Optimizing Storage and Handling of DNA Extracts[a]

S. B. Lee*
Forensic Science Program
Justice Studies Department
San Jose State University
San Jose, California
United States of America

C. A. Crouse
Forensic Biology Unit
Palm Beach County Sheriff's Office Crime Laboratory
West Palm Beach, Florida
United States of America

M. C. Kline
Human Identity Project Team
National Institute of Standards and Technology
Gaithersburg, Maryland
United States of America

TABLE OF CONTENTS

[a]Contribution of the U.S. National Institute of Standards and Technology. Not subject to copyright. Certain commercial equipment, instruments, and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments, or equipment identified are necessarily the best available for the purpose. Points of view in this document are those of the authors and do not necessarily represent the official position or policies of the U.S. Department of Justice.

1042-7201/22-02/July 2010/131–144/$14.00 • Copyright © 2010 Central Police University Press

Optimizing Storage and Handling of DNA Extracts

REFERENCE: Lee SB, Crouse CA, Kline MC: Optimizing storage and handling of DNA extracts; *Forensic Sci Rev* 22:131; 2010.

ABSTRACT: Nucleic acid sample storage is of paramount importance in forensic science as well as in epidemiological, clinical, and genetic laboratories. Millions of biological samples, including cells, viruses, and DNA/RNA, are stored every year for diagnostics, research, and forensic science. PCR has permitted the analysis of minute sample quantities. Samples such as bone, teeth, touch samples, and some sexual assault evidence may yield only low-quality and low-quantity DNA/RNA. Efficient storage of the extracted DNA/RNA is needed to ensure the stability of the sample over time for retesting of the CODIS STRs, mtDNA, YSTRs, mRNA, and other future marker-typing systems.

Amplification of some or all of these markers may fail because the biological material has been highly degraded, contains inhibitors, is too low in quantity, or is contaminated with contemporary DNA. Reduction in recovery has been observed with refrigerated liquid DNA extracts and also those exposed to multiple freeze-thaw cycles. Therefore, the development of optimal storage and amplification methods is critical for successful recovery of profiles from these types of samples since, in many cases, retesting is necessary.

This review is divided into three sections. The Introduction and Background covers forensic DNA storage, factors that influence DNA stability, and a brief review of molecular strategies to type non-optimal DNA. Section I covers the importance of DNA extract storage in forensic and non-forensic DNA databanks and the mechanisms responsible for loss during storage. Finally, Section II covers strategies and technologies being utilized to store DNA.

KEY WORDS: DNA storage, FTA, SampleMatrix, trehalose.

INTRODUCTION AND BACKGROUND

Forensic DNA Storage Issues

Millions of biological samples, including cells, viruses, and DNA/RNA, are stored every year for diagnostics, research, and forensic science. DNA extracts from forensic evidence samples such as hair, bones, teeth, and sexual assault evidence may contain less than 100 pg of DNA [31,32,61,71]. Low DNA yields may be due to damage [13,17,38] or degradation [23,35,38]; small cell numbers found in low copy number (LCN) or "touch" samples [7,14,31,32,34,38.42,44]; oligospermic [68] or aspermic perpetrators [66]; or low male DNA from extended interval post-coital samples in sexual assault cases [33]. Trace biological evidence (e.g., fingerprints and touch evidence) may provide low yields [7,42,45,65,75,79]. Biological evidence may be consumed with the result that the DNA extracts may be the only remaining genomic resource to retest and test with new technologies for retrospective and prospective testing. Optimal storage of DNA is therefore critical to retrospective (retesting) or prospective (downstream analysis with additional or new genetic markers) testing [16,46,50].

Factors Influencing DNA Stability

Degradation is a major factor in the ability to analyze low-quantity samples such as those derived from ancient or degraded bones and teeth and those from mass disasters [13,14,38,55,58]. Degradation results in the reduction or loss of the structural integrity of cells and the quantity and quality of genomic DNA. Many laboratories store DNA extracts frozen in Tris EDTA (TE) buffer. However, reduction in DNA recovery may occur with refrigerated liquid DNA extracts and those repeatedly frozen and thawed [20,67] or stored in certain microcentrifuge tubes [28,29,46].

Low yields or loss of DNA due to these factors may preclude or diminish the ability to test LCN crime scene samples using current STR methods; therefore, other methods such as mini-amplicon STRs [5,17,35,53,56,57] or less discriminating mtDNA testing [23,49] are typically dictated for low-quantity samples in advanced states of degradation. The quantity and quality of template DNA from many low-copy forensic samples falls below recommended thresholds (0.5 to 1.25 ng; [18]) and ineffective storage only exacerbates further sample loss. Poor sample quality and the presence of inhibitors may lead to incomplete genetic profiles or no profile, reducing the probative value of the results.

In addition to sample quantity and intrinsic differences in sample types resulting in differences in quality and quantity, extrinsic differences resulting from (a) the effectiveness of the extraction method utilized; (b) the type and effectiveness of preservatives and storage buffers (e.g., presence of antimicrobial agents and nuclease inhibitors in the storage matrices and buffers); (c) purity,

especially regarding the amount of nuclease contamination; (d) ionic strength; (e) tube material and quality; (f) exposure to UV light; (g) temperature and humidity range and duration in short- or long-term storage; and (h) exposure to multiple freeze-thaw cycles (as occurs with repeated sampling or unexpected power loss), may all lead to differences in the ability to recover and retest the samples.

Non-optimal Sample Typing Strategies

Modifications to existing amplification and typing protocols such as mini-amplicons, whole genome amplification (WGA) [6,34], and LCN protocols [14,31,32,61,71] to increase the DNA signal and consequently, the analytical success rate of challenged samples, are currently being investigated [62,64]. Other approaches have been adopted that include addition of more *Taq* polymerase and BSA (J. Wallin of California Department of Justice, personal communication) and increasing cycle number or injection time [26]. Amplifying DNA with over 28 cycles is widely used [26]; nested primer amplification and increased time and voltage for electrokinetic injection of samples have also improved profiling success [45]. Post-PCR purification to remove any ionic components that compete with PCR products during electrokinetic injection has also been used to enhance results [48].

New PCR enhancement reagents have also recently been reported [47]. PCRboost™ has been reported to enhance amplification of low-quality and low-quantity samples and those containing inhibitors such as hematin and humic acid [47] as well as indigo dye [78]. Although these approaches have resulted in some success, they have not been universally adopted by forensic DNA laboratories due to inconsistent results (especially on highly degraded, inhibited, or low-quantity samples), high cost, and/or additional validation requirements. The mini-amplicon multiplex AmpFlSTR® MiniFiler™ [53] has greatly improved the ability to amplify degraded samples; however, it does not contain all of the CODIS core loci. In addition, new STR multiplexes are continually being developed and optimized with the goal of enhancing amplification and improving results for highly degraded, inhibited, low-quantity samples.

These new developments in non-optimal DNA sample methodologies underscore the importance of DNA storage. New methods that push the lower limit of detection expand applications to extremely low-quantity and low-quality samples. Stable DNA storage and handling over time are therefore especially important when the amount of sample is limited.

I. IMPORTANCE OF SAMPLE STORAGE

A. Forensic DNA Databanks and Casework Samples

The importance of DNA storage is obvious in the global growth and expansion of forensic DNA databases and repositories. The Combined DNA Index System (CODIS) currently has 8,080,941 offender profiles and 311,560 forensic profiles as of March 2010 [25]. The European Network of Forensic Science Institutes that includes 36 countries reports 7,247,183 offender profiles and 800,854 "stains" as of June 2009 [24]. The Armed Forces DNA Identification Laboratory (AFDIL) provides worldwide scientific consultation, research, and education services in the field of forensic DNA analysis to the Department of Defense and other agencies. AFDIL provides DNA reference specimen collection, accession, and storage of United States military and other authorized personnel and processes thousands of samples in casework each year. Forensic DNA laboratories around the world also process thousands of samples each year. All of these samples need to be properly stored and maintained. In addition to these samples, many laboratories conducting forensic DNA casework and data banking also store casework extracts as well as dilutions of the extracted DNA samples. Finally, new international forensic DNA databases, expansion of database laws to include arrestees, missing persons databases, and additional DNA samples from new property crime casework programs collectively increase the rate of growth and expansion of the number of stored DNA extracts.

B. Non-Forensic DNA Databanks and Biobanks

In addition to the growth and expansion of forensic DNA databanks, several other types of DNA biobanks have been established. These include clinical biobanks to assist in the development of new medicines and drugs [63]. For example, the UK Biobank has set a goal to collect, store, and eventually distribute half a million samples with related medical information from 30 to 35 clinics in Great Britain [11]. Additional efforts are underway at the Vanderbilt University School of Medicine in Nashville, TN, where they are planning on a 250,000-person DNA study, and the Oakland, CA, Kaiser Permanente DNA Biobank of 500,000 samples [11].

Several DNA banks have been established for studying human evolution. These include the worldwide Genographic project run by Dr. Spencer Wells and the National Geographic Society [81].The goal of this study is to analyze historical patterns in DNA from participants around the world to better understand our human genetic roots [81]. Biodiversity DNA databanks have also been

established supporting research on global diversity in response to extinctions. One such group hosts the DNA Bank Network [82] with 10,448 taxa containing 32,532 DNA samples.

C. Mechanisms of DNA Loss

Understanding the different mechanisms of DNA loss provides a foundation for developing the most optimal methods for efficient storage of DNA. It is well known that DNA may be damaged with exposure to temperature fluctuations such as freeze-thaw cycles [20,67]. In addition, it is well known that both water and oxygen may damage DNA through hydrolysis and oxidative damage [12]. Many laboratories have therefore explored other options for storage, including dry state storage.

The assumption is often made that if nucleic acids are dried they are then stable for long periods of time. However, it is becoming increasingly evident that degradation can occur during storage that can irreversibly damage the samples. For example, Lindal [51] reviewed evidence that DNA can undergo chemical changes such as depurination, hydrolysis, and oxidation even at low moisture content. Hofreiter et al. [37] suggest that such chemical degradation might be responsible for the difficult recovery of DNA from aged samples. Although dried DNA is stable in the short term, it is nevertheless imperative to prevent detrimental chemical changes for optimal recovery.

More recently, dry-storage DNA damage has been studied and it was found that solid-state DNA degradation is greatly affected by atmospheric water and oxygen at room temperature [12]. DNA may be lost by aggregation. As pointed out by Bonnet et al. [12], loss by aggregation is highly significant since laboratory plastic tubes and plate seals generally are not airtight and therefore both water and oxygen may adversely react with DNA. In this study the authors also tested the stabilizing effects of the additive trehalose. In the presence of trehalose, solid-state natural DNA, heated to 120 °C, does not denature [83]. This stabilization effect of trehalose may be explained by its ability to block the negative charges on the phosphates (water replacement) or by hydrogen bonding between trehalose and DNA, which may reduce the DNA structural fluctuations (vitrification hypothesis) [2,83].

Mechanisms for DNA damage during storage have recently been reviewed [12]. In addition to chemical damage, loss may also occur by the co-extraction and then subsequent action of nucleases that may not have been removed in the purification procedures. This is an important consideration in crude DNA extraction procedures such as Chelex® [76] that are then stored over time. In addition, loss may also occur during any additional manipulations of the DNA via purification through additional phase separations and column purifications. Finally, dilutions of DNA and subsequent storage in dH_20 may also result in loss through damage by water [12].

II. DNA STORAGE AND HANDLING STRATEGIES

A. Tube Characteristics

It has been well documented that loss of DNA may occur due to the material and quality of the tubes used to store the samples [43,46]. Polypropylene plastic microcentrifuge tubes that are routinely utilized in forensic DNA laboratories may retain DNA [28,29,46] with the amount of adsorbed DNA as high as 5 ng /mm^2 of tube wall [28]. In addition, different tube lots from the same manufacturer have been reported to retain variable amounts (5% to 95%) of DNA [29]. The authors suggest the use of polyallomar tubes or introducing 0.1% detergent, Triton-X 100, to prevent the retention of DNA on polypropylene tubes [29].

Polytetrafluoroethylene (PTFE, known commercially as Teflon®) tubes have also been compared to polypropylene for DNA storage [43]. Kline et al. conducted an inter-laboratory blind quantification study and reported that recovery of the low-target [DNA] samples (50 pg), stored in PTFE tubes was 73% versus only 56% from samples stored in polypropylene. This suggests that at this low level [DNA], a significant proportion of the sample DNA binds to the polypropylene walls and greater DNA recovery can be achieved with storage in PTFE-coated tubes [43].

B. Cold Storage

Among the most common strategies for DNA preservation are cold and dry storage strategies that include: (a) 4 °C refrigeration; (b) –20 °C; (c) –80 °C; (d) –196 °C (liquid nitrogen); and (e) dry storage on a solid matrix. Protection in the "dry state" and cryopreservation at -196 °C both maintain the DNA in the glassy or vitreous state. In the glassy state, DNA and other molecules lose the ability to diffuse. This results in very little movement at the molecular level. In fact, "movement of a proton (the hydrogen ion) has been estimated to be approximately one atomic diameter in 200 years"; this in turn makes any chemical reactions highly unlikely over hundreds of years [9]. If, however, moisture is reintroduced to the "dry state" or an increase in temperature occurs above the glass transition temperature of water (nominally –135 °C), chemical reactions may start again resulting in DNA instability [9].

Storage at –20 °C to –80 °C may provide adequate conditions depending on the quality and quantity of DNA needed for further testing and the duration of storage. Most forensic DNA laboratories utilize –20 °C to –80 °C freezers for storage. Forensic DNA research efforts have focused on developing new methods of amplification and typing with low-quality and low-quantity samples due in part to the observation that current storage methods are not optimal. Neither –20 °C nor –80 °C conditions have been shown to provide long-term storage quality equivalent to maintenance at liquid nitrogen temperatures [9]. Unfortunately, the storage of all forensic DNA extracts at liquid nitrogen temperature is not practical with over 16 million samples in U.S. and European forensic databanks alone!

As stated by Baust, "There are few studies that provide definitive answers to the question of optimal storage conditions for DNA." [9] The National Institute of Standards and Technology (NIST) and the National Cancer Institute have published data that suggest that "colder is better" and NIST has shown humidity control to be an important factor in stable storage. This is consistent with the fact that cryopreservation and dry-state storage both reduce DNA chemical reactivity.

Forensic DNA scientists face additional variables in optimizing DNA storage protocols. These variables include the initial contaminants that might be co-extracted with the DNA from crime scene samples, different DNA purities and final dilution buffers utilized in DNA extraction methodologies, the integrity of storage conditions including exposure to different temperatures, humidity and light, the tube material and efficiency of the seal, and downstream sample requirements. According to Baust, "Dry matrix storage should be dry and devoid of changes in moisture content ... and cold conditions should rely on stable, noncycling temperatures." [9] That is, when storing samples, there should be no temperature fluctuations such as those found in frost-free cycles of most modern refrigerators and there is a need to conduct comparative tests on DNA storage methodologies on forensic DNA samples over time using different storage approaches.

C. Dry Storage Comparisons

1. Trehalose

In 2005, Smith and Morin conducted a comparison of different storage conditions with the addition of potential preserving agents [72]. Dilutions of known concentrations of human placental DNA, and gorilla fecal DNA, were stored under four conditions (4 °C, –20 °C, –80 °C, dry at room temperature), with three additives (TE buffer, Hind III digested Lambda DNA, and trehalose. The effectiveness of the different methods was tested periodically using qPCR and PCR assay of a 757-bp fragment. The highest quantity of DNA remained in samples stored at –80 °C, regardless of storage additives, and those dried at room temperature in the presence of trehalose [72]. DNA quality was best preserved in the presence of trehalose, either dried or at –80 °C; significant quality loss occurred with –20 °C and 4 °C storage [72]. These results indicate that dry storage with an additive such as trehalose may improve recovery of low-quantity and low-quality DNA versus traditional liquid extract freezer storage.

DNA storage tests under different conditions and a literature review has been conducted by the DNA Bank Network of Germany [82]. This organization was established in spring 2007 and is currently funded by the German Science Foundation (DFG) and was initiated by GBIF Germany (Global Biodiversity Information Facility). DNA bank databases of all their partners are linked and are accessible via a central Web portal [21] providing DNA samples of complementary collections (microorganisms, protists, plants, algae, fungi, and animals) to support biodiversity applications. In their reviews and the results of their tests they determined that long-term storage of DNA samples in buffer should be carried out at –80 °C or below. Furthermore, as expected, dried, lyophilized DNA must be stored at low relative humidity to avoid DNA aggregation [22]. They also determined that energy and environmental costs were the main reasons to support dry storage at ambient temperature [22].

2. FTA® Technology

FTA® Cards contain chemicals that lyse cells, denature proteins, and protect nucleic acids from nucleases, oxidative, and UV damage. Others have evaluated treated filter paper for collection and storage of buccal cells. The treated filter paper technology FTA® [69] is used in a room-temperature storage product, offered by GenVault (Carlsbad, CA). Following 7 years of storage, Sigurdsen et al. found only modest DNA yields and reduced recovery that was insufficient for WGA [69]. However, others have shown good recovery from FTA® paper for forensic DNA analysis [27,59,70,74].

3. SampleMatrix®/QiaSafe

Biomatrica Inc. has recently developed a proprietary technology for the dry storage of biological materials at ambient temperatures. The key component of this technology is SampleMatrix® (SM, also known as QiaSafe), a synthetic chemistry storage medium that was developed based on anhydrobiosis ("life without water"), a natural protective mechanism that enables survival of some multicellular organisms in extremely dry environments [19]. Such organisms can produce high concentrations of disaccharides, particularly trehalose, a non-

reducing disaccharide of glucose, to protect their cellular structures during prolonged droughts and can be revived by simple rehydration [19]. Recent evidence suggests that trehalose can preserve intact cells in vitro in the dry state [80]. Trehalose disaccharides are predicted to interact with DNA molecules through minor groove interactions based on hydrogen bonding (**Figure 1**A; [16]). Biomatrica has developed proprietary synthetic compounds that mimic the protective properties of anhydrobiotic molecules with additional improvements that are especially pertinent to protecting DNA during dry storage. SM, a much improved synthetic formulation, is predicted to form similar interactive patterns with DNA as naturally occurring anhydrobiotic molecules (Figure 1B [16]).

The protective properties of SM are based on its ability to form a stabilizing structure via glass formation at a higher temperature than natural disaccharides and therefore to provide improved protective properties as compared to trehalose [16].

Storage of samples at different amounts demonstrated the protective properties of SM on DNA when PCR amplicons were detected in essentially all SM-protected samples at 70 °C, whereas unprotected samples showed more variable results. This is especially apparent in samples containing limited amounts of DNA (≤10 ng). It is noteworthy that the 4-ng samples stored at –20 °C for 24 h did not amplify as well as an identical sample stored dry in SM at 70 °C (Figure 1C [16]).

Stabilization of low-concentration DNA samples in SM has also been observed over 1 year [1,50]. For this study, purified male and female DNA was extracted from buccal swabs using DNA IQ™ (Promega, Madison, WI) followed by quantification using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems: Foster City, CA). DNA samples from the male and female donors were serially diluted and added in replicates into SM multiwell plates and tubes for final DNA concentrations ranging from 4 ng to 0.0625 ng in a total of 20 µL of water. Replicate DNA samples ($n = 4$) at each concentration were applied into SM multiwell plates and then dried overnight in a laminar flow hood at room temperature. Samples were maintained inside a storage cabinet with desiccant included to create a humidity-controlled environment (SM+D samples). A separate set of samples was stored inside an identical storage cabinet without desiccant to assess the effects of uncontrolled

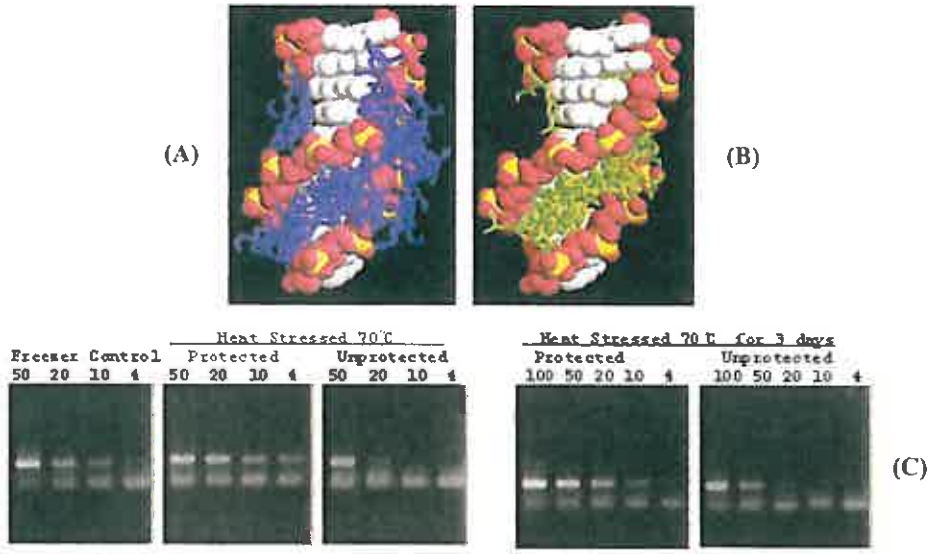
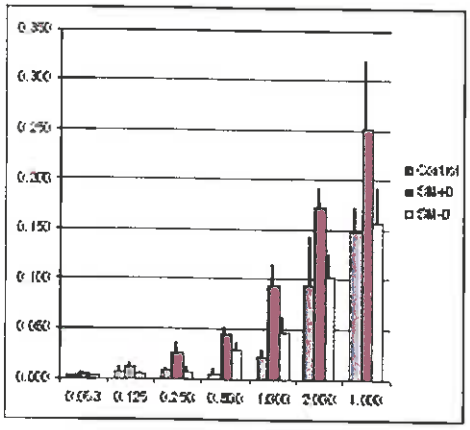


Figure 1. Protective properties of SM with nucleic acid molecules. (A) Three-dimensional depiction of trehalose disaccharides used in nature predicted to interact with nucleic acid molecules through minor groove interactions based on hydrogen bonding. (B) Three-dimensional depiction of SM as it is predicted to form similar interaction patterns as trehalose. (C) PCR results on human gDNA with and without SM protection, after 24 h at 70 °C or 3 d at 70 °C are shown. Various amounts of human genomic DNA (100 ng, 50 ng, 20 ng, 10 ng, and 4 ng) were applied in microcentrifuge tubes containing SM or into empty tubes (control samples). The samples were allowed to air dry overnight in a laminar flow hood. Samples were then placed on a heat block maintained at 70 °C. Samples were removed from the heat block at 24 h or 72 h and the DNA was hydrated in 10 µL water for 15 min on the benchtop prior to use in downstream applications without further purification. Aliquots of rehydrated samples were used to amplify the fibroblast growth factor 13 (FGF13) gene by PCR using 2.5 U Taq DNA polymerase (NEB), 3 µL 10× thermopol reaction buffer (NEB), 0.5 µL dNTPs (10 mmol/L each nucleotide), FGF13 forward (5'gaatgttaacaacatgctggc3') and FGF13 reverse (5'agaagctttaccaatgttttcca3') in a final volume of 30 µL. Cycling parameters were: 94 °C for 5 min followed by 40 cycles of 94 °C for 15 sec, 55 °C for 30 sec, and 72 °C for 30 sec. A 10-µL aliquot of each PCR reaction was run on a 0.8% agarose gel stained with ethidium bromide.

humidity on sample stability when stored in SM (SM-D samples). Identical samples were also aliquoted into empty polypropylene microcentrifuge tubes and stored frozen at -20 °C as standard in-house controls (Control) for comparison. The samples were processed immediately (0 d), 1 d, 1 week, 2 weeks, 1.5 months, 2 months, 3 months, 6 months, and 1 year prior to recovery and analysis by quantitative PCR followed by PowerPlex©16 STR analysis. Samples stored dry in SM were rehydrated with 20 µL of water and used directly in downstream applications without further purification to remove matrix components [1,50].

Recovered samples were quantified to determine the yield of DNA following dry storage in SM versus frozen control reference samples and also compared to initial quantification values obtained from the original DNA stock solutions at the time of sample preparation (0 d). Based on these quantification values, the average yield of DNA recovered following dry storage in SM under controlled humidity (SM+D-Red) conditions was dramatically improved as compared to samples stored without humidity control (SM-D-Beige) and in-house control (Control-Blue) samples stored frozen for 1 year (**Figure 2**A [1,50]). Similar results were detected after 4 months of storage where recovery of SM-stored DNA was significantly improved versus conventional polypropylene microfuge tubes (Figure 2B [16]).

A consortium of DNA biodiversity laboratories also conducted research on DNA storage that focused on new and suitable protective substances, storage at higher temperatures, rehydration of lyophilized DNA, and the usage of special cryotubes [22]. A workshop for long-term storage of DNA samples was held August 12, 2009, at the Systematics 2009 conference in Leiden, Netherlands [22]. At this workshop, it was reported that the characteristics of four commercial dry-storage systems at ambient temperature were tested: GenPlate, QIAsafe also known as SampleMatrix®, GenTegra™, and DNAshell . It is worth noting that GenPlate storage is based on FTA® paper and GenTegra™ is designed to protect RNA samples (*see* http://www.genvault.com/html/products/gentegra-RNA.html) with both being products of the same manufacturer (GenVault). DNAshell is a product from Imagene. Preliminary results of DNA storage experiments based on q-RT PCR data were presented by the DNA Bank Network. QIAsafe/SampleMatrix™ shows good storage performance at ambient temperature but did better at –20 °C and –80 °C. Unbuffered DNA in water (RT, 4 °C and –20 °C) was subject to fast degradation as would be expected from hydrolysis. The samples stored in QIAsafe/SampleMatrix provided comparable results to the theoretical best practice of samples storage in liquid nitrogen [22].



(A)

(B)

Figure 2. DNA recovery from samples stored in SampleMatrix® versus –20 °C. (A) Average DNA Recovery of SampleMatrix®-stored DNA versus –20 °C frozen controls. Replicate DNA samples at seven different concentrations were stored at ambient room temperature in SampleMatrix® with dessicant (SM+D), without dessicant (SM–D), or at –20 °C as frozen liquid (TE) controls in polypropylene microfuge tubes. Quantification was performed utilizing the Quantifiler® Human DNA Quantification Kit as per manufacturer's recommendations. Recovery from SM+D stored samples at room temperature was higher than that of frozen controls for every concentration. Error bars show the standard deviation for each time point and concentration from four relicates. (B) 10 ng of Control DNA (K562) was stored in SM and microcentrifuge tubes (MT) with no SM for 4 months at room temperature and at –20 °C. 1% agarose gel electrophoresis in 1X TBE followed by EtBr staining and laser flatbed scanning with an FMBIO III plus was used to detect the recovered DNA.

4. Shipping Study

The ability of SM to protect samples during shipment and storage conditions was evaluated in studies performed at NIST (Gaithersburg, MD [16]). Aliquots of 20 µL of human genomic DNA samples at 1.0 ng/µL, 0.25 ng/µL, and 0.05 ng/µL in Tris EDTA (TE) buffer were placed into multiple individual wells in four separate 96-well plates containing SM. The plates were dried overnight in a laminar flow hood and then sealed with aluminum foil seals provided by the manufacturer [16].

Two plates were shipped continuously across the country for 208 d in a cardboard shipping container (with no insulation or cold source). The remaining two plates

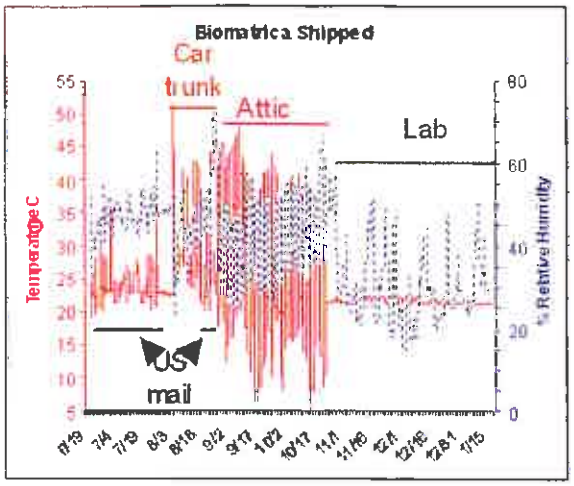
were maintained at laboratory ambient temperature conditions. A temperature and humidity monitor was included in the shipping container to continuously monitor the environmental conditions experienced during shipping and storage. Control DNA samples of identical concentration in TE were stored at 4 °C in PTFE containers.

The shipped samples were exposed continuously to environmental conditions (some extreme) during the summer and early fall of 2007. In total, the package traveled six times between Gaithersburg, MD, and San Diego, CA, via U.S. Postal Service. This series of shipping events was followed by an additional 14 d in a car trunk in Maryland. The package was then mailed across the country two more times and then maintained at ambient environmental conditions in a Maryland home attic for an additional 56 d including the month of August. The plates were then removed from the shipping container and stored at laboratory ambient conditions for the remainder of the study (208 d total).

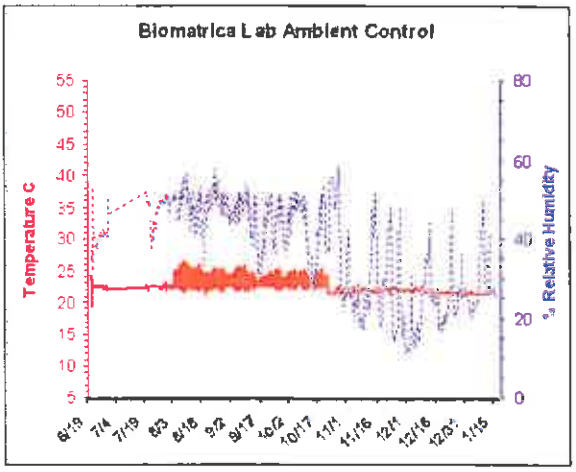
The other two plates were maintained at ambient temperature within the laboratory for the entire 208 d study without humidity control. During the summer, energy conservation efforts used to control the building HVAC system were detected and can be observed in the resulting temperature and humidity plots (Figure 3 [16]). Positive control DNA samples of identical concentration in TE were stored at 4 °C in PTFE containers for the duration of the study.

Data obtained from the temperature and relative humidity (RH) monitor indicate that the mailed samples were exposed to a maximum of 51.6 °C, 73% RH, and a minimum of 5.3 °C, 15% RH, with median and average conditions of 22.1 °C, 40% RH, and 23.6 °C. 39% RH, respectively (Figure 3). Laboratory ambient conditions reached a maximum of 26.4 °C, 58% RH, and a minimum of 19.4 °C, 11% RH; median and average conditions were 22.2 °C, 41% RH, and 22.4 °C, 38% RH, respectively. Energy conservation measures were implemented during the months of August through November 2007 (that caused the fluctuations seen in the ambient temperature and humidity plots in **Figure 3** [16]).

In order to assess any effects on DNA stability during long-term storage, DNA samples from four time points (0 d, 8 d, 23 d, and 56 d), were rehydrated and analyzed using quantitative PCR (Quantifiler® by Applied Biosystems). Identical control DNA samples that were stored dry in SM, but maintained at room temperature in the laboratory, were also quantified in the same manner. Results (comparison of Ct values) indicate that overall there was little to no difference in the amount of DNA recovered following storage and shipment in SM as compared with samples maintained dry in SM at room temperature (**Figure**



(A)



(B)

Figure 3. Temperature and humidity plots over 208 days of DNA storage (A) under various shipping conditions and (B) at ambient temperature in the laboratory.

4). These results indicate successful protection of dried DNA samples in SM during shipment in a standard cardboard shipping box without extra insulation or cold source.

To assess the yield of recovery following storage in SM for 208 d, quadruplicate DNA samples subjected to shipping stress were analyzed by qPCR as described above and compared with identical control samples stored in SM in the laboratory. Also included for analysis were the original DNA samples maintained in buffer and maintained at 4 °C in PTFE containers for the same time period.Results of recovery of DNA following shipment

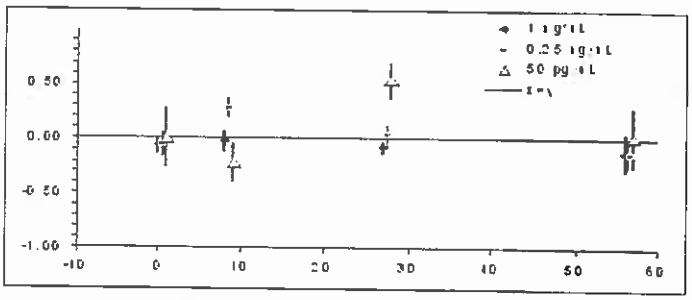


Figure 4. Delta Ct values from qPCR of shipped versus ambient-stored samples over 56 d. The X-axis displays time in days and the Y-axis displays the delta Ct value. Well-characterized DNA of a known concentration was diluted to target concentrations of 1.0, 0.25, and 0.05 ng/μL (50 pg/μL). TE^{-4} buffer was used as the fourth sample set. Data show that in general there is slight to no difference (displayed when Y = 0) in the relative value of DNA quantity and quality between the samples that were held at ambient temperature and humidity and the samples that were shipped and held in extreme environmental conditions.

and storage for 208 d indicate that the amount of DNA recovered from samples protected dry in SM following exposure to shipping stress and storage is comparable with identical DNA samples that were maintained at ambient temperatures in the laboratory for the same time period (**Table 1**). There was a slight decrease in the amount of DNA as compared to samples maintained in solution in PTFE containers and kept at 4 °C (Table 1). The use of PTFE containers is unfortunately not cost effective for the number of samples that need to be stored [16].

Samples subjected to shipping stress and those stored for the identical time period at ambient laboratory conditions were also analyzed by multiplex STR analysis using AmpF*l*STR Identifiler® (Applied Biosystems). Rehydrated samples were used directly in amplification reactions without further purification. Profiles obtained from DNA stored in SM (1 ng used for amplification) were compared with samples maintained in solution at 4 °C in PTFE-coated tubes. Slightly lower relative fluorescence units (rfu) were obtained from samples subjected to shipping stress while stored dry in SM. However, all samples gave complete Identifiler® profiles above 200 rfu, even samples amplified from 250 pg input DNA [16].

Table 1. Quantification results ($n = 4$) after 208 days of storage under various conditions

DNA (ng/μL)	Condition		
	Shipped[a]	Lab ambient	4 °C PTFE[a]
1.00	0.65 ± 0.06	0.69 ± 0.03	1.01 ± 0.02
0.25	0.18 ±0.03	0.20 ± 0.01	0.30 ± 0.01
0.05	0.04 ±0.00	0.04 ± 0.06	0.05 ± 0.00

[a] Shipped: in SampleMatrix; PTFE: polytetrafluoroethylene container.

D. Additional Practically Significant DNA Storage Modalities

Genomic DNA is also stored in variable number of tandem repeat (NVTR) restriction fragment length polymorphism (RFLP) Southern blot membranes, slot blots, and dot blots (both DQ alpha and PM), as well as in the liquid PCR products. Extraction and typing was recently reported from DNA extracted from VNTR-RFLP Membranes [73]. In addition, genomic DNA can be isolated from PCR products such as DQ Alpha products [36], PM, D1S80, and CTT STR products [60], and the isolated genomic DNA can be typed utilizing new genetic markers. It is expected that the new STR multiplex products can also be utilized in the same manner, that is, as a source of genomic DNA as the template is not consumed in the PCR reaction. This suggests that in cases where all the evidence and extracted DNA and dilutions have been consumed, the remaining PCR products may also be stored for future typing and retesting. Finally, it has been well documented that a portion of the DNA is lost in columns such as Centricon-100s and Microcon 100s [52]. If the entire sample is consumed in the extraction, then any remaining DNA extracted from the columns and eluates could also be stored under the most optimal conditions for future testing.

Finally, there are recently developed forensic methods that are based on RNA, indicating the need to optimize RNA storage as well as DNA. Different genetic expression patterns exist in different tissue types and detection of differential expression has been utilized to determine the origin of biological evidence based on determining relative abundance of messenger RNA. Body fluid identification has been reported based on their mRNA profiles [8,39-41,54,84]. In addition, estimating the age of a bloodstain

was reported using analysis of mRNA: rRNA ratios [4]. This type of information may be a useful tool in establishing when the evidence was deposited and may help in determining when a crime was committed. Advantages of the mRNA-based approach versus the conventional biochemical tests include greater specificity, simultaneous and semiautomatic analysis, rapid detection, decreased sample consumption, and compatibility with DNA extraction methodologies. The quantification of the amounts of the mRNA species relative to housekeeping genes is a critical aspect of the assays [8,40]. These relatively new RNA-based methods require sensitivity and a high degree of quantitative accuracy and highlight the need to stabilize the storage of both RNA and DNA in extracts. Stable dry storage of RNA for gene expression analysis out to 11 d [77] and for microarray expression analysis out to 4 weeks [30] has recently been reported.

The future of biological evidence analysis will continue to require the development, validation, and adoption of new methods and genetic markers [15]. The optimization of storage of nucleic acid extracts is a pivotal step toward maximizing the potential of the new methods and markers in future testing.

ACKNOWLEDGMENTS

The authors would like to thank the following individuals who have provided data and/or assisted in some way in the preparation of this document: Russ Miller, Taha Ahmad, and Karen Crenshaw of Palm Beach Sheriff's Office for assistance in performance and analysis of data shown in Figure 2a; Kimberly Clabaugh, Keri Larsen, Brie Silva, and Sal Murillo of San Jose State University for assistance in preparing data shown in Figure 2b; David L. Duewer of NIST for assistance with data presentation in Figures 3 and 4 and Table 1; and Dr. Rolf Muller of Biomatrica for data in Figures 1A–1C and support for research on SampleMatrix. Authors would also like to acknowledge *Forensic Science Review* editors Dr. Ray Liu and Dr. Jaiprakesh Shewale for their support, collaborations, and leadership in the field of forensic DNA; and Ines Iglesias-Lee for her many contributions in scientific discussions on this manuscript.

Research conducted at SJSU was supported in part by a California State University Program for Education and Research in Biotechnology Joint Venture grant to S. Lee and R. Muller, the National Science Foundation NSF-REU grant #DBI-0647160 to J. Soto, C. Ouverney and S. Lee of SJSU and by the California Association of Criminalists A. Reed and V. McGlaughlin Endowment Fund for scholarships to K. Clabaugh and B. Silva at SJSU and support provided by Chair, Dr. Mark Correia (Justice Studies Department, SJSU), Associate Dean Dr. Barbara Conry, and Dean, Dr. Charles Bullock (College of Applied Sciences and Arts, SJSU).

The shipping study described here was supported by the National Institute of Justice through interagency agreement 2003-IJ-R-029 to the Office of Law Enforcement Standards, National Institute of Standards and Technology.

REFERENCES

1. Ahmad T, Miller RW, McGuckian AB, Conover-Sikorsky J, Crouse CA: Biomatrica DNA SampleMatrix®: A new prospect for forensic DNA sample storage; *Proceedings —Auunal Meeting of the American Academy of Forensic Sciences*; Denver, CO; p 108; February 2009.
2. Alkhamis KA: Influence of solid-state acidity on the decomposition of sucrose in amorphous systems (I); *Int J Pharm* 362:74; 2008.
3. Allison SD, Chang B, Randolph TW, Carpenter JF: Hydrogen bonding between sugar and protein is responsible for inhibition of dehydration-induced protein unfolding; *Arch Biochem Biophys* 365:289; 1999.
4. Anderson S, Howard B, Hobbs GR, Bishop CP: A method for determining the age of a bloodstain; *Forensic Sci Int* 148:37; 2005.
5. Asamura H, Fujimori S, Ota M, Oki T, Fukushima H: Evaluation of miniY-STR multiplex PCR systems for extended 16 Y-STR loci; *Int J Legal Med* 122:43; 2008.
6. Ballantyne KN, van Oorschot RA, Mitchell RJ: Comparison of two whole genome amplification methods for STR genotyping of LCN and degraded DNA samples; *Forensic Sci Int* 166:35; 2007.
7. Balogh MK, Burger J, Bender K, Schneider PM, Alt KW: STR genotyping and mtDNA sequencing of latent fingerprint on paper; *Forensic Sci Int* 137:188; 2003.
8. Bauer M: RNA in forensic science; *Forensic Sci Int Genet* 1:69; 2007.
9. Baust JG: Strategies for the storage of DNA; *Biopreservation and Biobanking* 6:251; 2008 (http://www.liebertonline.com/doi/pdfplus/10.1089/bio.2008.0604.lett; accessed February 28, 2010).
10. Belotserkovskii BP, Johnston BH: Denaturation and association of DNA sequences by certain polypropylene surfaces; *Anal Biochem* 251:251; 1997.
11. Blow N: Biobanking: freezer burn; *Nat Methods* 6:173; 2009.
12. Bonnet J, Colotte M, Coudy D, Couallier V, Portier J, Morin B, Tuffet S: Chain and conformation stability of solid-state DNA: Implications for room temperature storage; *Nucleic Acids Res* 38:1531; 2010.
13. Budowle B, Bieber FR, Eisenberg AJ: Forensic aspects of mass disasters: strategic considerations for DNA-based human identification; *Leg Med* (Tokyo) 7:230; 2005.
14. Budowle B, Eisenberg AJ, van Daal A: Validity of low copy number typing and applications to forensic science; *Croat Med J* 50:207; 2009.
15. Budowle B, van Daal A: Extracting evidence from forensic DNA analyses: Future molecular biology directions; *BioTechniques* 46:339; 2009.

16. Clabaugh K, Silva S, Odigie K, Fourney R, Stevens J, Carmody G, Coble M, Loreille O, Scheible M, Kline M, Parsons T, Pozder A, Eisenberg A, Budowle B, Lee SB: Storage of DNA samples at ambient temperature using DNA-SampleMatrix; *Presentation—18th International Symposium on Human Identification*; Hollywood, CA; October 2007.
17. Coble MD, Butler JM: Characterization of new miniSTR loci to aid analysis of degraded DNA; *J Forensic Sci* 50:43; 2005.
18. Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA: Developmental validation of a single-tube amplification of the 13 CODIS STR Loci, D2S1338, D19S433 and amelogenin; The AmpFlSTR Identifiler PCR amplification kit; *J Forensic Sci* 49:1265; 2004.
19. Crowe JH, Carpenter JF, Crowe LM: The role of vitrification in anhydrobiosis; *Annu Rev Physiol* 60:73; 1998.
20. Davis DL, O'Brien EP, Bentzley CM: Analysis of the degradation of oligonucleotide strands during the freezing/thawing processes using MALDI-MS; *Anal Chem* 72:5092; 2000.
21. DNA Bank Network (http://www.dnabank-network.org/Index.php; accessed February 17, 2010).
22. DNA Bank Network: Long Term DNA Storage Workshop Proceedings (http://www.dnabank-network.org/publications/Workshop_Long-term_DNA_storage-Summary_and_Abstracts.pdf; accessed February 15, 2010).
23. Eichmann C, Parson W: 'Mitominis': Multiplex PCR analysis of reduced size amplicons for compound sequence analysis of the entire mtDNA control region in highly degraded samples; *Int J Legal Med* 122:385; 2008.
24. European Network of Forensic Science Institutes: Survey on DNA-databases in Europe June 2009 (http://www.enfsi.eu/page.php?uid=98; accessed February 26, 2010).
25. Federal Bureau of Investigation: Combined DNA Index System Statistics (http://www.fbi.gov/hq/lab/html/codis1.htm; accessed February 28, 2010).
26. Forster L, Thomson J, Kutranov S: Direct comparison of post-28-cycle PCR purification and modified capillary electrophoresis methods with the 34-cycle "low copy number" (LCN) method for analysis of trace forensic DNA samples; *Forensic Sci Int Genet* 2:318;2008.
27. Fujita Y, Kubo S: Application of FTA technology to extraction of sperm DNA from mixed body fluids containing semen; *Leg Med* (Tokyo) 8:43; 2006.
28. Gaillard C, Strauss F: Avoiding adsorption of DNA to polypropylene tubes and denaturation of short DNA fragments: Elsevier Technical Tips on-line 1998 (http://frstrauss.free.fr/reprints/gaillard_TTO98.pdf; accessed July 3, 2004).
29. Gaillard C, Strauss F: Eliminating DNA loss and denaturation during storage in plastic microtubes; *International Biotechnology Laboratory* 6; August 2000 (http://frstrauss.free.fr/reprints/gaillard_IBL00.pdf; accessed February 14, 2010).
30. Hernandez GE, Mondala TS, Head SR: Assessing a novel room-temperature RNA storage medium for compatibility in microarray gene expression analysis; *BioTechniques* 47:667; 2009.
31. Gill P: Application of low copy number DNA profiling; *Croat Med J* 42:229; 2001.
32. Gill P, Whitaker J, Flaxman, C, Brown N, Buckleton J: An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA; *Forensic Sci Int* 112:17; 2000.
33. Hall A, Ballantyne J: Novel Y-STR typing strategies reveal the genetic profile of the semen donor in extended interval post-coital cervicovaginal samples; *Forensic Sci Int* 136:58; 2003.
34. Hanson EK, Ballantyne J: Whole genome amplification strategy for forensic genetic analysis using single or few cell equivalents of genomic DNA; *Anal Biochem* 346:246; 2005.
35. Hill CR, Kline MC, Coble MD, Butler JM: Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples; *J Forensic Sci* 53:73; 2008.
36. Hochmeister MN, Budowle B, Borer UV, Dirnhofer R: A method for the purification and recovery of genomic DNA from an HLA DQA1 amplification product and its subsequent amplification and typing with the AmpliType PM PCR Amplification and Typing Kit; *J Forensic Sci* 40:649; 1995.
37. Hofreiter M, Serre D, Poinar HN, Kuch M, Paabo S: Ancient DNA; *Nat Rev Genet* 2:353; 2001.
38. Irwin JA, Leney MD, Loreille O, Barritt SM, Christensen AF, Holland TD, Smith BC, Parsons TJ: Application of low copy number STR typing to the identification of aged, degraded skeletal remains; *J Forensic Sci* 52:1322; 2007.
39. Juusola J, Ballantyne J: Messenger RNA profiling: a prototype method to supplant conventional methods for body fluid identification; *Forensic Sci Int* 135:85; 2003.
40. Juusola J, Ballantyne J: mRNA profiling for body fluid identification by multiplex quantitative rt-PCR; *J Forensic Sci* 52:1252; 2007.
41. Juusola J, Ballantyne J: Multiplex mRNA profiling for the identification of body fluids; *Forensic Sci Int* 152:1; 2005.
42. Kita T, Yamaguchi H, Yokoyama M, Tanaka T, Tanaka N: Morphological study of fragmented DNA on touched objects; *Forensic Sci Int Genet* 3:32; 2008.
43. Kline MC, Duewer DL, Redman JW, Butler JM: Results from the NIST 2004 DNA Quantitation Study; *J Forensic Sci* 50:570; 2005.
44. Kloosterman A, Kersbergen DP: Efficacy and limits of genotyping low copy number (LCN) DNA samples by multiplex PCR of STR loci; *J Soc Biol* 197:351; 2003.
45. Lagoa AM, Magalhães T, Pinheiro MF: Genetic analysis of fingerprints—Could WGA or nested-PCR be alternatives to the increase of PCR cycles number? *Forensic Sci Int Genet* 1:48; 2008.
46. Larsen K, Lee S: Optimization Strategies for DNA storage; *Presentation—16th International Symposium on Human Identification*; Grapevine, TX; September 2005.
47. Le L, Clabaugh KC, Chang A, Meinenger M, Coble MD, Loreille O, Scheible M, Demara R, Muller R, deRozieres S, Kline M, Lee SB: Recovering DNA profiles from low quantity and low quality forensic samples; *Presentation—19th International Symposium on Human Identification*; Hollywood, CA; October 2008.
48. Lederer T, Braunschweiger G, Betz P, Seidl S: Purification of STR-multiplex amplified microsamples can enhance signal intensity in capillary electrophoresis; *Int J Legal Med* 116:165; 2002.

49. Lee HY, Kim NY, Park MJ, Yang WI, Shin KJ: A modified mini-primer set for analyzing mitochondrial DNA control region sequences from highly degraded forensic samples; *BioTechniques* 44:555; 2008
50. Lee SB, Clabaugh KC, Silva S, Odigie KO, Fourney RM, Stevens J, Carmody GR, Coble MD, Loreille O, Scheible M, Parsons TJ, Pozder A, Eisenberg AJ, Budowle B, Ahmad T, Miller RW, McGuckian AB, Conover-Sikorsky J, Crouse CA: Assessing a novel room temperature DNA storage medium for forensic biological samples; *Forensic Sci Int Genet* (Submitted 2010).
51. Lindahl T: Instability and decay of the primary structure of DNA; *Nature* 362:709; 1993.
52. Meinenger MRD, Barloewen B, Jones J, Lee SB: Evaluation of purification columns for forensic DNA extraction; *Presentation—19th International Symposium on Human Identification*; Hollywood, CA; October 2008.
53. Mulero JJ, Chang CW, Lagacé RE, Wang DY, Bas JL, McMahon TP, Hennessy LK: Development and validation of the AmpFlSTR MiniFiler PCR Amplification Kit: AMiniSTR multiplex for the analysis of degraded and/or PCR inhibited DNA; *J Forensic Sci* 53:838; 2008.
54. Nussbaumer C, Gharehbaghi-Schnell E, Korschineck I: Messenger RNA profiling: A novel method for body fluid identification by real-time PCR; *Forensic Sci Int* 157:181; 2006.
55. O'Rourke DH, Hayes MG, Carlyle SW: Ancient DNA studies in physical anthropology; *Annu Rev Anthropol* 29:217; 2000.
56. Opel KL, Chung DT, Drabek J, Butler JM, McCord BR: Developmental validation of reduced-size STR Miniplex primer sets; *J Forensic Sci* 52:1263; 2007.
57. Opel KL, Chung DT, Drabek J, Tatarek NE, Jantz LM, McCord BR: The application of miniplex primer sets in the analysis of degraded DNA from human skeletal remains; *J Forensic Sci* 51:351; 2006.
58. Paabo SP, Poinar H, Serre D, Jaenicke-Despres V, Hebler J, Rohland N, Kuch M, Krause J, Vigilant L, Hofreiter M: Genetic analyses from ancient DNA; *Annu Rev Genet* 38:645; 2004.
59. Park SJ, Kim JY, Yang YG, Lee SH: Direct STR amplification from whole blood and blood- or saliva-spotted FTA without DNA purification; *J Forensic Sci* 53:335; 2008.
60. Patchett KL, Cox KJ, Burns DM: Recovery of genomic DNA from archived PCR product mixes for subsequent multiplex amplification and typing of additional loci: Forensic significance for older unsolved criminal cases; *J Forensic Sci* 47:786; 2002.
61. Phipps M, Petricevic S: The tendency of individuals to transfer DNA to handled items; *Forensic Sci Int* 168:162; 2007.
62. Prinz M, Schiffner L, Sebestyen J, Bajda E, Tamariz J, Shaler R, Baum H, Caragine T: Maximization of STR DNA typing success for touched objects; In Amorim A, Corte-Real F, Morling N (Eds): *Progress in Forensic Genetics 11* (21st Congress of the International Society for Forensic Genetics); Elsevier: Amsterdam, The Netherlands; p 651; 2006.
63. Roden DM, Pulley JM, Basford MA, Bernard GR, Clayton EW, Balser JR, Masys DR: Development of a large-scale de-identified DNA biobank to enable personalized medicine; *Clin Pharmacol Ther* 84:362; 2008.
64. Roeder A, Elsmore P, Greenhalgh M, McDonald A. Maximizing DNA profiling success from sub-optimal quantities of DNA: A staged approach; *Forensic Sci Int Genet* 3:128; 2009.
65. Schulz MM, Reichert W: Archived or directly swabbed latent fingerprints as a DNA source for STR typing; *Forensic Sci Int* 127:128; 2002.
66. Shewale, JG, Sikka, SC, Schneida, E, Sinha, SK: DNA profiling of azoospermic semen samples from vasectomized males by using YPLEX 6 amplification kit; *J Forensic Sci* 48:127; 2003.
67. Shikama K: Effect of freezing and thawing on the stability of double helix of DNA; *Nature* 207:529; 1965.
68. Sibille I, Duverneuil C, Lorin de la Grandmaison G, Guerrouache K, Teissiere F, Durigon M, and de Mazancourt P: Y-STR DNA amplification as biological evidence in sexually assaulted female victims with no cytological detection of spermatozoa; *Forensic Sci Int* 125:212; 2002.
69. Sigurdson AJ, Ha M, Cosentino M, Franklin T, Haque KA, Qi Y, Glaser C, Reid Y, Vaught JB, Bergen AW: Long-term storage and recovery of buccal cell DNA from treated cards; *Cancer Epidemiol Biomarkers Prev* 15:385; 2006.
70. Smith LM, Burgoyne LA: Collecting, archiving and processing DNA from wildlife samples using FTA databasing paper; *BMC Ecol* 4:4; 2004.
71. Smith PJ, Ballantyne J: Simplified low-copy-number DNA analysis by post-PCR purification; *J Forensic Sci* 52:820; 2007.
72. Smith S, Morin PA: Optimal storage conditions for highly dilute DNA samples: A role for trehalose as a preserving agent; *J Forensic Sci* 50:1101; 2005.
73. Steadman SA, McDonald JD, Andrews JS, Watson ND: Recovery and STR amplification of DNA from RFLP membranes; *J Forensic Sci* 53:349; 2008.
74. Tack LC, Thomas M, Reich K: Automated forensic DNA purification optimized for FTA card punches and identifiler STR-based PCR analysis; *Clin Lab Med* 27:183; 2007.
75. van Oorschot, RA, Jones, MK: DNA fingerprints from fingerprints; *Nature* 387:767; 1997.
76. Walsh PS, Metzger DA, Higuchi R: Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material; *BioTechniques* 10:506; 1991.
77. Wan E, Akana M, Pons J, Chen J, Musone S, Kwok PY, Liao W: Green technologies for room temperature; *Curr Issues Mol Biol* 12:135; 2009.
78. Wang C, Trogdon C, Le L, Meinenger M, Lee SB: Comparison of different amplification reagents for alleviating inhibitory effects of Indigo Dye in PCR; *Proceedings—Auunal Meeting of the American Academy of Forensic Sciences*; Seattle, WA; p 102; February 2010.
79. Wickenheiser, RA: Trace DNA: A review, discussion of theory, and application of the transfer of trace quantities of DNA through skin contact; *J Forensic Sci* 47:442; 2002.
80. Wolkers WF, Tablin F, Crowe JH: From anhydrobiosis to freeze-drying of eukaryotic cells; *Comp Biochem Physiol A Mol Integr Physiol* 131:535; 2002.
81. Zalloua PA, Platt DE, El Sibai M, Khalife J, Makhoul N, Haber M, Xue Y, Izaabel H, Bosch E, Adams SM, Arroyo E, López-Parra AM, Aler M, Picornell A, Ramon M, Jobling MA, Comas D, Bertranpetit J, Wells RS, Tyler-Smith C: Genographic Consortium. Identifying genetic

traces of historical expansions: Phoenician footprints in the Mediterranean; *Am J Hum Genet* 83:633;2008.
82. Zetzsche H, Dröge G, Gemeinholzer B: DNA Bank Network—Webkatalog und Referenzdatenbank für organismische DNA; *GfBS Newsletter* 22:4; 2009 (http://www.dnabank-network.org/Index.php; accessed February 4, 2010).
83. Zhu B, Furuki T, Okuda T, Sakurai M: Natural DNA mixed with trehalose persists in B-form double-stranding even in the dry state; *J Phys Chem B* 111:5542; 2007.
84. Zubakov D, Hanekamp E, van IJken W and Kayser M: Stable RNA markers for blood and saliva identification revealed from whole genome expression analysis of time-wise degraded stains; *Int J Legal Med* 122:135; 2008.

ABOUT THE AUTHORS

S. B. Lee;
C. A. Crouse; M. C. Kline

Steven B. Lee holds a B.S. degree in biology with a minor in chemistry from the State University of New York (Binghamton, NY), an M.S. degree in molecular biology and population genetics from New York University (New York, NY), and a Ph.D. in molecular and physiological plant biology from the University of California, Berkeley (Berkeley, CA). Dr. Lee conducted postdoctoral research in molecular evolution at the University of Georgia, Athens (Athens, GA). Dr. Lee holds several concurrent positions including professor and director of the forensic science programs at San Jose State University (San Jose, CA), visiting scholar in the Plant and Microbial Biology Department at the University of California, Berkeley, and an adjunct professor appointment in the Department of Biological Sciences at San Francisco State University (San Francisco, CA).

Dr. Lee was formerly (1994–2000) the director of R&D at the California Department of Justice's DNA Laboratory, where he served as an expert witness in DNA, spearheaded the implementation of robotics and STRs, and conducted formal DNA training courses for criminalists and students through the California Criminalistics Institute. From 2000 to 2004, Dr. Lee served as the director of R&D at MiraiBio Inc., Hitachi Software Engineering America, Ltd. (Alameda, CA).

Dr. Lee is a full member of the American Association for the Advancement of Science and the California Association of Criminalists, and a fellow in the Criminalistics Division of the American Academy of Forensic Sciences. He also serves as a certified inspector for the Laboratory Accreditation Board of the American Society of Crime Laboratory Directors. Dr. Lee served on the FBI Technical Working Group on DNA Analysis Methods group from 1994–2000 and serves on the Department of Defense Armed Forces DNA Identification Laboratory Quality Assurance Board.

Cecelia A. Crouse earned a B.S. degree in biology from Michigan State University (East Lansing, MI) and a Ph.D. in microbiology and immunology from the University of Miami (Miami, FL). She is currently the chief scientific officer of the Palm Beach County Sheriff's Office Crime Laboratory and manager of the Palm Beach County Sheriff's Office Forensic Biology Unit.

Dr. Crouse was a postdoctoral fellow at the Bascom Palmer Eye Institute of the University of Miami Health System (Miami, FL) and then worked as a plant geneticist at Eli Lilly & Co. (Indianapolis, IN). Dr. Crouse has also been actively involved in the development of forensic DNA over the last 18 years. She served 7 years as a United States American Prosecutors Research Institute DNA Faculty Member for "DNA: Witness to the Truth" and a member of the National Institute of Science and Technology white paper "Forensic Science Summit: Roadmap to the Year 2000". She was a member of the working group for Attorney General John Ashcroft's Initiative on DNA Laboratory Analysis Backlogs and the Janet Reno National Commission for the Future of DNA Evidence Laboratory Funding Group, the FBI Scientific Working Group on DNA Analysis, the NIJ's DNA Technical Working Group, the NIJ Technical Working Group for the Defense, and the NIJ Advisory Board for DNA Expert Systems.

Dr. Crouse served on the Scientific Working Group on DNA Analysis and Methods (SWGDAM) and is also a member of the editorial review board of the Journal of Forensic Science. Dr. Crouse has participated as a board member for ChoicePoint's DNA Advisory Board for the National Rape Evidence Project and also served on the Department of Defense Armed Forces DNA Identification Laboratory (AFDIL) Advisory Board. She is also on the Scientific Committee for the International Society for Applied Biological Sciences.

Margaret C. Kline holds a B.S. degree (magna cum laude) in animal science/veterinary science from the University of Maryland (College Park, MD) and an M.S. degree in food science from the University of Maryland. She is currently a research biologist in the Applied Genetics Group, Chemical Science and

Technology Laboratory, in the Biochemical Science Division, National Institute of Standards and Technology (Gaithersburg, MD).

Ms. Kline conducts research using the polymerase chain reaction (PCR) for human identity purposes and optimizes electrophoretic separations and sequencing of the various genetic loci studied. She has designed and conducted PCR/DNA-based interlaboratory studies leading to the development and production of standard reference materials that are used in virtually all forensic DNA laboratories. She regularly designs, prepares, distributes, and collects data from interlaboratory studies involving human identity projects including STR Kit evaluations, DNA quantitation issues, and mixture interpretation issues and has served as the quality manager for Division 831 since April 2005.

Ms. Kline is a member of the American Chemical Society and the Association for Molecular Pathology.