Green Technologies for Room Temperature Nucleic Acid Storage

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Abstract

Maintaining the long-term integrity of nucleic acids in the laboratory has traditionally required the use of freezers. However, novel nucleic acid stabilization technologies may allow for the storage of DNA and RNA at room temperature in a cost-effective, environmentally friendly manner. In this study, we evaluated two novel products for room temperature DNA storage: Biomatrica's DNA SampleMatrix technology and GenVault's GenTegra DNA technology. We compared the integrity and quality of DNA stored using these products against DNA stored in a -20°C freezer by performing downstream testing with short range PCR, long range PCR, DNA sequencing, and SNP microarrays. In addition, we tested Biomatrica's RNAstable product for its ability to preserve RNA at room temperature for use in a quantitative reverse transcription PCR assay.

Introduction

The rapid growth of genomics research has led to an unprecedented need for the storage of large numbers of biological specimens, including DNA, RNA, proteins, cells, and tissues. The traditional method for long-term storage of such specimens has been through laboratory freezers at -20°C, -80°C, or in liquid nitrogen. However, emerging technologies may offer the opportunity to store these samples at room temperature, thus reducing the carbon footprint associated with freezers. Two companies, Biomatrica (San Diego, CA) and GenVault (Carlsbad, CA), have developed products to store nucleic acids at room temperature.

Biomatrica's DNA SampleMatrix is based on a glass polymer that "shrink-wraps" and protects DNA from heat and UV light through a mechanism similar to that used by extremophiles, small organisms that can survive in dry environments for up to 120 years (Crowe et al., 1998).. The polymer is dispensed as a dissolvable coating on the inner surface of individual tubes or 96- or 384-well plates. After the addition of DNA in solution, the sample is dried down for room temperature storage. When ready for use, DNA is recovered by the addition of water or buffer. Similarly, Biomatrica's RNAstable product relies on a synthetic matrix that forms a thermostable barrier around RNA during the drying process. Characteristics of Biomatrica's DNA SampleMatrix and RNAstable are presented in Table 1.

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GenVault's GenTegra DNA is an inorganic mineral matrix with oxidation protection and antimicrobial activity for storage of purified DNA at room temperature. GenTegra DNA is supplied as a transparent coating at the bottom of each GenTegra DNA tube. Purified DNA is added to the GenTegra tube and dried down in a laminar flow hood or GenVault's FastDryer, a boxed enclosure with built-in fans. Recovery of DNA occurs with the addition of water. GenVault also offers GenPlates, a product consisting of FTA paper elements placed into a multi-well plate. Whole blood or buffy coat is aliquoted onto the paper elements, allowing for room temperature storage and downstream DNA recovery. Characteristics of GenTegra DNA are listed in Table 1. As the cellulose FTA paper in GenPlates does not represent a new technology (Hsaio et al., 1999; Smith and Burgoyne, 2004), additional information on this technology is not shown in Table 1.

In this study, we evaluated the integrity and quality of DNA stored for 3 weeks at room temperature using Biomatrica and GenVault products against DNA stored at -20°C. Our downstream testing included short range PCR, long range PCR, DNA sequencing, and SNP microarrays. In addition, we tested Biomatrica's RNAstable product for its ability to preserve RNA at room temperature for use in a quantitative reverse transcription PCR assay. Finally, we tested the ability of GenVault's GenPlates to store whole blood samples at room temperature.

Materials and Methods

Human genomic DNA from 8 different whole blood samples was extracted using Machery-Nagel Nucleospin Blood XL Kits according to the manufacturer's instructions. The concentration of the DNA was determined using a NanoDrop 1000. 1-5 μg of DNA in 20 μl water was aliquoted into Biomatrica SampleMatrix wells or GenTegra tubes, with the remaining DNA kept frozen at -20°C. For SampleMatrix, drying was performed overnight in a laminar flow hood. After drying, the DNA plate was sealed in aluminum foil and stored at room temperature in a desiccant dry box. DNA was stored at room temperature 1 week for GeneChip analysis and 3 weeks for all other assays. For GenTegra, drying was performed overnight in the GenVault FastDryer unit. GenTegra tubes were capped and stored at room temperature in a drawer for 3 weeks. DNA was recovered by the addition of 20 µl water. The concentration of rehydrated DNA was measured using a NanoDrop 1000.

DNA recovered from room temperature storage and -20°C frozen storage was run on a 2% agarose gel. Both types of DNA were subjected to four downstream applications:

1) Short Range PCR, 531 bp product from *ZNF750* gene, forward primer 5'-AATACTGTGCCTCCCAGGGTAT-3', reverse primer 5'-GTACTTACCAGAGGTGGGCAGTG-3', PCR conditions: 95°C for 5 min; 34 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1.5 min; 72°C for 10 min

Table 1. Characteristics of Biomatrica and GenVault nucleic acid storage platforms. *Manufacturer specifications, not tested here.

	Biomatrica	GenVault	
DNA Storage			
Product Name	DNA SampleMatrix®	GenTegra™ DNA	
Product Formats	1.7 ml Snap-Cap Individual Tubes, 96 and 384 Well Plates	96 Well Cluster Tubes	
Max DNA Per Tube or Well*	30 µg	50 µg	
Minimum Recoverable DNA*	5-10 pg	50 ng	
Drying Method	Air Dry, Speed Vacuum	GenVault FastDryer, Air Dry	
Storage Environment	Desiccating Box, Moisture-Barrier Bag	GenVault Storage Cabinet, Desiccating Box	
Room Temp Storage Time*	26 Months Real Time Tested, 30 years Simulated at 60°C	4 Months Real Time Tested, 10 Years Simulated at 76°C	
Recovery	Add Water or TE Buffer	Add Water	
Reusability*	Up to 3 Rehydration/Drying Cycles	Up to 4 Rehydration/Drying Cycles; May also perform additional cycles by transferring to a new GenTegra tube	
Sample Tracking	Barcoded Tubes or Racks	Barcoded Tubes or Racks	
RNA Storage			
Product Name	RNAstable™	NA	
Product Formats	1.5 ml Screw-Cap Tube, 96-Well Plate	NA	
Max RNA Per Tube or Well*	100 µg	NA	
Minimum Recoverable RNA*	10 pg (using RNAconcentrator)	NA	
Drying Method	Air Dry, Speed Vacuum	NA	
Storage Environment	Desiccating Box, Moisture-Barrier Bag	NA	
Room Temp Storage Time*	18 Months Real Time Tested, 10 Years Simulated at 50°C	NA	
Recovery	Add Water or Aqueous Buffer	NA	
Reusability*	Up to 3 Rehydration/Drying Cycles	NA	
Sample Tracking	Barcoded Tubes or Racks	NA	

- 2) Long Range PCR, 15.1 kb product from *PLAT* gene, forward primer 5'-CCTTCACTGTCTGCCTAACTCCTT-3', reverse primer 5'-ACTGTGCTTCCTGACCCATGGCAG-3', PCR conditions: 94°C for 2 min; 29 cycles of 94°C for 15 sec, 68°C for 12 min; 68°C for 17 min
- DNA Sequencing, ZNF750 gene from short range PCR, using ABI 3730xl sequencer and Sequencher 3.8 software
- 4) Affymetrix GeneChip Analysis, Human Mapping 500K Array Set for SampleMatrix DNA and Genomewide Human SNP Array 6.0 for GenTegra DNA, both according to manufacturer's protocol

Total RNA was extracted from skin tissue samples using a ProScientific homogenizer and Qiagen RNeasy extraction kit. RNA was quantified using NanoDrop 1000. RNA (500 ng) was aliquoted into the Biomatrica RNAstable tubes and the remaining RNA stored at -80°C. The room temperature RNA

was dried down overnight in a laminar flow hood, sealed with aluminum foil, and stored at room temperature in a desiccant dry box for 11 days. RNA was rehydrated by adding 20 μ l DEPC water to the dried sample in the RNAstable tube and incubated at room temperature for 15 minutes. RNA quality and concentration of frozen and room temperaturestored samples were then determined using Agilent 2100 Bioanalyzer. RNA (100 ng) from both sets of samples was used to synthesize cDNA with a SuperScript VILO kit (Invitrogen). Quantitative real-time PCR of the GAPDH gene (forward primer 5'-GAAGGTGAAGGTCGGAGTC-3' and reverse primer 5'GAAGATGGTGATGGGATTTC-3') (Gutala and Reddy, 2004) was performed using Power SYBR Green PCR master mix (Applied Biosystems) and 5 ng cDNA per reaction. All reactions were carried out in triplicate, including a no template control, with PCR conditions set at 95°C for 10 min and 40 cycles of 95°C for 15 sec and 60°C for 1 min. The qPCR was performed on an Applied Biosystems

Table 2. Estimated DNA yields, measurement of A₂₆₀/A₂₈₀, and measurement of A₂₆₀/A₂₃₀ following room temperature storage using Biomatrica and GenVault platforms.

Biomatrica Sample	Expected Conc. (ng/ L)	Measured Conc. (ng/ L)	% DNA Recovery	A ₂₆₀ / A ₂₈₀ (Before / After)	A ₂₆₀ / A ₂₃₀ (Before / After)
1	250	249.0	99.6	1.88 / 1.91	1.57 / 0.73
2	250	259.8	103.9	1.88 / 1.87	1.76 / 0.77
3	250	334.7	133.9	1.89 / 1.89	1.74 / 0.87
4	250	254.7	101.9	1.90 / 1.92	1.70 / 0.75
5	250	227.2	90.9	1.86 / 1.89	1.74 / 0.73
6	250	243.6	97.4	1.86 / 1.84	1.87 / 0.75
7	250	281.7	112.7	1.89 / 1.91	1.95 / 0.82
8	250	264.8	105.9	1.87 / 1.85	1.99 / 0.83
			Median: 103	Mean: 1.88 / 1.89	Mean: 1.79 / 0.78
GenVault Sample	Expected Conc. (ng/ L)	Measured Conc. (ng/ L)	% DNA Recovery	A ₂₆₀ / A ₂₈₀ (Before / After)	A ₂₆₀ / A ₂₃₀ (Before / After)
1	100	94.6	94.6	1.88 / 1.83	1.57 / 0.33
2	100	138.7	138.7	1.88 / 1.85	1.76 / 0.38
3	50	61.5	123.1	1.89 / 1.85	1.74 / 0.18
4	50	47.9	95.7	1.90 / 1.86	1.70 / 0.15
5	100	108.9	108.9	1.86 / 1.86	1.74 / 0.28
6	100	200.5	200.5	1.86 / 1.88	1.87 / 1.94
7	100	170.9	170.9	1.89 / 1.88	1.95 / 0.39
8	50	44.9	89.9 Median: 116	1.87 / 1.85	1.99 / 0.14

7900HT machine and analyzed with SDS software V2.3 (Applied Biosystems) to determine the threshold cycle (C_a) for each sample. The relative standard curve of the average Cq values plotted against the log-scale of the RNA quantities showed a linear relationship with a r² of 0.998 and a qPCR efficiency of 105%.

For GenPlates testing, 8 whole blood samples previously stored at -20°C were thawed to room temperature and 10 µl of blood was aliquoted into each well of a 384-well GenPlate. After 8 GenPlates were placed inside a desiccant bag for 48 hours, the plates were sealed with aluminum foil and stored at room temperature for 4 weeks. Whole blood DNA recovery from the GenPlate FTA paper elements was performed using a GenSolve kit according to the manufacturer's instructions and DNA quantitation performed using an Invitrogen Qubit fluorometer.

Results

DNA Quality and Yield

To assess DNA quality, samples stored for 3 weeks at room temperature with SampleMatrix and GenTegra and at -20°C were run on a 2% agarose gel to compare band intensity and size (Figure 1). The results showed that room temperature stored DNA did not degrade and remained in good condition compared to the frozen controls. To estimate DNA yield, DNA concentration was measured using a Nanodrop 1000 before and after room temperature storage. The median percent DNA recovery of SampleMatrix and GenTegra stored samples was excellent at 103% and 116%, respectively (Table 2). The greater than 100% recovery in some samples is most likely due to variances in Nanodrop measurement. Room temperature storage did not alter the A260/A280 ratio, but lowered the A₂₆₀/A₂₃₀ ratio (Table 2). This can be explained by the fact that both Biomatrica's and GenVault's DNA-preserving compounds show strong absorbance at the 230 nm wavelength, but minimal absorbance at 260 nm and 280 nm. Therefore, the decreased A₂₆₀/A₂₃₀ reflects a spectrophotometric property of the inorganic preservative compounds rather than unknown contaminants.

Short Range and Long Range PCR

Short range PCR (531 bp) and long range PCR (15.1 kb) were performed on DNA stored using the SampleMatrix and GenTegra systems. For both room temperature storage methods, DNA samples showed comparable band intensity and size with the frozen controls (Figure 2). For short range PCR, the identities of all PCR products were confirmed by DNA sequencing (next section). In a few of the GenVault samples (Figure 2b, lanes 3, 5, 9, and 13), we observed a

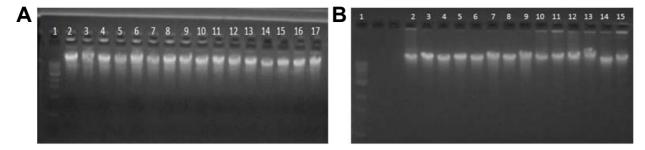


Figure 1. Room temperature stored genomic DNA versus -20°C stored genomic DNA run on 2% agarose gels. (a) Biomatrica. Lane 1: 100 bp DNA ladder; Lanes 2, 4, 6, 8, 10, 12, 14, 16: SampleMatrix stored samples; Lanes 3, 5, 7, 9, 11, 13, 15, 17: Corresponding frozen controls. (b) GenVault. Lane 1: 100 bp DNA ladder; Lanes 2, 4, 6, 8, 10, 12, 14: GenTegra stored samples; Lanes 3, 5, 7, 9, 11, 13, 15: Corresponding frozen controls.

very slight downward shift in the gel bands, possibly reflecting a slight change in the DNA's charge or conformation when bound by the mineral matrix stabilizer.

DNA Sequencing

DNA sequences obtained from short range PCR of the *ZNF750* gene using SampleMatrix and GenTegra stored DNA were compared to frozen control DNA using Sequencher software. The traces of the room-temperature stored samples showed clear peaks with very low background noise. Of 480 base calls passing quality control on Sequencher, there were no mismatches between the 8 DNA samples stored with SampleMatrix compared with the corresponding 8 frozen samples. Similarly, there were no mismatches between the 8 samples stored with GenTegra compared with the corresponding 8 frozen samples.

Affymetrix 500K or 6.0 DNA Microarrays

Biomatrica stored DNA samples and corresponding frozen DNA samples were run on Affymetrix 500K Nsp and Sty

microarrays. For the 2 samples tested, the mean call rate was 96.5% and the mean concordance rate with frozen controls 99.2% (Table 3). GenTegra stored DNA samples were run in tandem with their matching frozen controls on Affymetrix 6.0 chips. For the 6 samples tested, the mean call rate was 99.0% and the mean concordance rate with frozen controls 99.8% (Table 3). These results confirm that both Biomatrica and GenVault platforms yield high quality DNA suitable for whole genome applications.

RNA Quality, Yield, and Quantitative Real Time PCR
After 11 days of Biomatrica-based room temperature storage, RNA quality and yield of 3 samples was compared to -80°C frozen controls using an Agilent 2100 bioanalyzer. As compared to pre-stored RNA samples, frozen and Biomatrica stored samples had similar RNA Integrity Number (RIN) values, indicative of high quality RNA (Table 4). In addition, the bioanalyzer tracings of the RNA samples, before and after Biomatrica storage, exhibited distinct 18S and 28S peaks, which was representative of high quality

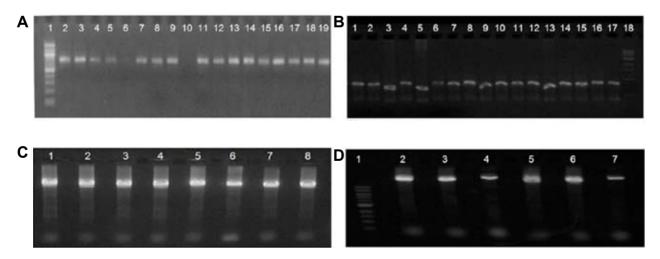


Figure 2. Short and long range PCR of room temperature stored DNA in comparison with frozen DNA. (a) Short range PCR of Biomatrica stored samples. Lane 1: 100 bp DNA ladder; Lanes 2-9: SampleMatrix stored samples; Lanes 12-19: Corresponding frozen controls; Lane 10: No template negative control; Lane 11: Reference DNA positive control. (b) Short range PCR of GenVault stored samples. Lanes 1, 3, 5, 7, 9, 11, 13, 15: GenTegra stored samples; Lanes 2, 4, 6, 8, 10, 12, 14, 16: Corresponding frozen controls; Lane 17: Reference DNA positive control; Lane 18: 1 kb DNA ladder. (c) Long range PCR of Biomatrica stored samples. Lane 1-4: SampleMatrix stored samples; Lanes 5-8: Corresponding frozen controls. (d) Long range PCR of GenVault stored samples. Lane 1: 1 kb DNA ladder; Lanes 2-4: GenTegra stored samples; Lanes 5-7: Corresponding frozen controls.

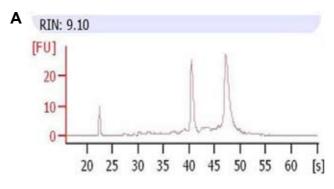
Table 3. Affymetrix 500K or 6.0 SNP microarray call rates for room temperature stored DNA, and concordance rates with corresponding frozen DNA controls. Genotyping performed with BRLMM algorithm for 500K Arrays and Birdseed algorithm for 6.0 Arrays.

Biomatrica Sample	# SNPs Called on Affy 500K Array (Call Rate %)	# Concordant SNPs with Frozen Sample (Concordance %)
1	484278 (96.8%)	480715 (99.3%)
2	480474 (96.1%)	476176 (99.1%)
MEAN	482376 (96.5%)	478446 (99.2%)
GenVault Sample	# SNPs Called on Affy 6.0 Array (Call Rate %)	# Concordant SNPs with Frozen Sample (Concordance %)
1	899044 (99.2%)	897375 (99.8%)
2	898765 (99.1%)	897137 (99.8%)
3	897304 (99.0%)	895539 (99.8%)
4	899481 (99.2%)	898062 (99.8%)
5	897242 (99.0%)	895248 (99.8%)
6	004040 (00 70/)	004044 (00 00/)
U	894619 (98.7%)	891341 (99.6%)

RNA (Figure 3). Good RNA yields of the Biomatrica stored samples were obtained with a mean RNA recovery of 113% (Table 4). Following cDNA synthesis of frozen and Biomatrica stored RNA samples, qPCR for expression of the housekeeping gene, GAPDH, was performed to compare RNA sample quantity between the two storage formats. The qPCR amplification plots demonstrated similar amounts of RNA between the frozen and Biomatrica stored samples. and the Cq values were comparable between the two storage conditions (Figure 4). The Cq for the no template control (NTC) did not cross the threshold for two of the NTC triplicate wells and had a Cq value of 30 for one NTC triplicate well, which was greater than ten cycles away from sample Cq values (Figure 4) (Bustin and Nolan, 2004).

Whole Blood DNA Recovery

After 4 weeks of room temperature storage of whole blood samples on GenPlates, we attempted DNA recovery from the FTA paper elements using the GenVault GenSolve



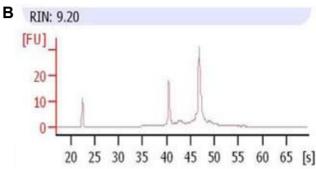


Figure 3. Representative bioanalyzer tracings of RNA samples before (A) and after (B) Biomatrica room temperature storage. There were two distinct peaks corresponding to 18S and 28S RNA, demonstrating high quality RNA.

manufacturer kit. During this process, it was noted that the FTA paper elements containing the blood samples had not dried completely in the 48 hours allotted for the drying step (minimum 16 hours recommended by GenVault). The incomplete drying led to lower than expected DNA yields (data not shown). No further downstream application testing was performed on this DNA as interpretation of results from incompletely dried samples would be difficult.

Discussion

In this study, we report an evaluation of two DNA room temperature storage products: Biomatrica's SampleMatrix technology and GenVault's GenTegra technology. Our results demonstrate that DNA stored at room temperature for up to 3 weeks using either platform performed equivalently to DNA kept frozen at -20°C with regard to downstream applications such as short range PCR, long range PCR, DNA sequencing, and SNP microarray analysis. The workflow for both platforms was straightforward, involving the addition of

Table 4. RNA Integrity Number (RIN) and yield of RNA samples stored for 11 days at room temperature using the Biomatrica storage platform. RIN values above 7.0 are indicative of high quality.

Sample	RIN Before Storage	RIN Freezer (-80°C)	RIN Biomatrica	Expected RNA Conc. (ng/ l)	Measured RNA Conc. (ng/ l)	% RNA Recovery Biomatrica
1	9.1	8.9	9.2	75	85	106
2	7.4	7.6	7.6	25	35	136
3	8.7	8.2	8.8	25	24	96
MEAN	8.4	8.2	8.5			113

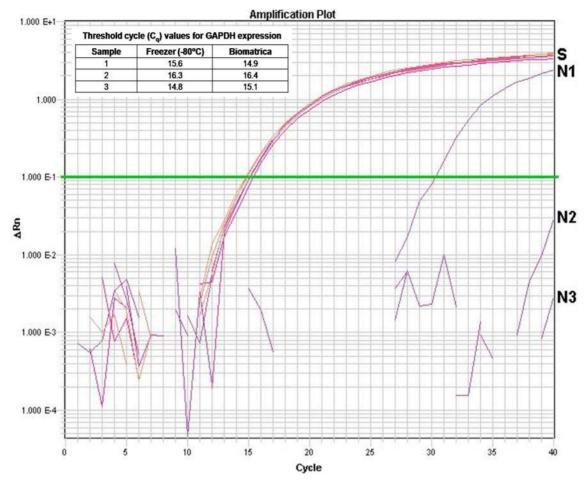


Figure 4. Representative quantitative PCR amplification plot for *GAPDH* using RNA from frozen storage (yellow lines, S) compared to Biomatrica room temperature storage (red lines, S). The Cq for the no template controls (NTC) did not cross the threshold for two of the NTC triplicate wells (N2 and N3) and one NTC triplicate well (N1) had a Cq value of 30. Inset: Threshold cycle (Cq) values for *GAPDH* gene expression of RNA samples stored at -80°C or room temperature (Biomatrica). Cq values in table are the average of three replicates.

DNA to the product tubes, overnight drying, storage in a dry environment, and recovery with water. No further clean-up or purification was required after rehydration. Importantly, the proprietary ingredients for each DNA stabilization platform did not appear to interfere with any of the downstream applications, even though they did affect the A_{260}/A_{230} ratio.

For RNA, Biomatrica's RNAstable product maintained the integrity of RNA stored at room temperature for 11 days. This is an impressive result, given the well known propensity for RNA to degrade at room temperature.

For whole blood, GenVault's GenPlates system resulted in lower than expected DNA yields due to incomplete drying of the blood on the FTA paper elements, despite exceeding the manufacturer's recommended drying time. In addition, we found the process of aliquoting 10 ml tubes of whole blood into 384-well plates time-consuming when performed by hand. Robotic automation would be a requirement for any high throughput operation. A disadvantage to storing whole blood on FTA paper is the loss of the ability to assay for biomolecules other than DNA, such as RNA and proteins, which are preserved in a -80°C environment.

Limitations of the present study were that we did not test room temperature DNA or RNA storage beyond 3-4 weeks, although both manufacturers report internal tests showing successful storage for many years (Table 1). Also, we did not test recovery of DNA or RNA at the lower or upper limits of the recommended range per tube. Finally, we did not evaluate the ability of these products to undergo multiple cycles of drying and rehydration, although both companies report that at least 3 such cycles is possible (Table 1).

Table 5 compares the features of room temperature nucleic acid storage with the features of freezer storage. Overall, there are many advantages to room temperature nucleic acid storage over freezers, including a reduction in space, energy usage, power outage risk, and long-term cost. Room temperature nucleic acid storage also greatly facilitates transfer and shipping of samples. However, due to the slightly longer time and effort that the drying process requires compared to freezing, room temperature storage technologies in their current form may be best suited for long-term storage of stock and archival samples, whereas freezers may be better suited for day-to-day use of working samples.

Feature	Freezer (-80°C)	Room Temperature Nucleic Acid Storage (Biomatrica, GenVault)	
Footprint	Large, Typically in a Separate Lab Area / Room	Small to Large, Desktop Storage Possible	
Energy Consumption	7,000 kWh/year	0	
Annual Energy Cost	Approx \$1,000	0	
Fluorocarbon and CO ₂	Yes, Some Models	No	
Risk of Sample Loss with Power Outage	Yes	No	
Sample Shipping	Dry Ice or Cold Packs	Room Temperature	
Sample Storage Ease	Freeze-Thaw within Minutes	aw within Minutes Overnight Drying; Instant Rehydration	

Table 5. Comparison of features for room temperature nucleic acid storage and freezer storage.

In conclusion, our testing demonstrated that both Biomatrica and GenVault offer products which are able to maintain the integrity of nucleic acids stored at room temperature, with no loss of sample quality in downstream applications such as short and long range PCR, DNA sequencing, DNA microarray analysis, and real time quantitative PCR. These new platforms offer an opportunity for laboratories to store their samples in an environmentally friendly manner, with reduced long term costs in comparison to freezer storage.

Acknowledgements

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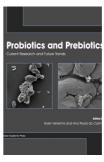
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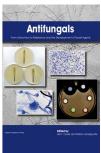












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