Identification of Forensic Samples via Mitochondrial DNA in the Undergraduate Biochemistry Laboratory

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During the last decade, DNA evidence has played an increasingly greater role in the criminal justice system. Not only can DNA help to convict those accused of violent crimes, but it can also determine the actual innocence of those who have been wrongly convicted. The use of DNA in criminal investigations is an application that is of great interest to the general public. An analysis of forensic samples to solve a dastardly crime such as that described below provides an exciting motivating factor in the undergraduate biochemistry laboratory while exposing students to manipulation of DNA and cutting-edge technology.

> Toddler M was removed from her bedroom during a cocktail party at her parents' estate in Maine. A subsequent ransom note, containing a single tooth, demanded one million dollars for her return. As a member of the Colby Forensic Analysis Facility, you will be provided with a number of samples related to this horrific crime in an attempt to aid in this tragic kidnapping case. First, is the tooth actually that of Toddler M? Various samples collected from Toddler M's bathroom will be available for comparison. Second, is the cigarette butt found outside Toddler M's window related to the crime, or simply from a littering party guest? Third, was the abandoned car found at the Augusta airport used by the kidnapper? A sample of Toddler M's favorite brand of chewing gum was retrieved from the back seat, as well as a biological sample from the driver's seat. Fourth, does the envelope containing the ransom note provide a clue as to the kidnapper's identity?

We have selected the relatively simple forensic approach of mitochondrial DNA (mtDNA) sequencing *(1–3)* for this exercise. MtDNA has three characteristics that render it useful forensically *(4)*: it contains a hypervariable noncoding region that accumulates mutations approximately ten times faster than nuclear DNA; it is present in several hundred copies per cell; and it is maternally inherited. MtDNA is most useful for identification of human remains when the only reference material available is from maternal relatives or when the samples are badly degraded *(5).* For example, when the remains of the Romanovs, the Russian Imperial family executed in 1918 by the Bolsheviks, were excavated in 1991, positive identification was made via mtDNA sequencing and comparison to England's Prince Philip, whose maternal great-grandmother was the mother of the Czarina, Alexandra Romanov *(6).*

Experimental Procedure

We perform this laboratory exercise over a two-week period in the first-semester biochemistry course, comprised largely of junior and senior chemistry and biology majors concentrating in biochemistry. Students select a single biological sample related to the above scenario and then collectively pool their data to solve the crime. The first laboratory period requires about 1.5–2 hours and consists of preparing mtDNA for the polymerase chain reaction (PCR). Samples are digested with proteinase K and then boiled in Chelex 100 *(7),* which binds released metal ions that inhibit PCR. PCR using the primers F15971 and R16410 *(8)* is then performed overnight on an aliquot of each sample to amplify a hypervariable subregion, HV1, of human mtDNA. Samples are frozen until the second laboratory period, which requires 2–3 hours. Successful amplification is verified via 1.5% agarose gel electrophoresis in TBE buffer (0.045 M Tris-borate/0.001M EDTA) followed by staining with ethidium bromide or methylene blue. After the second laboratory period, we perform in-house cycle sequencing using an automated sequencer (Applied Biosystems Instruments 310 Genetic Analyzer) and post the data for the students to analyze within 2–3 days. Alternatively, the Sequencing Service of the Dolan DNA Learning Center at the Cold Spring Harbor Laboratory will generate mtDNA sequences using the F15971 primer for college and high school classes and post the results online. This service is free and takes about ten business days to complete. Data analysis consists of sequence comparisons via CLUSTAL W *(9),* also available at the Cold Spring Harbor Laboratory's Dolan DNA Learning Center Sequence Server web page *(10).*

Hazards

Ethidium bromide is a potent mutagen and should be handled with care. Gloves, goggles, and a laboratory coat should be worn at all times when manipulating ethidium-bromide-containing solutions or gels. Staining solutions can be decontaminated using activated charcoal *(11),* which along with the stained gels should be treated as hazardous chemical waste and appropriately discarded. Eye protection opaque to ultraviolet light must be used with transilluminators that do not have build-in safety shields. Methylene blue staining, while less sensitive, is a viable alternative that we have used with our nonmajors and does not require decontamination nor produce hazardous waste. Because of the possibility of blood-borne pathogens, crime scene samples containing human blood should be avoided. Teeth can be soaked in ~10 mL household bleach (~5% sodium hypochlorite solution) for 10–15 minutes to minimize biological hazards. The bleach should then be diluted with copious amounts of water (~1 L) in the fume hood prior to flushing down the drain. Eye protection, laboratory coat, and nitrile rubber gloves should be worn when handling bleach *(12).*

Results and Discussion

We have found that the 440 base pair (bp) fragment of the HV1 region of human mtDNA flanked by the primers F15971 and R16410 is extremely amenable to amplification even in the hands of novices. Since the initial DNA extraction steps are considered crucial to the success of subsequent PCR, we have adapted a simple kit (Carolina Biologicals #21-1236) designed to amplify mtDNA from hair and cheek cells. Proteinase K and Chelex 100 are used for DNA isolation in lieu of traditional organic extraction techniques (phenol–chloroform) for samples such as teeth *(13, 14),* hair *(15),* saliva *(16),* and fingernails *(17).* We have had good success, as demonstrated via 1.5% agarose gel electrophoresis, with these items as well as cigarette butts, envelope flaps, Q-tips, toothbrushes, and chewing gum (Figure 1). Advance treatment of teeth with bleach, to minimize the potential for contact with remnants of biohazardous material, does not adversely affect amplification. It is also not necessary to use teeth for this experiment; hair could easily be substituted. During typical student laboratory conditions this year, we had 20 out of 24 samples yield sufficient PCR product for sequencing—two of the failures visibly suffered from inaccurate volumes during the amplification stage and one was an eyelash (an attempt to push the limits of amplification).

Following verification of the 440 bp PCR product, cycle sequencing can be performed either in-house or via the Sequencing Service of the Dolan DNA Learning Center at the Cold Spring Harbor. The associated web site *(10)* is also an outstanding resource that allows facile manipulation of sequence data (*http://www.bioservers.org/html/sequences/sequences.html,* accessed Dec 2002) as well as providing a wealth of information about mtDNA at the genetic origins link (*http://www.geneticorigins.org/geneticorigins/*, accessed Dec 2002).

For those sending samples out for sequencing, the equipment and supplies necessary for this exercise are standard for most undergraduate biochemistry laboratories. The possible exception is the PCR thermocycler, but this technology is becoming more widespread and could perhaps be accessed at a friendly biology department nearby.

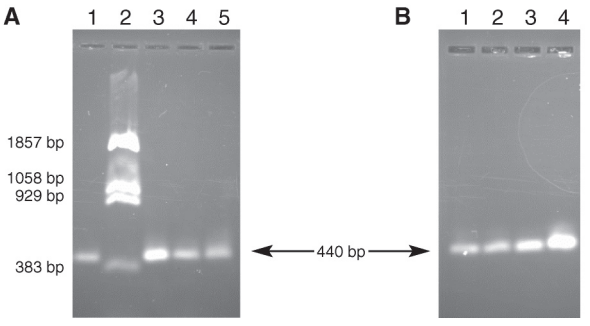

Figure 1. Agarose gel electrophoresis of PCR products resulting from crime scene samples. Panel A: Lane 1, toothbrush; Lane 2, size markers (fragment lengths listed on left); Lane 3, fingernail; Lane 4, chewing gum; Lane 5, Q-tip,. Panel B: Lane 1, tooth (bleach-treated); Lane 2, cigarette; Lane 3, envelope flap; Lane 4, hair.

Comments from students who performed this exercise were extremely positive. The collaborative nature of the project, the relevance to real life and criminal investigations, and the element of mystery involved were particularly enjoyed. Donors of biological samples, mostly nonscientists, were also delighted to be included in the effort and appreciated a lesson in the power of forensic biochemistry. Without fail, everyone involved was amazed to find that we all leave our DNA trail behind wherever we go for those astute enough to follow it.

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[W]Supplemental Material

Instructions for the students and notes for the instructor are available in this issue of *JCE Online*.

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