Evaluation of Biomatrica™ DNAstable® Room-Temperature DNA Storage System

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Introduction

Real time PCR and STR analysis are customary genetic methods commonly employed in evaluating genomic DNA from crime scene samples. These samples sometimes contain low amounts of genomic DNA. This is especially true of DNA samples recovered from many crime scenes requiring forensic DNA analysis. Biomatrica's DNAstable reagent is a novel bio-stability product developed to stabilize and preserve DNA against degradation at ambient room temperature. DNA extracts from forensic samples are typically analyzed by real time PCR and STR assays, as well as microarray experiments. DNAstable was tested and validated in comparison tests against conventional -20°C freezers. This study was performed to evaluate the reproducibility and reliability of DNAstable to protect low amounts of DNA samples at room temperature. In this study, DNA was purified from semen, buccal swabs and blood stains. The DNA samples were stored at room temperature (DNAstable; dry-matrix) and at -20°C for 45 months. Samples were rehydrated and analyzed without further purification necessary.

Materials and Methods

<u>Buccal swab, blood stain, and semen sample collection</u>: All samples were collected from human donors. Semen was placed onto a cotton swab and allowed to dry. The bloodstain was from a venous blood draw and spotted onto filter paper and dried. The buccal swab was collected onto a cotton swab. The method for genomic DNA isolation for all samples is using both: Qiagen EZ1 BioRobot, using the Tissue Extraction Kit and using Beckman Coulter Biomek 2000 with the DNA IQ Extraction Kit. Samples were quantified by using Applied Biosystems Human Quantifiler Assay on the ABI Prism 7500 Real-Time PCR system. The following amounts of purified genomic DNA was stored in DNAstable; 48ng of semen, 120ng of buccal, and 105ng of blood. Samples were dried in a laminar flowhood for approximately 24 hours. Samples were rehydrated at 12 month and 45 month timepoints and quantitated using real time PCR. Subsequently, STR analysis was used on the ABI Identifiler PCR/STR Typing Kit and the ABI-310 Genetic Analyzer.

Results Real time PCR:

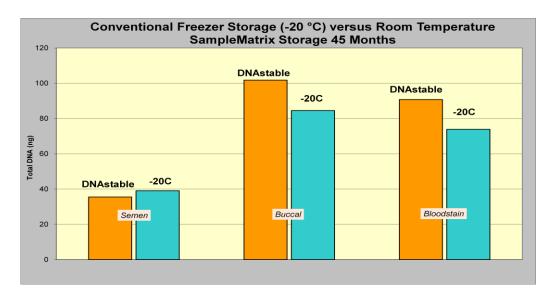


Figure 1: (A) Results from Applied Biosystems' Human Quantifiler Assay. Graph shows recovery of DNA samples extracted from semen, buccal and bloodstains and stored either in DNAstable (room temperature) or frozen (-20C) are comparable after 45 months.

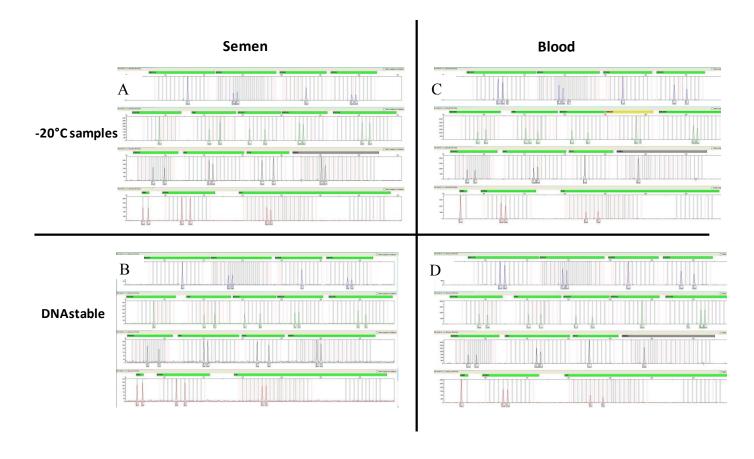


Figure 2. DNAstable maintains amplification and recovery of genetic profiles from blood and semen in comparison to the corresponding freezer controls. (A) DNA extracted from semen stored at -20°C. (B) DNA from semen stored in DNAstable at room temperature. (C) DNA isolated from blood and stored at -20°C. (D) DNA from blood stored in DNAstable at room temperature.

Discussion

As Figure-2 shows, DNAstable allows for long-term stabilization of forensic DNA samples at room temperature. DNA analysis of amplification and STR profiles show complete preservation of DNA stored in DNAstable similar to those stored in freezers (the loci from freezer samples are similar to those stored in DNAstable). More importantly, there are no allelic dropouts when comparing the freezer samples to DNAstable samples. Biomatrica's DNAstable is an excellent alternative bio-specimen storage technology complimentary to or as a replacement for -20°C freezers. The recovered DNAstable samples can be used directly in downstream applications such as PCR, STR, etc without the need for further purification.

Conclusions

The study described here provides data from approximately 45 months of room temperature storage of purified DNA extracts from different samples – bloodstains, buccal cells, and semen compared to storage in a -20°C freezer. Sample recovery and quality after storage using STR analysis showed that room temperature-stored samples (DNAstable) provided a slightly better performance than cold-stored samples (-20°C freezer). There were no discernible difference nor allelic drop-outs in STR-analyzed samples that were stored dry at room temperature compared to those stored frozen at -20°C. Our study finds that forensic DNA samples can be safely stored at room temperature in DNAstable as an alternative storage technique to traditional -20°C freezers, without risk of loss of sample quality or yield.