Saving Your SAMPLES

Room temperature storage and stabilization of DNA and RNA

by Steven B. Lee and Katherine A. Roberts

Successful forensic DNA analysis depends on the ability to properly collect, analyze and preserve biological evidence. Stabilization of biological evidence in cells and following DNA extraction is particularly important, since testing is generally not performed immediately after sample collection and retesting may be needed on stored DNA extracts. Many forensic DNA samples contain minute quantities of DNA that may be damaged or degraded (i.e., trace evidence or low copy number samples). Current storage systems may exacerbate the difficulties in obtaining reliable and useful results from such samples if further loss or damage occurs during storage. Therefore, reliable DNA storage is of paramount importance in the forensic science community.

Micrograms to Picograms

Forensic DNA testing was initially performed using DNA fingerprinting and RFLP analysis, developed in 1985 by Sir Alec Jeffreys at the University of Leicester. In the past, microgram amounts of DNA were needed for optimal results; unfortunately, many forensic samples collected at crime scenes did not yield biological samples in sufficient amounts for high-quality DNA analysis. However, the advent of PCR technology allowed for testing of smaller amounts of DNA; labs now routinely work with nanogram to picogram amounts of DNA, with as little as 1 pg needed for optimal analysis.

Currently, there are two major arenas for DNA testing in the forensic community: databanking and casework. Databanking involves collecting the DNA of mainly convicted felons, generating genetic profiles and then entering the profiles into a database, called the Combined DNA Index System (CODIS). In the past, states were only performing DNA testing on felony cases involving murder and rape. Recently, though, testing has expanded to include assaults and burglary. Forty-six states now have laws mandating more comprehensive testing, with 11 having enacted laws to collect DNA from arrestees.[1] For example, the San Diego County Sheriff's Department has established a team of forensic biologists within its Regional Crime Laboratory devoted to handling DNA evidence collected exclusively from street crimes.[2] At the national level, the U.S. Department of Justice is currently completing rules to allow the collection of DNA from people arrested or detained by federal authorities.[3]

England has undertaken the world's most aggressive DNA gathering effort, gathering close to 3.4 million samples in its DNA database, covering approximately six percent of the population.[4] Australia's military recently announced that it will ask 90,000 soldiers for DNA blood samples to help identify troops as the country prepares for more casualties in Iraq and Afghanistan.[5] With law enforcement and military agencies recognizing the power of DNA evidence for identification purposes, the number of samples being collected and submitted for testing has grown massively. Finding easy-to-use and reliable collection, storage and shipping technologies is rapidly becoming a priority as current methodologies are proving too cumbersome to handle the high volume of samples being generated.

Compromised Sample Integrity

Damage and degradation are major obstacles in the characterization of biological evidence. For successful forensic analysis, sample stability after long-term storage is critical, especially when the amount of DNA is limiting. Sample retesting is a vital component of forensic work, where trace evidence can lead to the exoneration of the innocent or identification of a suspect or victim. The need to retest the sample is always an important consideration to confirm or refute previous findings with the same or a new technology that may permit analysis of previously recalcitrant samples. Poor sample handling and storage, particularly across jurisdictions, may lead to further loss or degradation of the samples and in turn lead to less informative results. Factors compromising sample integrity include degradation, damage from UV exposure, temperature fluctuations, repeated freeze-thaw cycles and other sub-optimal storage conditions that occur not only during transport, but also in storage facilities. Samples that must be maintained under cold storage conditions (i.e., liquid nitrogen, -80°C, -20°C, or 4°C) are especially vulnerable and can also create significant logistical problems during transport. Consequently, samples may be compromised before analysis can occur.

Typically in forensics work, casework samples are stored for various lengths of time (most commonly months or years), with no standard time limit defined. For

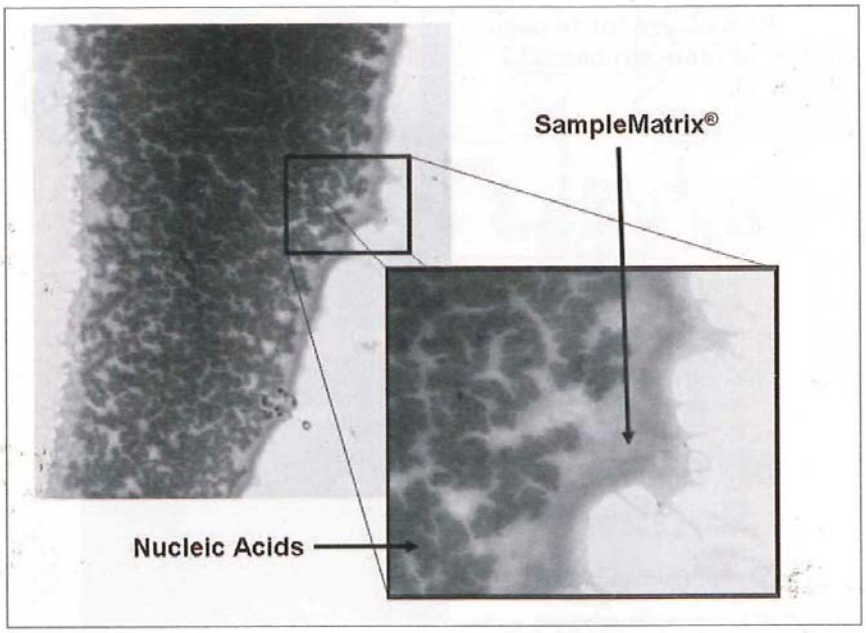

Figure 1: Electron micrograph of the SampleMatrix barrier. Electron microscopy shows the thermo-stable, glass-like shell that forms around nucleic acid molecules, which stabilizes and helps prevent degradation.

example, some samples are kept indefinitely, while others are stored only until the appellate process is complete. There is also no standard on where or how the DNA samples are stored and maintained; some labs store all their DNA samples in-house, while other labs return samples to the submitting jurisdiction for long-term storage. Given the current undefined nature of forensic casework, there exists a critical need to develop alternative handling, storage and shipping strategies to supplement or even replace current cold storage procedures. Implementing consistent and reliable protocols for sample storage is important for maintaining sample integrity over time. One technology that has been utilized is a paper-based, solid-phase collection technology. Blood samples collected on FTA paper are used as the starting material for DNA isolation, and analysis is performed with PCR-based technology. Samples can also be obtained from buccal swabs in the form of a foam applicator that are used for specimen collection. The applicator is then rubbed onto FTA paper for long-term storage of the dried cellular lysate sample. An alternative method uses a cotton swab for collection of buccal cells, the tip of which is then cut off and used directly for DNA extraction and subsequent PCR reaction. A vast majority of liquid DNA samples are stored in cold environments, and while DNA can also be stored dried for short periods of time, degradation can occur during storage that can irreversibly damage the samples.

Crime scene samples and liquid extract derivatives, obtained from samples collected using solid-based technologies or other methods, are routinely stored frozen until thawed at the time of analysis. Forensic scientists have also been able to utilize the expression profiles of biological samples to assist in the interpretation of tissue sources using RNA.[6] While the DNA profile of all tissues from an individual is essentially identical, the messenger RNA (mRNA) spectrum made by the different cells in each tissue type is diverse. Each tissue or cell type is composed of a unique expression profile of mRNAs, some specific for only that tissue or cell type. Therefore, analysis of the RNA expression profile in a sample may uniquely identify the fluid of origin. Thus, the ability to enhance stability of RNA, a highly labile molecule prone to degradation even under carefully controlled conditions, may provide more reliable analysis of expression profiles for this purpose.

Preventing Degradation

To address the need to stabilize and prevent the degradation of biological materials, Biomatrica, Inc. has developed a platform technology to store samples at room temperature without degradation, as a potential solution to the variables associated with biological sample collection, transport and long-term storage. SampleMatrix technology was designed by combining extremophile biology (long-term survival in extremely dry environments in an arrested life form) and synthetic chemistry. DNA SampleMatrix is a synthetic dissolvable matrix with unique thermo-stable properties that works by forming a protective shield around the sample as it dries, to prevent further damage and degradation (Figure 1). Using the same principles, Biomatica has also developed RNAstable for the room temperature storage of precious labile RNA samples (Figure 2).[7] Sample recovery is achieved through simple rehydration; recovered samples can be used directly without further purification in downstream assays, including PCR (i.e., real-time PCR, multiplex analysis, quantitative PCR and end-point PCR), genotyping, electrophoresis, bioanalyzer analysis, microarray analysis, STR analysis (i.e., PowerPlex 16, Identifiler and Profiler Plus), restriction analysis, transformation and cDNA synthesis (Figure 3). Studies indicate that fluctuating and inconsistent temperatures commonly experienced during shipment do not damage nucleic acids stabilized dry in DNA SampleMatrix or RNAstable. Rather, samples were protected from degradation, even at elevated temperatures of 50°C.

To evaluate the feasibility of developing and using Biomatrica's technology for forensics work, an international consortium of leading forensic laboratories evaluated using DNA SampleMatrix for the collection, storage and shipment of forensic source DNA samples. The preliminary results were recently presented at the 18th International Symposium on Human Identification. Data collected from the study indicate that genomic and mitochondrial DNA can be safely stored in DNA SampleMatrix without degradation or loss as compared to control samples. Recovered samples were used successfully in a variety of downstream applications, including quantitative PCR, STR analysis, electrophoresis and multiplex PCR, without inhibition or interference from the matrix. Studies are currently underway to evaluate the

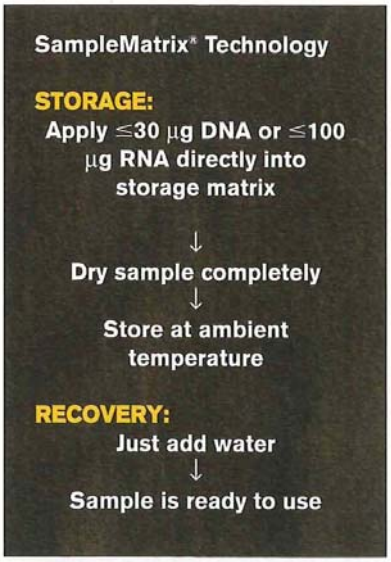

Figure 2: Protocol for DNA or RNA storage. DNA or RNA samples are applied directly into the appropriate storage matrix, dried and then stored at ambient temperatures. Sample recovery requires one-step rehydration, and the DNA or RNA is ready for use in downstream applications without the need for further purification.

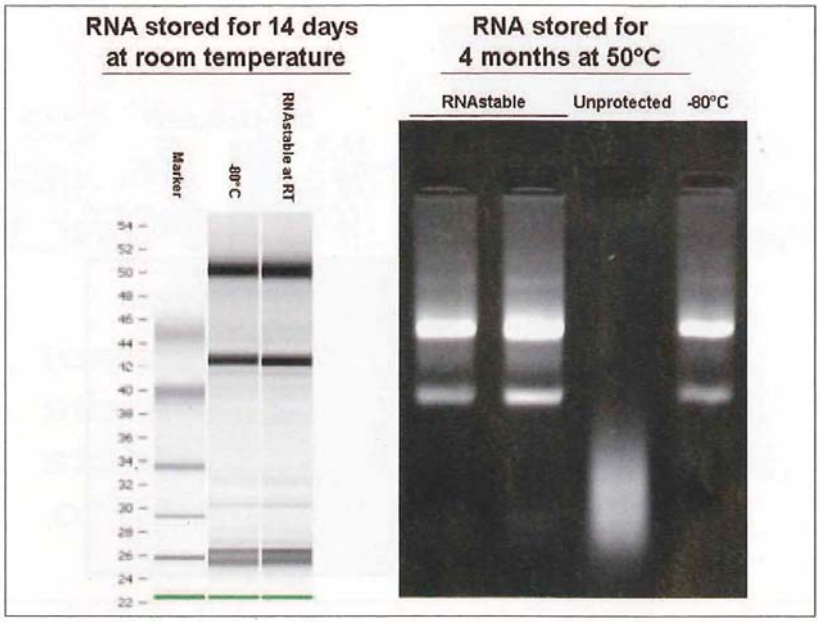

Figure 3: RNAstable protects RNA from degradation and damage from environmental insults. RNA samples stored dry either at room temperature or at elevated temperatures for long time periods are stabilized and protected from degradation. Results from bioanalyzer analysis indicate no detectable degradation of samples stored in RNAstable for two weeks at room temperature as compared to control samples (left). Samples stored in RNAstable were protected from degradation for four months at 50°C, while RNA stored unprotected was completely degraded.

use of DNA SampleMatrix to prevent the degradation of low copy number samples, samples previously extracted and typed from proficiency tests, degraded DNA samples, DNA extracted from bones and teeth, and samples subjected to multiple freeze-thaw cycles.

Future Testing

The National Institute of Justice recently awarded a grant to the School of Criminal Justice and Criminalistics at California State University, Los Angeles, as well as Biomatrica, to evaluate and develop SampleMatrix technology for use in collecting and storing biological evidence obtained from crime scenes. Various methods are used to collect biological material from crime scenes and impounded evidence, but the majority of samples are obtained by swabbing. The standard procedure in forensics laboratories for the storage of swabs is to first air-dry the samples, then package them in paper (ventilated) containers that are stored at 4°C or less until ready for analysis. The multiple freeze-thaw cycles typically associated with sample handling results in significant sample loss, thus hampering the ability to obtain reliable consistent results. The joint grant will allow testing and validation of SampleMatrix room temperature storage technology for use in stabilizing cell morphology, protein structure and enzymatic activity; retaining the integrity of cell morphology; and also reducing the exogenous degradation of DNA by minimizing the adverse effects of hydrolysis and stresses associated with multiple freeze-thaw cycles. Investigations will also focus on the stabilization of trace quantities of nuclear DNA, as would be derived from touch samples and environmentally challenged samples recovered from crime scenes using swabs. The development of room temperature storage of biological samples will have a significant impact on forensic analysis, as well as other fields of biological research and analysis, if it can be found to eliminate some of the detrimental variables associated with sample collection, transport and storage.

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