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Novel Nucleic Acid Stabilization and Enhancement Technologies for PCR Analysis

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ABSTRACT

We have developed several novel technologies designed to improve upon life science research. One technology stabilizes and protects nucleic acid samples from degradation during storage at room temperature (RT). We have also developed a highly effective PCR enhancer reagent that improves amplification during PCR-based analysis. The objective of this study is to evaluate these technological developments and assess their usefulness in cell and molecular biology research applications. The development of ambient temperature stabilization technologies provide a useful alternative to current cold storage methods, and has particular application for sample shipments to core facilities. We will also present data on enhancing PCR-based analysis for compromised or limited sample types.

The stabilization technology is based on extremophile biology that allows some organisms to survive while in a dry state for >100 years. The synthetic medium forms a thermo-stable barrier during the drying process to protect samples from degradation during storage at RT. Results demonstrate stabilization of DNA for >2 years RT, an equivalent of >14 years under accelerated aging conditions. Bacterial cultures or whole blood samples were also stored in the medium and results indicate stabilization of DNA, even when contained in the unpurified samples. Purified total RNA was successfully stabilized for >8 months at RT and even at 50°C for long time periods. DNA and RNA samples were recovered using a one-step rehydration protocol and used directly without further purification in downstream applications. Data will also be presented demonstrating improved PCR amplification with inclusion of a polymerase enhancer, particularly for compromised and difficult to amplify templates. Studies to evaluate the stabilization of other protein-based samples dry at RT are on-going. Advances in sample preservation that prevent degradation at ambient temperatures and enhanced PCR-based detection will have a significant impact for improving genomic technologies used in biological research.

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).

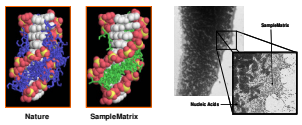


Figure 1. Structural Prediction of SampleMatrix interacting with Nucleic Acids. Molecular modeling prediction of interactions of SampleMatrix with nucleic acid molecules. Trehalose disacchrides are predicted to interact with nucleic acid molecules through minor groove interactions based on hydrogen bonding (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).*

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 100 µg of RNA. The steps involved in using SampleMatrix for DNA or RNA storage are outlined in Figure 2. By adding nucleic acids in either water or buffer, DNA SampleMatrix or RNAstable is rehydrated and mixes with the sample. Through its natural affinity to DNA or RNA, the matrix associates with the nucleic acid in the liquid phase. Air-drying of the mixture results in a stabilizing glass that serves to protect nucleic acids from degradation. Once completely dried, samples can be stored at room temperature and relative humidity conditions ≤50%.

Sample recovery requires simple rehydration using water or a buffered solution. Furthermore, since the re-hydration volume can be chosen between 10-100 µl, storage of DNA or RNA in the matrix 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. Recovered rehydrated samples can be used directly in downstream applications (such as reverse transcription, cDNA synthesis, PCR, gel electrophoresis, bioanalyzer and microarray analysis) without inhibition or interference.

Materials and Methods

Sample Preparation and Storage of Nucleic Acids: For purified DNA or RNA samples, aliquots of human genomic DNA or total RNA prepared from human 293T cells were applied to DNA SampleMatrix, RNAstable or RNAconcentrator in individual tube formats and allowed to dry at room temperature in a laminar flow hood (DNA samples) or vacuum concentrator (RNA samples). Non-protected control samples (NP) were prepared by drying identical aliquots into an empty tubes using appropriate conditions. Dried samples were then stored at room temperature or at elevated temperatures to simulate accelerated aging conditions for various times with relative humidity of <50%. Samples were rehydrated by adding water and incubating for 10 min. Rehydrated samples were used immediately in downstream applications without further purification. Frozen reference samples (positive control) were kept at -20°C for identical time periods.

TaqMan® One-Step RT- PCR: Following dry storage for various times at room temperature or elevated temperatures (50°C or 60°C), DNA or RNA samples were rehydrated in treated water to a final concentration of 20 ng/µl for each sample. Serial dilutions were performed as needed to a final concentration of 0.2 ng/µl. A 5 µl aliquot of each sample was used as template for expression of the 18s rRNA gene or RNase P gene using TaqMan® One-Step RT-PCR (ABI) reagents and appropriate primers. A final concentration of 400 mM was used for each forward and reverse primer in the reaction. A 250 mM final concentration of the 18s rRNA or RNase P probe was used (5' labeled with FAM and 3' labeled with TAMRA). Reactions were prepared in a 25 µl final volume in triplicate.

Results

Long-term Storage of Genomic DNA

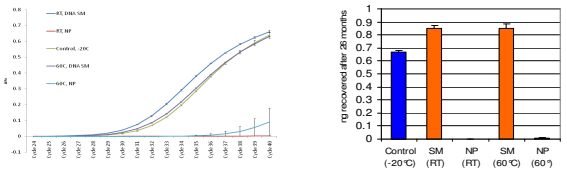


Figure 2. Quantitative PCR traces of triplicate experiments with standard deviation displayed. A theoretical total concentration of 1 ng per reaction was quantified using a primer/probe set specific for the RNaseP gene (triplicate reactions) to determine the final amount of gDNA remaining in each well after 26 month of storage (TaqMan® One-Step RT-PCR; ABI) *(left).* Amount of DNA recovered (adjusted to final input concentration) *(right).* Samples were stored in DNA SampleMatrix (SM) or left unprotected (NP) for 26 months at either ambient room temperature (RT) or under accelerated aging conditions **equivalent to 30 years of room temperature storage** (60ºC). An identical aliquot of control DNA was stored at -20ºC for the same time period. Results of qPCR analysis indicate significant recovery of the original gDNA sample stored dry in DNA SampleMatrix, which significantly outperforms performance of the -20°C freezer control after an equivalent storage time interval.

Protection of DNA Under Extreme Conditions (Autoclaving)

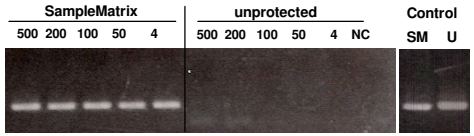


Figure 3. DNA SampleMatrix protects sample even after autoclaving. Various amounts (500, 200, 100, 50 and 4 ng) of human genomic DNA were applied into DNA SampleMatrix or empty tubes (unprotected) and allowed to dry overnight in a laminar flow hood. The samples were then subjected to a dry autoclave 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*).

Enhanced PCR-based Analysis

PCRBoost™ is a reagent that enhances end-point and reverse transcription PCR performance by improving sensitivity and specificity during amplification of difficult templates. PCRboost is used in place of water in a standard reaction, eliminating the need to optimize cycling conditions and reaction parameters.

Improved Sensitivity and Yield

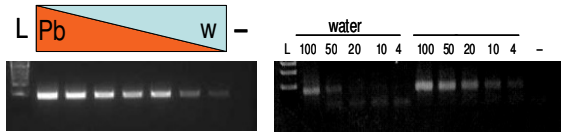


Figure 4. Addition of PCRboost improves sensitivity and yield during amplification of human genomic DNA templates. Aliquots (50 ng) of gDNA were amplified using human β-actin primers in decreasing PCRboost (Pb) and increasing water (w); (-) no template control; (L) ladder (*left*). Aliquots of 100, 50, 20, 10 or 4 ng of gDNA were used for PCR amplification in water or PCRboost (Pb); (-) control; (L) ladder (*right*).

Compatibility with Commercial *Taq* Polymerases

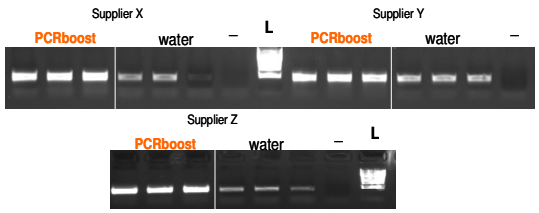


Figure 4. Aliquots (10 ng) of human gDNA were amplified with human β-actin primers in PCRboost or water using Taq polymerases from 3 different suppliers (X, Y and Z); (-) no Taq control; (L) ladder.

Protection of RNA at Room Temperature in RNAstable

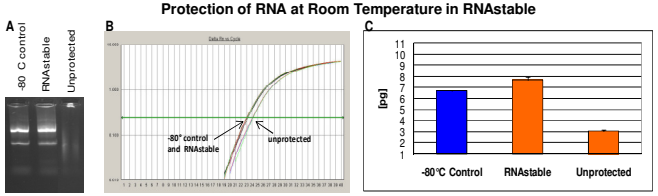
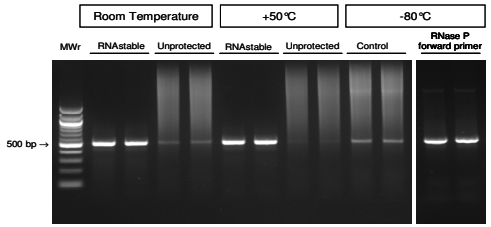


Figure 6. (**A**) Aliquots for total RNA were stored at either -80°C or at room temperature protected in RNAstable or unprotected for **14 months**. The integrity of the RNA following storage was analyzed using agarose gel electrophoresis. (**B**) Aliquots of the stored RNA samples were quantified using the 18S rRNA gene in a TaqMan One-Step RT-PCR reaction (ABI). Three samples were amplified for each storage condition and the results from the 9 reactions are overlaid on the graph. (**C**) Recovery levels (pg) for total RNA stored under different conditions.

RNAstable protects poly(A) mRNA after 14 months storage



Oligo dT used in 1st strand synthesis

Figure 7. Aliquots (500 ng) of total RNA purified from 293T cells was stored dry in RNAstable and kept at room temperature, 50°C, and -80°C for 14 months. Samples were rehydrated with 5 µl water and the entire volume was used in reverse transcription reactions to generate cDNA. A 5 µl aliquot of the cDNA reaction product was used to amplify the RNaseP amplicon (517 bp). Results indicate that RNAstable is protecting full-length poly(A) mRNA transcripts from degradation during long-term storage better than conventional freezer storage.

RNAconcentrator Prevents Sample Loss

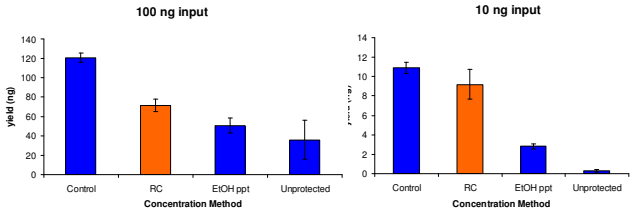


Figure 8. Various amounts of input purified total RNA from 293T cells were concentrated using RNAconcentrator (RC), a product specifically formulated for concentrating low quantity RNA samples without significant sample loss. RNA was applied into tubes containing RNAconcentrator and dried with a vacuum concentrator. Samples were recovered by rehydrating in a desired volume and added directly to quantitative RT-PCR reactions to asses yield of recovered RNA. Traditional ethanol precipitation (EtOH ppt) was also performed with identical amounts of input RNA. Unprotected samples were dried into empty tubes and rehydrated per RNAconcentrator samples. For control reactions, samples were removed directly from the stock RNA preparation and used directly without further concentration. Results indicate improved recovery using RNAconcentrator to concentrate low amounts of input RNA as compared to traditional ethanol precipitation where multiple wash steps can contribute to sample loss.

Conclusion

- SampleMatrix technology protects biological samples from degradation at ambient temperatures, and even at extremely elevated temperatures during long-term storage with relative humidity ≤50%.
- Sample recovery requires a simple one-step rehydration protocol – just add water.
- Recovered samples can be used immediately without further purification and are compatible with downstream applications.
- Sensitivity and yield are improved with the addition of PCRboost in standard PCR reactions, particularly with difficult to amplify templates.
- Results indicate that technology advances in sample preservation can prevent the degradation process of biological samples in life science research.
- Products are currently being developed for stabilizing proteins, complex samples and bacterial cells at convenient temperatures to facilitate efficient sample storage, processing and transport for the life sciences community.

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