

SAMPLES STABILIZATION AT ROOM TEMPERATURE : FROM COLLECTION TO STORAGE



Trisha Dowling¹, Rolf Muller¹, Soheila de Rozieres¹, Austin Jacobsen¹, Peggy Ho Faix¹, Laurent Coulon¹ and Judy Muller-Cohn¹

ABSTRACT

Successful biobanking requires effective and efficient sample storage technologies that function to protect the integrity of collected specimens during long-term storage and transport. The challenge is to identify and implement innovative new technologies that enhance sample collection quality and manipulation. Biomatrixa has developed a technology, SampleMatrix[®], to preserve DNA from various complex samples at room temperature, such as tissues, blood and hair. The stabilization at room temperature is crucial and allows for the stabilization of DNA from the collection site through storage in the biorepository. SampleMatrix[®] technology can be adapted to robotic platforms and high-throughput work flows due to its ability to accommodate a variety of storage device formats, thereby facilitating efficient sample transfer from existing collections or the creation of new ones. A recent pilot study conducted by a large American University demonstrates that the university could cut usage of electricity by forty million kilowatt-hours, reduce its carbon footprint and save \$16 million dollars in operating costs over the next ten years by transferring biological samples from frozen storage to room temperature storage. Likewise, the benefits of utilizing room temperature stabilization technology in a biobanking environment can also be derived from the pilot study. Integration of alternative technologies into biobanking processes and workflows will provide significant cost-savings by reducing reliance on current cold-storage practices for sample storage and transport. Ambient temperature storage also provides a cost effective solution for secondary offsite back-up to adequately insure valuable sample collections.

INTRODUCTION

From Nature to the Lab

SampleMatrix[®] is a novel platform technology that directly preserves and stabilizes biological samples at ambient and elevated temperatures and prevents their degradation. SampleMatrix is based on the natural principles of anhydrobiosis (meaning "life without water"), a biological mechanism employed by some multicellular organisms that enables their survival while dry for up to 120 years. Anhydrobiotic organisms can protect their nucleic acids, proteins, membranes and cellular systems for survival and can be revived by simple rehydration. SampleMatrix technology transfers the molecular principles of anhydrobiosis to a synthetic chemistry-based stabilization science that works by forming a thermo-stable barrier to securely "shrink-wrap" samples such as nucleic acids, and provide protection against degradation (Fig. 1).

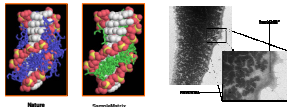


Figure 1. Structural Prediction of SampleMatrix interacting with Nucleic Acids. Molecular modeling prediction of interactions of SampleMatrix with nucleic acid molecules. Trehalose disaccharides are predicted to interact with nucleic acid molecules through minor groove interactions based on hydrogen bonding (Nature left). SampleMatrix is predicted to form similar interaction patterns as trehalose (SampleMatrix center). Electron microscopy shows the thermo-stable barrier that forms around nucleic acid molecules, which stabilizes and helps prevent degradation (right).

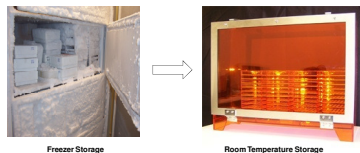


Figure 2. Biomatrixa's Innovative Room Temperature Storage System. SampleMatrix technology is designed to prevent the degradation of biological materials at room temperature and eliminate the need for conventional cold storage.

¹Biomatrixa, Inc., 5627 Oberlin Drive, Suite 105, San Diego, CA 92121

TRANSPORT

DNAgard™ was developed for biostability of DNA in complex sample using SampleMatrix® Technology. Complete ambient temperature sample management and flexible workflow

Tissue Stabilization at room temperature – biostability for DNA

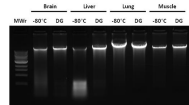


Figure 3. Preservation of tissue sections at room temperature. Tissue sections of small mouse brain, heart, liver, lung, muscle, and spleen were submerged directly in 500 µl DNAgard (DG) or frozen at -80°C as control samples. Tissue sections were harvested to be approximately of equivalent sizes, but not empirically weighed. Tissue sections submerged in DNAgard were stored at room temperature for 30 days. DNA was then isolated using DNA extraction columns and run on a 0.8% agarose gel stained with ethidium bromide. DNA isolated from tissues stored in DNAgard remained intact even after storage for 30 days at room temperature as compared to frozen control samples. Results indicate that tissue sections can be harvested and submerged in DNAgard for storage without compromising the quality or integrity of DNA obtained after subsequent DNA isolation.

Blood Stabilization at room temperature – for DNA

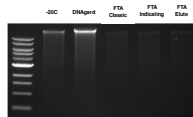


Figure 4. Preservation of whole blood at room temperature. Abouts 50 µl of whole blood were applied to 500 µl DNAgard or onto three different types of FTA cards. An identical aliquot was stored frozen at -20°C as a reference control sample. Samples were then allowed to incubate overnight at room temperature. DNA was then isolated using DNA extraction columns and run on a 0.8% agarose gel stained with ethidium bromide. DNA isolated from blood stored in DNAgard remained intact as indicated by the presence of high molecular weight DNA, compare to control sample stored frozen. Blood samples stored dry on FTA paper for identical time periods showed significant degradation and loss of DNA. In addition, storage in DNAgard significantly improved the yield of recovered full length intact cellular DNA.

Hair Stabilization at room temperature

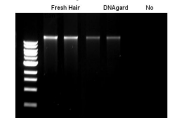
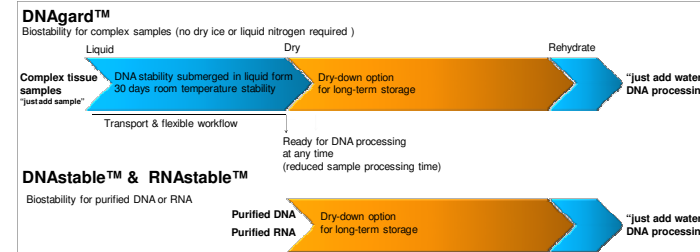


Figure 5. Preservation of DNA from hair. Two strands of hair with follicles attached were applied into tubes containing 500 µl DNAgard or water. Although the number of hairs preserved in each sample were identical, DNA amounts contained in the follicles cannot be empirically determined. Samples were then stored for an equivalent of 1.75 months at room temperature (-80°C for 7 days under accelerated aging conditions). A hair ball sample was then used for comparison during subsequent DNA extraction using column-based purification kits. DNA isolated from hair follicles stored submerged in DNAgard remained intact as indicated by the presence of high molecular weight DNA, compare to hair ball samples. In contrast, no DNA was isolated from hair follicles stored in water for the identical time period.



PROCESSING

SampleMatrix® Technology is compatible with most of the automation systems available on the market.

A variety of samples can be transported and stored at room temperature:

- DNA samples
- RNA samples
- Protein samples
- Buffy coat
- Complex samples

Open Platform Compatibility

Biomatrixa SampleMatrix[®] can be arrayed into any tube or multi-well plate. SampleMatrix[®] technology can also be adapted to robotic platforms and high-throughput work flows due to its ability to accommodate a variety of storage device formats, thereby facilitating efficient sample transfer from existing collections or the creation of new ones.



Biomatrixa technology allows flexibility to utilize the best automation system for your needs:

- Nexus Biosystems
- RTS
- Custom solutions

Large scale automated sample management at more convenient temperatures

Advantages:

- Protection during prolonged power outage
- High integrity sample management
- Cost effective automation
- No freeze/thaw
- Scalable Biobanking
- Easy cherry picking
- Green solution
- User configurable
- Easily expandable

STORAGE

Large Scale Room temperature Pilot Project Case Study

Background: Researchers have assembled large collections of biological samples from clinical and field studies, representing an enormous scientific and financial value for the researcher and the university.

A major research university in California recently completed a large scale pilot project to evaluate and to transfer their biosamples to Biomatrixa's room temperature storage technology.

Goal: Reduce reliance on current methods ultra-cold storage for storing and protecting its biological samples.

To transfer biological samples from freezers to room temperature storage technology within a diverse group of university research laboratories and evaluate environmental, financial and additional benefits.

Cost, Savings and Sustainability Results:

- The pilot data provided the basis for using the model to make a conservative estimate of the total number of addressable samples on campus.

One campus	Accumulative 10 years Savings
Cost Savings	\$16-20 Million
Energy Reduction	>220,000 Million BTU
Carbon Footprint Reduction	18,000 Metric Tons of CO2

Additional Results:

- Reduced risk of sample loss
- Integration of the complete Biomatrixa's technology in research workflow results in significant cost savings
- Effective solution also for secondary offsite back-up
- Protect valuable sample collections from freezer breakdown and natural disasters

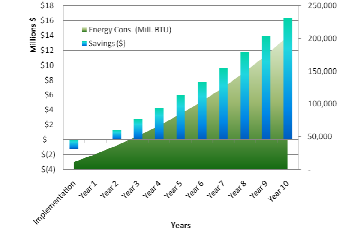


Figure 7. Total Accumulated Energy and Cost Savings.

CONCLUSION

Complex Sample Stability

- DNA gard™ - DNA stability for whole tissue samples**
- >Stabilize DNA immediately in complex samples and during subsequent purification.
 - >**Submerged - samples** are stable for **30 days** at room temperature without degradation. Ideal for shipping facilitated workflow.
 - >Unique **dry-down** feature allows convenient long-term storage without impact on workflow

Large Scale Pilot Study a Major Research University

- >Significant cost savings for large scale operations
- >Risk management with reduced sample loss
- >Large energy savings and reduced carbon footprint