Biomatrica
THE BIOSTABILITY COMPANY

Room temperature preservation of gDNA quality in tissue specimens and culture cells with DNAgard™

Steven Wilkinson,[1] Sohela de Rozieres, Rolf Muller, Laurent Coulon
Biomatrica Inc., 5627 Oberlin Dr, #120, San Diego, CA 92121.

Introduction

Current methods for storing, shipping and preserving high quality genomic DNA of human and animal tissue or cells for clinical, forensic and academic research needs are costly and/or insufficient for reliable molecular diagnostics. Shipping of animal tissues commonly depends on dry-ice or liquid nitrogen, which is costly, associated with quality degradation due to multiple freeze/thaw cycles, and often not practical for the collection of samples in the field. Another common method of tissue preservation, formalin fixation paraffin embedding (FFPE), is also impractical for field collection and can result in damage to nucleic acids. DNAgard™, developed by Biomatrica, is a new medium for stabilizing genomic DNA in tissues and culture cells at ambient room temperature or elevated temperatures that can be encountered during tissue sample collection or shipment. The technology applies the molecular mechanisms by which certain extremophiles protect cellular macromolecules during exposure to adverse environmental conditions. DNAgard provides a convenient, cost-effective means to transport and store animal tissue samples and culture cell lines – while maintaining genomic DNA integrity.

Materials and Methods

Tissue storage: The kidney from a fresh, euthanized rat was harvested and fragmented (using a scalpel) into segments weighing approximately 25 mg. The exact weights of the kidney fragments were recorded and three samples were immediately processed for DNA isolation, providing “time zero DNA”. The remaining samples were submerged in 500 µl of DNAgard or water (non-protected). A fraction of the samples were stored in liquid DNAgard or water, protected from light. The remaining samples were processed for dry storage: DNAgard and non-protected kidney samples were lysed with 0.75 mg/ml proteinase K overnight at 56°C and then spotted on 96-well plates for drying in a sterile laminar flow hood. Dried samples were stored in heat-sealed pouches with desiccant.

Tissue culture cell storage: Human embryonic kidney (293) cells were grown up and harvested. Cell density was determined and volumes of $1x10^6$ cells were aliquoted into microfuge tubes. The cells were pelleted down and the supernatant removed. DNA was immediately isolated from three cell pellets using a commercially available column purification technology (“Time 0 DNA”). The remaining cell pellets were resuspended in either 500ul DNAgard or 500ul water. Half of the samples were stored in liquid and the other half were aliquoted into a 96 well plate and dried in a sterile laminar flow hood. The dried samples were heat sealed in a moisture barrier bag containing a desiccant pack.

Simulated shipping and sample recovery: All samples were subjected to a simulated shipping process using the following temperature cycles: 3 days at 45°C, 3 days at room temperature, 2 days at -20°C, 2 days at room temperature, and 3 days at 45°C. After the simulated shipping

[1] Author to whom correspondence should be addressed (swilkinson@biomatrica.com).

cycles, dry samples were rehydrated in 500 µl of water. DNA was isolated from all samples using a commercially available column purification technology (QIAamp DNA Mini Kit, Qiagen). DNA yield and integrity was analyzed on a 0.8% 1xTAE agarose gel by directly comparing DNA recovery from 0.75 mg of kidney tissue or 10^4 cells with samples processed at the time of experimental set-up (T0) (Figures 1 & 2).

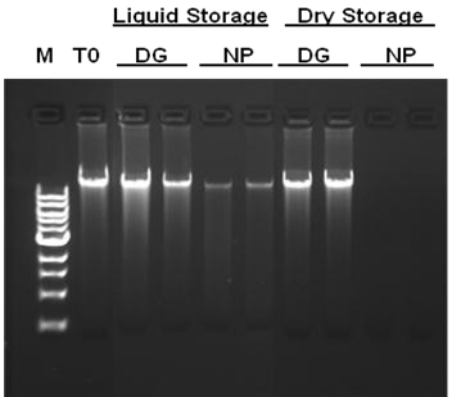

Figure 1: DNAgard protects genomic DNA in tissue samples during shipping. Rat kidney samples were stored in DNAgard (DG) in either the liquid or dry storage format and exposed to a 13 day simulated shipping cycle with temperature fluctuations between -20°C and 45°C. For comparison, samples were also stored in water (NP; non-protected). The DNA recovery corresponding to 0.75 mg of tissue was analyzed for each sample. (M = 1 kb ladder; T0 = DNA at time zero).

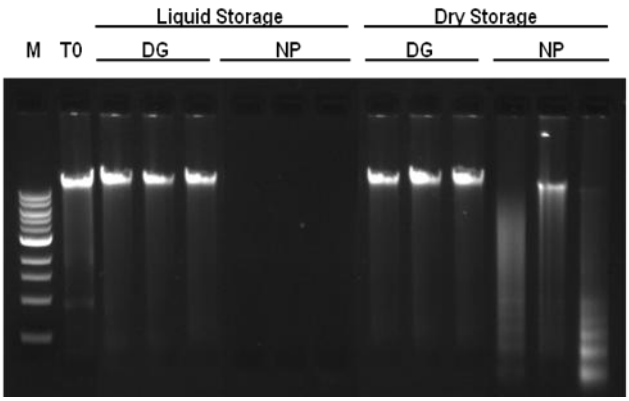

Figure 2: DNAgard protects genomic DNA in tissue culture cells during shipping. 293 cell samples were stored in DNAgard (DG) in either the liquid or dry storage format and exposed to a 13 day simulated shipping cycle with temperature fluctuations between -20°C and 45°C. For comparison, samples were also stored in water (H_2O). The DNA recovery from 10^4 cells was analyzed for each sample. (M = 1 kb ladder; T0 = DNA at time zero).

Results and Discussion

DNAgard, in both liquid and dry storage formats, was tested as a shipping medium for animal tissue or culture cells by exposing rat kidney tissue and 293 cell samples stored in DNAgard to a temperature cycle that fluctuated between -20°C and 45°C. The integrity of genomic DNA was preserved in tissue samples and culture cell samples stored in DNAgard in both liquid and dry formats as determined by comparison with DNA isolated from rat kidney and culture cell pellets at the time the tissue and cells were harvested (Figure 1&2). In contrast, samples stored in water (NP; non-protected) resulted in reduced genomic DNA yield or were completely degraded. These results demonstrate that DNAgard protects genomic DNA in complex tissue samples and culture cells that are exposed to fluctuating temperatures of extreme heat and cold that may be encountered during sample transport.

Conclusions

DNAgard preserves gDNA in tissues and cells at room temperature allowing these biosamples to be shipped and/or stored without degradation of sample quality. DNAgard is ideally suited for long-term storage of samples at ambient temperatures or for the shipment of samples without the need for cold packaging. Samples stored in DNAgard are protected from the extreme fluctuations in temperature that can occur during sample shipping, thus eliminating the use of expensive storage and shipping formats such as liquid nitrogen or dry-ice.