guilty from the innocent in many criminal cases [1]. It has been used to clear wrongly accused and incarcerated individuals and has provided virtually irrefutable evidence in the conviction of many criminals. In addition, this technique has also been utilized extensively in establishing paternal relationships in many civil cases. The method is very precise and can work on minute trace quantities of DNA extracted from blood, semen, urine, hair, and various body cells and fluids (*e.g.* epithelial cheek cells and saliva). Such evidence found at crime scenes often becomes key evidence in court cases involving murders and rapes, and this type of evidence can also be involved in child support disputes. Due to the increasing role DNA fingerprinting plays in many areas of society, it is important for students to be exposed to the chemical principles of this technique and to learn the skills necessary to perform accurate and rigorous DNA analysis.

The main motivation for this project was to develop a DNA fingerprinting module for an upper division undergraduate biochemistry laboratory course. The learning objective is to teach students the biochemical principles at the root of DNA fingerprinting analysis and to provide a greater appreciation and understanding of its benefits and extensive applications in today's criminal justice system. A secondary learning objective was to teach students the importance of the proper and careful laboratory techniques that are essential for successful application of this method and for the correct statistical analysis of the generated data. We also believe that the actual materials, protocols, and standards utilized by laboratories employed by law enforcement agencies have matured to the point where they can be investigated and applied in upper division undergraduate laboratory course work.

Our approach builds on previously reported laboratory exercises in that many of the protocols can be completed with commercially available kits [2–6]. With the methods reported here, three of the 13 short tandem repeat (STR)[1] loci that are required by the FBI CODIS (Combined DNA Index System) data base were amplified, analyzed, and resolved via polyacrylamide gel electrophoresis and standard silver staining protocols. STRs are length polymorphisms (*i.e.* the DNA molecules differ in length rather than sequence) that contain repeating units of between 3 and 7 bp [7]. The most useful of these STRs for genetic

[S] The on-line version of this article (available at http://www.bambed.org) contains additional details concerning the protocols described as well as a supplemental figure.

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[1] The abbreviations used are: STR, short tandem repeat; FBI CODIS, Federal Bureau of Investigation Combined DNA Index System.

This paper is available on line at http://www.bambed.org

identity determinations vary between 5 and 21 repeats depending upon the locus. In the United States, a set of 13 core loci have been standardized, chosen because the alleles are well distributed among the population, and when amplified with specific primers, the products range from 150 to 350 bp [8, 9]. Allele frequency tables for each locus (there are up to two at each site, barring mutations and contamination) are used to determine the genotypic frequency of an individual's alleles at each locus, and the frequencies for each locus are multiplied to determine the probability that a given pattern will match that of a particular individual [1]. A more detailed description of the statistics and probabilities used in forensics analysis can be found in the Supplemental Materials (Section C). In a forensic laboratory, a set of alleles found or linked to a crime scene may be submitted to the CODIS data base, which contains genetic profiles of convicted offenders, suspects, and casework samples where the DNA did not match a victim or suspect [10]. The data base can be searched to determine whether crimes may be linked followed by reanalysis of samples to confirm matching profiles.

Our students utilized the methods and procedures (on a subset of loci) that are used by criminal justice laboratories such as the FBI. Specifically, this laboratory exercise teaches students how to use sterile methods to isolate human epithelial cheek cells, extract genomic DNA from the cells, amplify the allele sets at three STR loci (*i.e.* CSF1PO, TPOX, THO1), perform agarose and polyacrylamide gel electrophoresis, and silver-stain the gel. Students were instructed on how to statistically analyze the results in the context of human population genetics and how to calculate the probability that their DNA would match a sample obtained from a theoretical crime scene.

An additional test of these methodologies entailed the collection of genomic DNA samples from a family in New York. After shipping the samples cross-country, the subsequent analysis of the three amplified STR loci provided direct experimental evidence of the genetic paternal and maternal relatedness of each family member.

EXPERIMENTAL PROCEDURES

Introductory Materials

The laboratory takes a minimum of three full laboratory periods to accomplish, with two lectures before the first laboratory period and additional brief lectures interspersed between laboratory activities. Due to the complexity of the steps, the laboratory may be stretched to accommodate 4–5 laboratory periods (especially in larger laboratories, to accommodate the greater number of students using equipment). The two lectures cover basic theory and application of the following topics: gel electrophoresis using agarose and acrylamide (both native and denaturing gels); the theory of PCR, STRs, and multiplexing; isolation of DNA using the Promega DNA-IQ kit; the analysis of samples; avoidance of contamination of samples during the collection of evidence; the 13 loci of the CODIS system; silver staining of acrylamide gels; and the determination of genetic identity. The proper background for statistical analysis of the data (assuming Hardy-Weinberg equilibrium) was described to the students with appropriate reference to literature sources.

To successfully complete this protocol, students must be able to accurately pipette specific volumes, have background information on the structure/charge of DNA, understand the process of PCR, and have some experience with gel electrophoresis. Proficiency in these methods and appropriate knowledge of the above concepts are necessary, and therefore it is suggested that these modules be reserved for upper division biology, biochemistry, and forensics laboratory courses. In addition, laboratory assistants are especially encouraged to provide appropriate guidance when working with the relatively large polyacrylamide gels.

Materials

The following materials are necessary for the full analysis of the three described STR loci: latex (or neoprene) gloves, laboratory coats, Pipetman (model numbers p2, p20, p200, and p1000), sterile pipette tips, buccal swabs (Puritan sterile foam-tipped applicators, catalog number 25-1506 1PF), 1.5 ml, and PCR microcentrifuge tubes, PCR thermocycler, gel electrophoresis power unit, glass plates/clamps/tape/spacers for large polyacrylamide gel (31.0 cm $\times$ 38.5 cm $\times$ 0.4 mm), Promega DNA IQ^{TM} System (catalog number DC6701), Promega MagneSphere® technology magnetic separation stand, two-position (catalog number Z5332), Promega Multiplex for the following human STRs: CSF1PO, TPOX, TH01 (catalog number DC6001); and Promega DNA silver staining system (catalog number Q4132). In addition, materials necessary for each specific step are listed in the Supplemental Materials section.

Methods

Notes for Students—All safety precautions must be strictly adhered to for the safety of the students and the instructors. In addition, it is essential to work under sterile conditions when appropriate and that all sample precautions are strictly adhered to so as to prevent external contamination and/or cross-contamination. Students should, at all times, wear eye goggles and latex or neoprene gloves to avoid contamination of samples with DNA from fingers and to avoid exposing skin to potentially toxic chemicals and reagents. Use appropriate heat protection and additional eye protection (*e.g.* a full-face plastic shield) when handling hot agarose. Students must wear eye protection that is rated to completely block UV radiation when visualizing ethidium-stained agarose gels (a UV rated full-face plastic shield is strongly recommended).

Potential Hazards—Acrylamide in the powder and liquid form is a neurotoxin and must be handled accordingly. An airtight facemask must be worn when weighing out acrylamide (it is recommended that either the instructor or the teaching assistants handle the powder form of acrylamide). Ethidium bromide is a suspected carcinogen and should also be handled accordingly (*i.e.* carefully and only when wearing gloves).

RESULTS

Prior to implementing this laboratory module in an upper division biochemistry laboratory, we wanted to ensure its feasibility, and therefore we performed two trial fingerprinting runs on the three STR loci used exclusively in this project (*i.e.* CSF1PO, TPOX, THO1). The objective of performing the trial runs was to ensure that the module could be completed in a timely manner and that all associated technical aspects were not too demanding for an undergraduate laboratory. The two trial runs were followed by an actual application in an upper division biochemistry laboratory course taught here at San Diego State University (SDSU) (CHEM567). The two trial runs (with appropriate modifications to protocols) and the actual application proved to be highly successful in achieving our learning objectives. All modifications to the protocols listed in the Promega technical bulletins are described in detail in the Supplemental Materials section.

The first trial run entailed the initial implementation of the DNA fingerprinting and took place in the laboratory of Dr.

TABLE I
Allelic numbering for each individual's banding pattern at each loci

The band numbers for each allele for each individual at the three loci are listed in the respective column headed with the loci label. The ethnic group used for allele frequencies (included in the Promega manual) are listed in the fifth column. The sixth column contains the numerical likelihood that that an individual would randomly match the particular combination of alleles of a sample left at a potential crime scene.

Column no.	CSF1PO	TPOX	THO1	Promega table used	1/( )[a]
1	10, 11	9, 10	8, 9	African-American	3074.11
2	10, 11	10, 11	8, 9.3	African-American	3966.10
3	11, 12	8	9, 10	Caucasian-American	11347.44
4	12	8, 11	6, 10	Caucasian-American	12919.38
5	11, 13	8, 11	7, 10	Caucasian-American	60759.79
6	9, 12	8, 11	6, 9	Caucasian-American	2083.77
7	11	11	9.3	Hispanic-American	3904.81
8	12	8, 12	6, 7	Hispanic-American	515.84
9	10	8, 11	6	Caucasian-American	942.27
10	10, 13	8, 11	9	Caucasian-American	4607.86
11	11, 13	8	6, 7	Caucasian-American	1378.96
12	12	8, 11	6, 9.3	Caucasian-American	195.16
13	11, 12	8	7, 9	Caucasian-American	383.36
14	11, 12	8	9.3	Caucasian-American	160.54
15	10, 12	8, 12	9.3	Caucasian-American	1410.14
16	12	8, 11	7	Caucasian-American	1397.87
17	9, 10	8, 9	9.3	K562 control	5610.21

[a] The chance is 1/(calculated value) that an individual would randomly match the particular combination of alleles of a sample left at a potential crime scene.

Love at SDSU. Sterile buccal swabs were used to collect epithelial cells from 16 individuals whose genomes were subsequently DNA-fingerprinted for the three STR loci. The 16 individuals were chosen in an attempt to sample a wide ethnic diversity, and the different races included: African-American, Asian (Chinese, Taiwanese, Filipino, Middle Eastern, Indian), and Caucasian. Of the 16 individuals sampled, none had identical allelic banding patterns across all three loci, and all were analyzed using allelic frequencies (provided in Promega Technical Manual Number D004) and standard statistical analysis. The results are listed in Table I.

The second trial run entailed the collection of epithelial cells from a family in New York (father, mother, and two daughters) and subsequent DNA fingerprinting of the collected genomes in Dr. Love's laboratory in California. The main objective of the second trial run was to examine the feasibility of offsite (nationwide) collection of DNA samples with analysis performed at SDSU and also to determine the feasibility of exploring familial relatedness by analyzing only three STR loci. The initial step of this trial (the collection of epithelial cheek cells) had to be repeated as the first collection, which was stored at 4 °C for ~1 month, did not produce viable PCR product. We hypothesize that bacteria or fungi endemic to the human mouth may have degraded the genomic DNA of the test individuals during the month-long storage at 4 °C. To obtain viable genomic DNA, epithelial cells were again collected in New York, sent to the Love Laboratory overnight via FedEx, and directly analyzed upon receipt. The fresh epithelial cheek cells and associated genomic DNA proved to be as viable as those collected directly at SDSU and ultimately gave rise to viable PCR product that was analyzed via PAGE (see Fig. 2).

The genetic (paternal and maternal) relatedness of the two daughters to each parent is readily apparent upon viewing the resulting band patterns listed in Table II for each of the three loci. It is clear that since the father is the only parent with a CSF1PO allele that had 13 STR repeats, the older daughter inherited that allele from him, and therefore the allele with 12 repeats must have originated from the mother. Conversely, the younger daughter inherited her CSF1PO allele with 11 repeats from her mother, and therefore she inherited her father's other allele (the allele with 12 STR repeats). The TPOX loci analysis is more straightforward as the mother has only one allele at that position (allele with eight repeats), and therefore it is obvious that the father donated his 11 repeat allele to the older daughter and his 10 repeat allele to the younger daughter. Finally, for the THO1 loci, the mother only has one allele (the 9 repeat allele), and the father split his alleles between his daughters: the 6 repeat allele to the older daughter and the 9.3 repeat allele to the younger daughter. This comparison provides direct evidence that analyzing just three loci is sufficient to demonstrate familial relatedness.

TABLE II
Allelic numbering for each family member and the student control at each locus

The band numbers for each allele for each individual at the three loci are listed in the respective column headed with the loci label.

Column no.	Individual	CSF1PO	TPOX	THO1
1	Positive control	9, 10	8, 9	9.3
2	Student control 1	10, 12	9, 11	7, 9
3	Older daughter	12, 13	8, 11	6, 9
4	Father	12, 13	10, 11	6, 9.3
5	Mother	11, 12	8	9
6	Younger daughter	11, 12	8, 10	9, 9.3
7	Uncle	11, 12	8	7, 9
8	Student control 2	12	8, 11	6
9	Unknown	12, 13	10, 11	6, 9.3
10	Student control 3	11, 12	8, 12	7, 9.3

In addition to exploring the feasibility of offsite DNA collection, we wanted to explore the possibility of simulating the pattern recognition inherent to the analysis of crime scene DNA (*i.e.* an unknown suspect). Therefore we in-

structed the family to provide buccal swabs for all family members and to repeat for one individual without indicating their identity. As can be readily observed in Fig. 2, the unknown suspect was beyond doubt (at least in the case of this particular family) the father as his banding pattern and the unknown's are 100% identical and unique relative to all individuals analyzed.

Having successfully met the objectives for the two DNA fingerprinting trials described above, we implemented the module in an upper division biochemistry laboratory that had a total of 18 students, most of whom were juniors and seniors and chemistry majors. Although the entire protocol is relatively lengthy, undergraduates were able to complete the protocol and properly analyze the resulting data in three laboratory periods. The first laboratory period entailed collection of the DNA samples, isolation of DNA, and PCR amplification of the three STR loci. During the second laboratory period, PCR products were visualized on an agarose gel (Fig. 1 in Supplemental Materials) followed by the preparation and pouring of six large polyacrylamide gels by groups of students. Since as many as ~30 samples can be run per gel, it is only necessary to run one large acrylamide gel, and therefore the best gel was selected for loading all student samples. During the third period, students loaded and ran the samples in the large gel and performed the silver staining procedure immediately afterward (data not shown).

For the statistical analysis, the students were instructed to use the formula $P = 2pq$ for heterozygous loci, where P is the genotype frequency at a locus, p is the allele frequency in the specific population of the first amplified set of STR repeats, and q is the frequency of the second set [1]. For homozygous loci, the formula $P = p^2$ was used. Alleles with a low frequency were given a minimum frequency of 0.002 to avoid inflating the probability due to the presence of a rare allele. To obtain the allele frequency used for p and q, the band numbers for each individual's alleles are first determined by comparing them to the CTT ladder bands on the acrylamide gel. For the CSF1PO loci, the smallest number of repeats is seven, and therefore the fastest migrating band (the lowest) in the CTT ladder is assigned the number seven. To obtain the allelic frequency, one looks up the population frequency for each band in the Promega-supplied allelic frequency table (Technical Manual Number D004). Following calculation of probability at each locus, the product rule was applied to calculate the probability of a match across all three loci [9, 11]. A detailed example calculation for individual number 1 from Fig. 1 is supplied on page 12 of the Supplemental Materials.

As part of the experimental discussion, students were reminded that the population must be assumed to be in Hardy-Weinberg equilibrium and were led to understand that the formula 2pq is derived from the fact that there are two ways to obtain the pq alleles: p from the father and q from the mother or the reverse. The frequency tables used by the students were provided by Promega, and students could choose from African-American, Caucasian-American, or Hispanic-American tables. Unfortunately, at the time, there were no comprehensive data sets of population frequencies available for Asians, and therefore we used the values from the Caucasian-American table for Asian students or controls. This did not present a large conceptual problem as the frequencies, across the three provided race categories, were relatively similar for corresponding alleles.

DISCUSSION

Overall, this project proved to be highly successful in establishing a biochemistry laboratory protocol for DNA fingerprinting at the undergraduate upper division level. The main learning objectives were achieved as the students were very successful at 1) isolating their genomic DNA from their epithelial cheek cells, 2) using PCR to amplify the allele sets at the target STR loci (*i.e.* CSF1PO, TPOX, THO1), 3) resolving the allele sets with polyacrylamide gel electrophoresis and silver staining, and 4) learning how to properly analyze the results in the context of human population genetics and how to calculate the probability that their DNA would match a sample obtained from a theoretical crime scene. Of particular noteworthiness, students commented that their motivation was increased knowing that they were performing the actual protocols used by law informant agencies and courts in determining guilt or innocence in criminal cases. In addition, the student's use of their own DNA in the protocol increased their interest by providing direct experimental evidence of the allelic nature of their genes.

Although we managed to incorporate all elements of the total procedure in three laboratory periods, it may be more convenient to spread the laboratory over 4–5 periods. Regarding the particular steps and protocols, we found that the collection of DNA samples via buccal swabs by the students was straightforward, as was the subsequent DNA isolation procedure using the Promega MagneSphere® technology magnetic separation stand and associated materials. PCR amplification of the three STR loci was also straightforward and provided the students with the opportunity to get hands-on experience with this important method that is utilized extensively in many areas of scientific research. The most challenging aspect of this protocol is the preparation of the glass plates and pouring of the large acrylamide gel for PAGE resolution of the STR bands. Prior instructor and teaching assistant expertise and experience will be needed for the students to be properly instructed in pouring, loading, and running of the gel. Preparation of the large gels may be accomplished at the end of the second period. Alternatively, it may be more realistic for the instructor and teaching assistants to prepare and pour the large acrylamide gel themselves prior to the third period as this is the most technically demanding step.

If the exercise is completed according to the protocol, the DNA fingerprints can be visualized quite clearly (Figs. 1 and 2). The polyacrylamide gel offers high resolution of the DNA fragments, and the silver staining procedure creates high contrast for direct visualization of the allelic bands. The alleles corresponding to the different STR loci are distinguishable relative to the CTT ladders, and the differing DNA fingerprint patterns are resolvable (Tables I and II). Determination of the numbering for most of the alleles, upon comparison with the ladders, was

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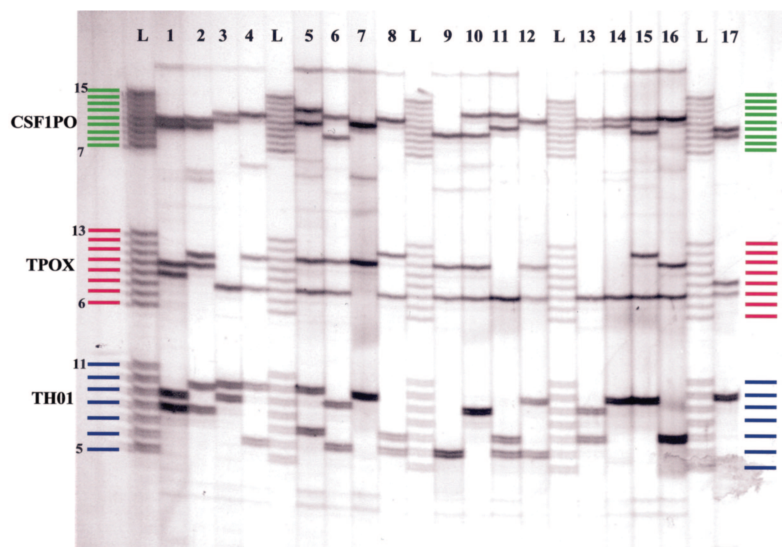

Fig. 1. **PAGE resolution of STR DNA from three loci: CSF1PO, TPOX, THO1.** The PCR amplification products corresponding to 16 individuals were resolved on a 6% polyacrylamide gel. The lane corresponding to each individual is numbered above, and the lanes with CTT allelic ladders are indicated with the letter *L*. In addition, the STR repeats of the allelic ladders are illustrated on the *left* and *right* by *colored bars*. The bars corresponding to the CSF1PO repeats (*topmost*) are colored green, those for TPOX (*middle*) are colored *pink*, and those for THO1 (*bottom*) are colored *blue*. The amplified alleles (*i.e.* STR repeats) from control K562 were run in *lane 17*. To obtain the STR repeat band numbers for each individual, the band patterns were compared with the CTT ladders.

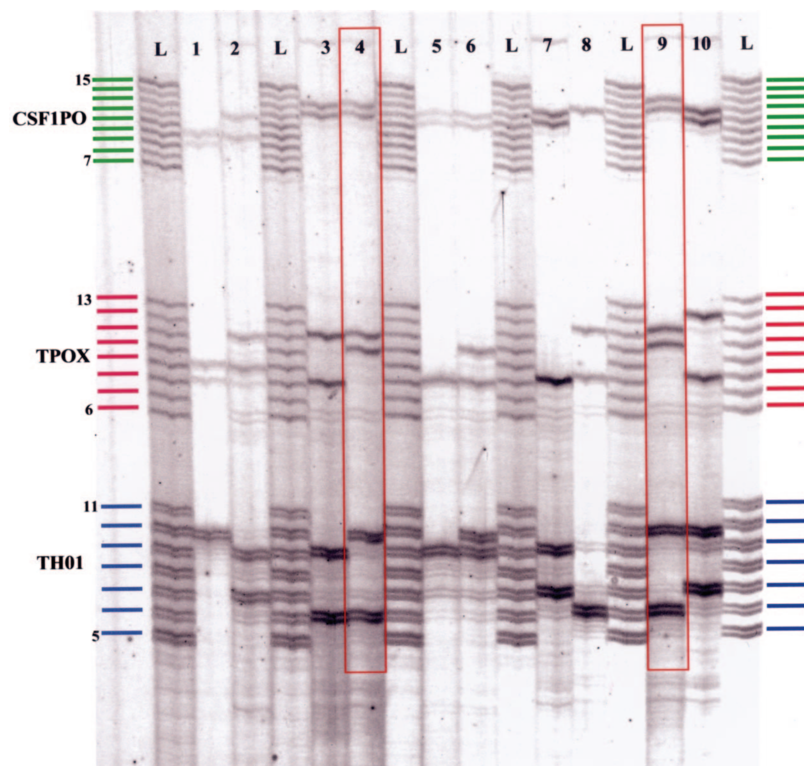

Fig. 2. **PAGE resolution of STR DNA from a family with one unknown sample.** This gel resolved the PCR amplification products that correspond to the three loci from a father, mother, two daughters, uncle, student controls, and an unknown. The unknown sample was run in *lane 9*, and the corresponding individual's DNA can clearly be identified in *lane 4* (the father). Both are illustrated by a *red bounding box*. The lanes with CTT allelic ladders are indicated with the letter *L*.

straightforward, but it is important to note that, due to normal drift across the gel, there should be no more than four sample lanes between ladder lanes. It should also be noted that the THO1 bands may appear as doublets, but this fact does not interfere with analysis as comparison with the CTT allelic ladder allowed for relatively unambiguous assignments. The only allele that posed any assignment problem was allele 9.3 for the THO1 loci, but upon careful comparison with the ladder alleles and the positive control, the correct assignments were made for each individual.

Although the FBI uses 13 specific loci for purposes of profiling suspects, analysis of the three loci that we utilized (CSF1PO, TPOX, THO1) proved sufficient to observe the differences between DNA fingerprints from different individuals. Of the 25 individuals analyzed (one individual was common to both gels), none had identical banding patterns. The values listed in Table I, rightmost column, correspond to the probability of a random match between a "crime scene" sample and a particular individual. The values ran from the low hundreds (*e.g.* lowest = 1 in 160.54) up to tens of thousands (*e.g.* highest = 1 in 60,759.79). This value range indicates that the likelihood of obtaining a random match between a "crime scene" sample and an unrelated individual in a laboratory class is relatively small. Therefore the use of a pseudo-criminal's crime scene DNA (*e.g.* a random student's DNA or the professor's DNA) should not pose a problem upon statistical comparison with students' DNA. In addition, the analysis of the allelic STR banding patterns of a family (father, mother, and two

daughters) proved that it is straightforward to dissect out which alleles are inherited from each parent (Fig. 2 and Table II), and thus paternity was demonstrated (yet not necessarily statistically proved) in the case of this particular family.

Overall, the successful results of the three implementations and the subsequent positive feedback from the students indicate the feasibility of introducing the DNA fingerprinting methods and technologies used by law enforcement agencies into an undergraduate laboratory class. The experience gained and knowledge acquired will assist those students who plan to pursue careers in the growing fields of forensic science as well as help all students be better informed individuals when performing their civic duty as jury members.

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