Innovative technology to stabilize DNA at room temperature in tissues and cells

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INTRODUCTION

Current methods for storing and shipping human and animal tissue or cells for clinical, forensic and biomedical research needs are costly and can be insufficient for reliable molecular diagnostics requiring preservation of high-quality genomic DNA. Animal tissues are commonly shipped on dry-ice or in liquid nitrogen which are costly and often not practical for the collection of samples in the field. Another common method of tissue preservation, formalin fixation followed by paraffin embedding (FFPE), is also impractical for field collection and often results in damage to nucleic acids. We have combined synthetic chemistry (Figure 1) with the natural principles of anhydrobiosis[1] (the capacity of some organisms to protect their molecular integrity and survive extreme desiccation for hundreds of years) to stabilize genomic DNA in animal tissue and mammalian tissue culture cells at room temperature.

Biomatrica has created a novel formulation, DNAgard® that preserves the integrity of genomic DNA in tissue and cells stored for at least 6 months at room temperature.

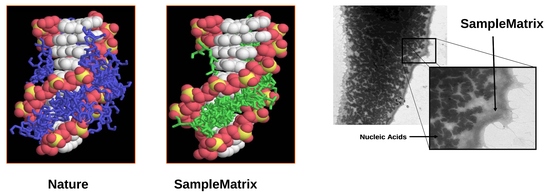


Figure 1. Structural Prediction of SampleMatrix™ interacting with Nucleic Acids. Molecular modeling prediction of interactions of SampleMatrix with nucleic acid molecules. Trehalose disacchrides are predicted to interact with nucleic acid molecules through minor groove interactions based on hydrogen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

MATERIALS AND METHODS

Sample Preparation and Storage in DNAgard: Upon harvesting, rat organs were fragmented, weighed (approx. 25 mg samples), immediately submerged in DNAgard or water (non-protected) and stored at room temperature. Control samples were frozen and stored at -80°C. Samples were then stored at room temperature for various times. Total DNA was recovered from the stored samples using commercially available column purification technology.

DNA purification:

DNA was isolated from tissue fragments and cultured cells using the QIAamp DNA Mini Kit (QIAGEN). The QIAamp protocol for extracting DNA from tissue samples was modified to minimize reagent volumes, as described in the DNAgard Tissue & Cells Handbook (Appendix A)[2].

Analysis:

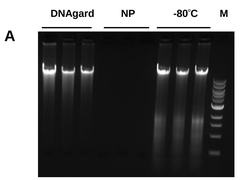
DNA yield and integrity was analyzed on a 0.8% agarose gel by directly comparing the recovery from equal mass amounts of tissue.

Real-time PCR: Genomic DNA isolated from rat kidney tissue was quantified using real-time PCR amplification of the β-actin gene.

***Sequencing*: .** After 70 days, total DNA was recovered from the stored samples using a commercially available column purification technology. A 296 bp portion of the rat β-actin gene was sequenced[3] (dye-terminator sequencing) from DNA isolated at time zero and from specimens stored for 70 days frozen or in DNAgard.

RESULTS

No degradation after 6 months at room temperature



B

DNAgard	NP	-80°C
245 45	ND	245 35

Figure 3. A: Genomic DNA in rat kidney is stabilized for over six months in liquid DNAgard. Rat kidney fragments were weighed (approx. 25 mg each) and immediately submerged in DNAgard solution or water (NP) and stored at room temperature. Control samples were frozen and stored at -80°C. After 195 days, total DNA was recovered from the stored samples using a commercially available column purification technology. DNA yield and integrity was analyzed on a 0.8% agarose gel (M = 1 kb ladder). **B: Picogreen quantification indicates equivalent DNA recovery from DNAgard samples and frozen samples.** Yield is presented as ng of DNA per mg tissue for the animal tissue samples

DNAgard® Technology

DNAgard is designed for the immediate stabilization of DNA in mammalian cells and tissues with the convenience of room temperature shipping, processing and storage. The liquid storage reagent rapidly permeates cell membranes to stabilize and protect genomic DNA. An optional dry-down feature permits storage at room temperature for at least one year, eliminating the need for freezers or liquid nitrogen. Dried samples are recovered by simple rehydration and are ready for subsequent DNA isolation using standard extraction techniques.

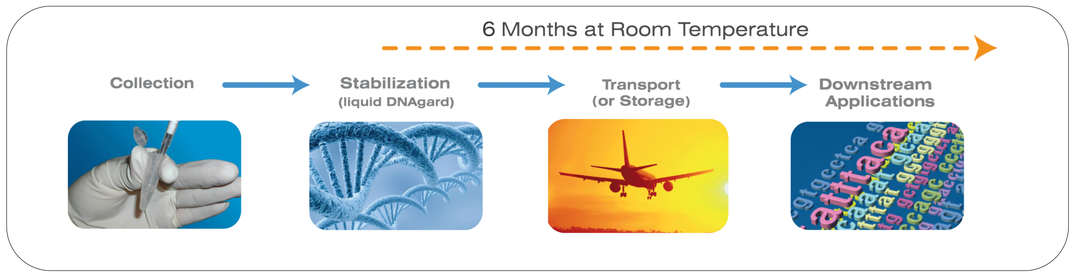


Figure 2. DNAgard workflow process.

DNA Integrity in a variety of tissues

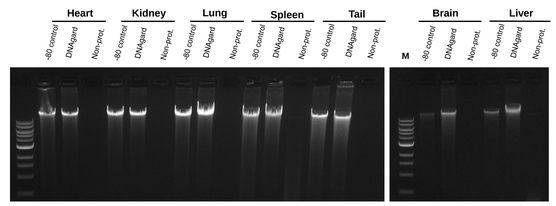


Figure 4. DNAgard stabilizes genomic DNA at room temperature from a variety of rat tissue types similar to conventional freezing. 25 mg samples of rat organs were submerged in DNAgard or water (non-protected) and stored at room temperature. Control samples were frozen and stored at -80°C. After 70 days, total DNA was isolated from the stored samples using a commercially available kit purification technology. DNA yield and integrity was analyzed on a 0.8% agarose gel by directly comparing the recovery from equal mass amounts of tissue.

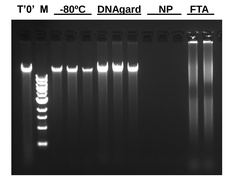


Figure 5. Liquid DNAgard protects genomic DNA in mammalian tissue culture cells at room temperature as well as storage at -80°C. 10^6 293T cells were pelleted and the supernatant (media) was removed. Cell pellets were resuspended in 500 µl of DNAgard or water (non-protected). For comparison, 10^6 cells were spotted on Whatman FTA paper and dried. As a positive control, 10^6 cells were stored at -80°C in 10% DMSO. DNAgard, Whatman and non-protected samples were stored at room temperature. After 84 days of incubation samples were processed for DNA isolation using a commercially available column purification technology. (The DNA was eluted from the Whatman FTA samples using Whatman's published protocol). The DNA yield and integrity from 5 x 10^4 293T cells was compared directly on a 0.8% agarose gel. (T'0' is DNA recovered at the time cells were harvested; M = 1 kb ladder).

DNA yield after 2 months storage

Sample	-80°C	DNAgard
Heart[1]	138	102
Kidney[1]	255	228
Liver[1]	51	194
Lung[1]	186	194
Spleen[1]	528	448
Tail[1]	103	145
293T cells[2]	2.8 µg	2.6 µg

Figure 6 . Representative DNA yields from DNAgard samples compared to frozen controls. [1] DNA was isolated from tissue fragments or 10^6 mammalian 293T cells stored in liquid DNAgard for 2 months. DNA yield was quantified using the Quant-iT PicoGreen fluorescent assay (Invitrogen). Yield is presented as ng of DNA per mg tissue for the animal tissue samples or total DNA for the 293T cell samples.

Successful downstream applications after 2 months

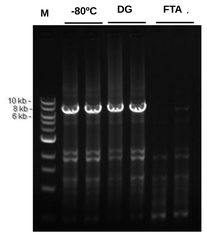


Figure 7: DNA stabilization and efficient long-range PCR from DNA recovered from DNAgard samples compared to FTA paper (FTA) . Long-range PCR amplification of a 7270 bp amplicon was performed on DNA from 0.1 mg of recovered rat kidney tissue that had been stored for 49 days at -80ºC or at 37ºC in DNAgard (DG) or on FTA paper (FTA). M = 1 kb ladder.

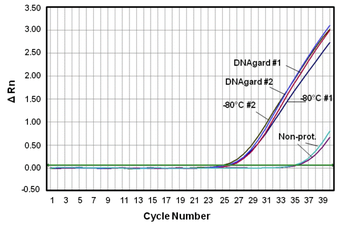


Figure 8. Yield of genomic DNA from tissue samples stored in DNAgard similar to frozen controls. Genomic DNA isolated from rat kidney tissue stored in duplicate for 71 days in DNAgard, water (non-protected; NP) or frozen at -80ºC was quantified using real-time PCR amplification of the β-actin gene. Real-time PCR traces demonstrate equal recovery of genomic DNA from DNAgard samples vs frozen controls.

Sequencing of rat β-actin from kidney samples stored 70 days in DNAgard

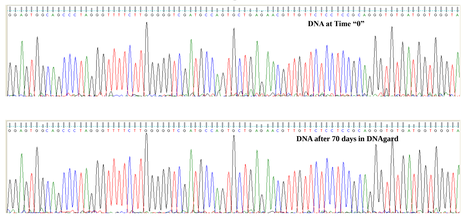


Figure 7 Genomic DNA in tissue specimens is stabilized by DNAgard as demonstrated by sequencing. A 296 bp portion of the rat β-actin gene was sequenced from DNA isolated at time zero and from rat kidney specimens stored for 70 days in DNAgard. The DNA sequence was identical in the two samples. Shown are corresponding, 80 nucleotide portions from the sequencing chromatograms of the time zero DNA and DNA from samples stored in DNAgard.

CONCLUSIONS

- DNAgard preserves DNA in tissues and cells at room temperature for at least 6 months at room temperature and even at elevated temperatures often experienced during sample shipment.
- This is a very practical solution for field collection of tissue samples where refrigeration is not available. With a 6 months time frame, the sample can be collected, shipped to the laboratory and stored for a period of time prior processing.

References

[1] Crowe, J.H., J.F. Carpenter, and L.M. Crowe. 1998. The role of vitrification in anhydrobiosis. Annu. Rev. Physiol. *60:73-103.*

[2] http://www.biomatrica.com/media/dnagard/dnagard_handbook.pdf

[3] http://www.etonbio.com/services.php