Room Temperature Storage of Biological Samples

Sohela De Rozieres, Sharron Ohgi, Peggy Ho Faix, Judy Muller-Cohn and Rolf Muller

ABSTRACT

We have developed a novel biological sample stabilization technology designed to protect complex samples (*e.g.* nucleic acids, bacteria, viruses, cells and blood) from degradation during dry storage at room temperature. The storage medium 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 >100 years. The objective of this study is to evaluate and develop an alternative handling, storage and shipping strategy to optimize complex biological sample stabilization for use in life science research. Results demonstrate stabilization of DNA (genomic, plasmids, oligonucleotides, PCR products, etc.) for >2 years at room temperature (RT) and for an equivalent of >13 years under accelerated aging conditions. Storage of crude bacterial cultures in the medium allows for rapid recovery of bacterial genomic and plasmid DNA that can be used directly in transformations, PCR, electrophoresis and restriction analysis. Many different sample types have been successfully stored in the medium. Data for the inhibition of degradation at even extremely elevated temperatures will be presented, including stability of total RNA purified from mammalian cells, blood, in addition to protein-based samples. Results indicate that recovered samples can be used directly in downstream applications without interference or inhibition following dry storage, including transformation, transfection, cloning, sequencing, PCR, restriction analysis, hybridization, electrophoresis, microarray analysis, STR analysis, whole genome amplification, genotyping and *in vitro* transcription. Results indicate that technology advances in sample preservation can prevent the degradation process of biological samples.

INTRODUCTION

Currently, collection, transportation and storage of complex biological samples such as DNA, RNA, proteins, bacteria, blood, and other biological molecules requires maintaining cold environments to prevent or at least reduce the rate of degradation. Maintaining cold storage environments, even for small laboratories, requires multiple expensive refrigeration and freezer units, all of which consume energy and limited laboratory resources. Current methods of sample collection and transport back to the laboratory are also problematic, as shipping frozen samples on dry ice is expensive, with shipments ranging up to hundreds of dollars due to bulky containers and expedited delivery costs. Unfortunately, even under carefully monitored cold storage and shipment environments, repeated freeze-thaw cycles and fluctuating temperatures only serve to promote degradation and compromise results.

Despite all the precautions taken to maintain what is typically referred to as cold chain logistics, the integrity of a sample is often compromised using current cold storage methodologies, particularly during long-term storage. For example, the average DNA sample lasts for about 10 years under cold storage conditions; unfortunately, this is not long enough if the sample itself is needed for future reference, as in the case of forensic samples or those used for diagnostics. There is also a critical need to develop technologies for simple and efficient collection, transport and storage of bacterial and blood samples that does not require cold chain logistics or complicated recovery protocols. For example, current methodologies for blood sample collection relying on paper-based technology requires lengthy protocols for recovery of genomic DNA at low yields. Until recently there were no products that stabilized complex biological samples at ambient temperatures as a means to eliminate the need for cold chain logistics and facilitate sample collection, transport and storage.

From Nature to the Lab

Biomatrica has developed a high-performing biostabilization technology to prevent the degradation of biological materials at room temperature. SampleMatrix® is Biomatrica's novel platform technology that directly preserves and stabilizes biological samples at ambient and elevated temperatures. 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. Biomatrica's technology transfers the molecular principles of anhydrobiosis to a synthetic chemistry-based stabilization science that works by forming a thermo-stable barrier securely "shrink-wrapping" samples, such as nucleic acids and providing protection against degradation (Fig. 1).

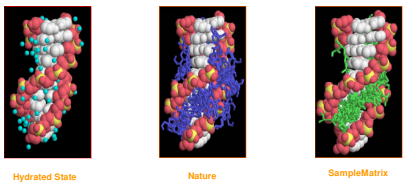

Figure 1. Structural Prediction of SampleMatrix interacting with Nucleic Acids. Molecular modeling prediction of interactions of SampleMatrix with nucleic acid molecules. Nucleic acid shown in hydrated form (Hydrated state; left). Trehalose disaccharides are predicted to interact with nucleic acid molecules through minor groove interactions based on hydrogen bonding (Nature; *middle).* SampleMatrix is predicted to form similar interaction patterns as trehalose (SampleMatrix; *right).*

SampleMatrix Technology

SampleMatrix is supplied as a dried matrix on the bottom of tubes or in a 96-well plate format. Each sample storage tube or well contains enough matrix to protect up to 30 µg of DNA, 10 µl of an overnight bacterial culture or ≤50 µl whole blood, depending on the specific SampleMatrix storage product. The steps involved in using SampleMatrix for storage of biological samples are outlined in Figure 2. By adding samples resuspended in water, buffer, growth media (*e.g.* LB media) or whole blood, SampleMatrix is rehydrated and mixes with the sample. Air-drying of the mixture results in a stabilizing glass that serves to protect the sample from degradation. Once completely dried, samples can be stored at room temperature or even elevated temperatures at relative humidity conditions ≤50%.

Sample recovery requires simple rehydration using water or a buffered solution. Furthermore, since the rehydration volume can be chosen between 10-100 µl, storage of samples in SampleMatrix also provides an easy method for sample concentration, eliminating the need for time-consuming salt precipitations and sample loss due to multiple wash steps or micro-concentration columns. Most samples recovered following storage in SampleMatrix can be used directly in downstream applications (such as reverse transcription, cDNA synthesis, PCR, gel electrophoresis, hybridization analysis, bioanalyzer and microarray analysis) without inhibition or interference; additional purification is required to recover genomic DNA following storage of whole blood in SampleMatrix optimized for blood storage.

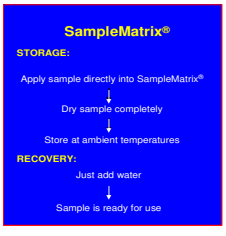

Figure 2. Protocol for sample storage in SampleMatrix. Samples are applied directly into SampleMatrix, dried, and then stored at ambient temperatures with ≤50% relative humidity. Sample recovery requires one-step rehydration, and the sample is ready for use in downstream applications without the need for further purification.

RESEARCH STATEMENT

The objective of this study is to evaluate and develop an alternative collection, shipping and storage strategy to faciliate biological research. Studies were designed to evaluate the feasibility of using SampleMatrix for the ambient temperature storage of biological samples such as DNA, bacterial cells and blood, and their subsequent use in downstream applications. Recovered samples were used directly without further purifying to assess any interference or inhibition in downstream applications including electrophoresis, PCR, quantitative PCR, tranformation and genomic DNA purification procedures.

MATERIALS AND METHODS

Sample Preparation and Storage: Aliquots of DNA (plasmids, genomic DNA or oligonucleotides) were applied to DNA SampleMatrix in the 1.7 ml standard microfuge tube format or 96-well plate format and allowed to dry overnight in a laminar flow hood before sealing and storage as described in each figure. Non-protected control samples (NP) were prepared by drying equivalent aliquots of DNA into an empty tube under identical conditions. Frozen control samples stored liquid (*i.e.* buffer or water) were kept at -20°C for identical time periods. Samples were rehydrated with water and used directly in various downstream applications without further purification. Aliquots of overnight bacterial cultures grown in LB media and 10 mg/ml ampicillin were applied directly into empty 1.7 ml standard microfuge tubes (unprotected) or tubes containing CrudE and allowed to dry overnight in a laminar flow hood before storage at room temperature for various times. Samples were rehydrated with water and used directly in downstream applications without further purification. Aliquots (10 µl or 50 µl) of whole blood were applied directly into SampleMatrix formulated and optimized for storing blood and allowed to dry overnight in a laminar flow hood before sealing and storage for various time periods. Samples stored in the matrix were rehydrated with 100 µl water and used directly for genomic DNA purification using the QIAamp DNA Blood Mini Kit following manufacturer's protocol. Identical samples were applied to Whatman FTA cards where described and stored dry for the same time periods. Frozen control blood samples were stored at -20°C until ready for use.

Transformations: Aliquots of plasmid DNA recovered following storage in DNA SampleMatrix were used directly to transform competent *E. coli* (DH5α or Stbl2) as described and following standard molecular biology techniques. Plasmid DNA recovered by rehydration following storage of bacterial cultures in CrudE were used directly for transformation into competent cells without further purification. Aliquots of transformed cells were plated on LB plates containing 10 mg/ml ampicillin and grown overnight at 37°C. Colonies were counted the following day.

Quantitative PCR analysis: Aliquots of genomic DNA recovered following storage of blood were used as template for QPCR to determine recovery yields. TaqMan® reagents were used for the QPCR amplification following manufacturer's instructions using primers specific to the human β-actin gene.

RESULTS

Long-term Storage

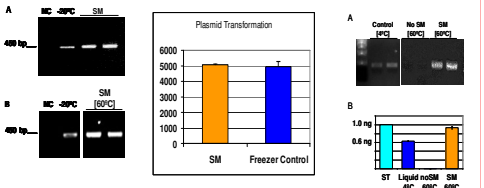

Figure 3: A 50 ng aliquot of pUC18 plasmid DNA stored in DNA SampleMatrix for (A) **2 years at ambient temperature** or (B) 10 months at 60°C under accelerated aging conditions **(equivalent to 11 years of room temperature storage),** were analyzed by PCR using pUC18 specific primers. Control DNA (50 ng) was stored in at -20°C, NC: negative control.

Figure 4: Comparison of colony counts of bacteria transformed with pCU18 stored for **1.5 years at room temperature** in DNA SampleMatrix (SM) compared to control plasmid DNA stored at -20°C.

Figure 5: Aliquots (20 ng) of human genomic DNA were stored in DNA SampleMatrix (SM) or left unprotected (No SM) and stored to 60°C for 10 months under accelerated aging conditions **(equivalent to 11 years of room temperature storage)**. An identical 20 ng aliquots of control DNA was stored at 4°C for 10 months. (A) PCR; (B) QPCR: 1 ng fraction of stored material; fresh DNA standard (ST = 1 ng).

Protection Under Extreme Conditions

Autoclaving

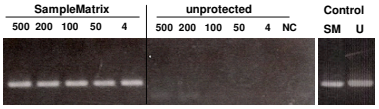

Figure 6. DNA SampleMatrix protects sample even after autoclaving. Various amounts (500, 200, 100, 50 and 4 ng) of human genomic DNA were applied into DNA SampleMatrix or empty tubes (unprotected) and allowed to dry overnight in a laminar flow hood. The samples were then subjected to a dry autoclave run at 250°F/121°C for 15 min at 15 lb/in² and dried for 30 min at 150°F/66°C. Cooled samples were rehydrated with 10 µl of water and used directly in PCR reactions to amplify the human β-actin gene. Aliquots (10 µl) of each PCR reaction were run on an 0.8% agarose gel containing ethidium bromide; control reactions of DNA stored at room temperature in SampleMatrix *(right)* or unprotected in the freezer are shown for comparison *(middle).* DNA protected in SampleMatrix was used successfully as templates for PCR reactions (*left*) while unprotected DNA sample failed to amplify following autoclaving (*middle*).

High Temperature (158°F/70°C)

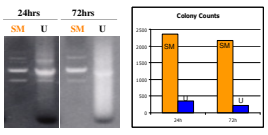

Figure 7. Aliquots (1 µg) of pUC18 plasmid were stored dry in SampleMatrix (SM) or unprotected (U) at 70°C for 24 or 72 h. Samples were then rehydrated in water and an aliquot was run on a 0.8% agarose gel containing ethidium bromide (*left*). Unprotected DNA was degraded after 72 h at high temperature. Aliquots of heat stressed DNA were used to transform bacteria; colonies were counted the next day. Plasmid DNA protected in SampleMatrix (SM) after exposure to high heat successfully transformed *E. coli* while the transformation efficiency of unprotected (U) samples were significantly reduced *(right).*

Rapid One-Step Recovery of Bacterial Genomic and Plasmid DNA

Bacterial cultures in selective growth media can be applied directly into CrudE SampleMatrix and dried for long-term storage of bacterial genomic and plasmid DNA. Bacterial DNA is recovered by a one-step rehydration procedure that results in DNA that can be used directly for PCR analysis and transformation without the need for purification, thus bypassing the need for DNA purifications (*e.g.* minipreps).

Recovered Bacterial DNA Following Long-Term Storage in CrudE SampleMatrix

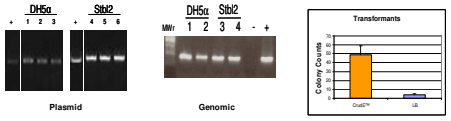

Figure 8. Aliquots of overnight bacterial cultures in selective growth media were applied directly into tubes containing CrudE and dried overnight in a laminar flow hood. Samples were then stored for 5 months at 50°C (**equivalent to 3 years at room temperature**). Bacterial DNA (genomic and plasmid) was recovered by rehydrating with 10 µl of water and aliquots were used directly in PCR reactions or transformation into competent *E. coli*. Amplification with plasmid specific primers resulted in amplicons of the expected size for pUC18 and pFIV plasmids (*left*; 490 bp (lanes 1-3) and 600 bp (lanes 4-6), respectively). Amplification with 16S ribosomal specific primers resulted in amplicons of the same size as the positive control frozen sample (*middle*: DH5α, lanes 1-2; Stbl 2, lanes 3-4; control lanes (-/+), indicating protection of bacterial genomic DNA in CrudE. Recovered plasmid DNA was used directly for transformation. Colony counts were compared to transformations using plasmids recovered from unprotected bacterial cultures stored under identical conditions (*right*). Results indicate protection of bacterial DNA during dry storage of bacteria cells in CrudE.

Room Temperature Storage of Blood Samples

SampleMatrix technology was used to develop a medium for the room temperature storage of whole blood in volumes up to 50 µl. Recovery of genomic DNA stored dry at room temperature protected in the matrix indicates improved sample protection and yield of recovery as compared to paper-based mediums (*e.g.* FTA-cards).

Improved Recovery of DNA Following Long-Term Storage of Blood

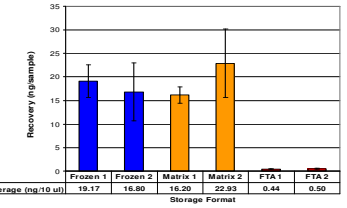

Figure 9. Aliquots of whole blood (10 µl) were stored dry in matrix or on FTA-cards for 6 months at room temperature. Control samples were stored at -20°C. Samples stored in the matrix were rehydrated and used directly without further purification for genomic DNA extraction with the QIAamp DNA Blood Mini Kit following manufacturer's protocol. Samples stored dry on FTA-cards were processed following instructions provided in the kit. QPCR analysis of recovered DNA indicates comparable levels of recovery following dry room temperature storage in the matrix as compared to frozen control samples, while recovery yields were significantly reduced following storage on FTA-cards.

Recovery of DNA From Blood After 6 Weeks Storage at Room Temperature

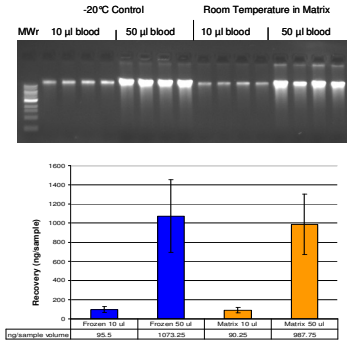

Figure 10. Aliquots of whole blood (10 µl and 50 µl) were stored frozen or applied into matrix and dried. Dried samples were then stored for 6 weeks at room temperature. Rehydrated samples were used directly for genomic DNA purification as described above. Aliquots were run on a 0.8% agarose gel and stained with ethidium bromide (*top*). DNA recovered from blood samples stored in the matrix were comparable to control samples. Aliquots of recovered DNA were also used as templates for QPCR analysis as described above. Results indicate comparable recovery levels (ng/sample volume) between samples protected in matrix at room temperature to frozen controls (*bottom*).

CONCLUSIONS

- SampleMatrix technology allows for long-term stabilization of biological samples at ambient temperatures with ≤50% relative humidity.
- Sample recovery requires a simple one-step rehydration – just add water.
- Recovered samples can be used directly without the need for further purification in downstream applications. Fluctuating and inconsistent temperatures during shipment will not damage samples stabilized dry in SampleMatrix.
- Sample stability is secured even at elevated temperatures.
- Studies are currently underway to evaluate the compatibility of SampleMatrix for storing other types of biological samples including cells, tissues and viruses. Long-term studies are ongoing for room temperature storage of blood in SampleMatrix.
- Results indicate that technology advances in sample preservation can prevent the degradation process of biological samples in life science research.