Forensic Science International: Genetics xxx (2011) xxx–xxx

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Assessing a novel room temperature DNA storage medium for forensic biological samples

Steven B. Lee [a,*], Kimberly C. Clabaugh [a], Brie Silva [a], Kingsley O. Odigie [a], Michael D. Coble [b,1], Odile Loreille [b], Melissa Scheible [b], Ron M. Fourney [c], Jesse Stevens [c], George R. Carmody [d], Thomas J. Parsons [e], Arijana Pozder [e], Arthur J. Eisenberg [f], Bruce Budowle [f], Taha Ahmad [g], Russell W. Miller [h], Cecelia A. Crouse [h]

[a] Forensic Science Program, Justice Studies Department, MacQuarrie Hall 521, San Jose State University, San Jose, CA 95192, United States
[b] Research Section, Armed Forces DNA Identification Laboratory, Armed Forces Institute of Pathology, 1413 Research Blvd., Bldg 101, Rockville, MD 20850, United States
[c] Biology Research and Development, Central Forensic Laboratory, Royal Canadian Mounted Police, Ottawa, ON, Canada K1G 3M8
[d] Department of Biology, Carleton University, 1125 Colonel By Drive, Ottawa, ON, Canada K1S 5B6
[e] Forensic Sciences Division, International Commission on Missing Persons, Alipašina 45a, 71000 Sarajevo, Bosnia and Herzegovina
[f] Forensic and Investigative Genetics and Institute of Investigative Genetics, University of North Texas Health Science Center at Fort Worth 3500 Camp Bowie Blvd., Fort Worth, TX 76107, United States
[g] Marshall University, One John Marshall Drive, Huntington, WV 25755, United States
[h] Palm Beach County Sheriff's Office, 3228 Gun Club Road, West Palm Beach, FL 33406, United States

A R T I C L E I N F O

Article history:
Received 15 November 2009
Received in revised form 21 December 2010
Accepted 17 January 2011

Keywords:
DNA storage
DNA recovery
Short tandem repeat analysis
Freeze–thaw
Trehalose
SampleMatrix
QIAsafe
DNA stability

A B S T R A C T

The ability to properly collect, analyze and preserve biological stains is important to preserving the integrity of forensic evidence. Stabilization of intact biological evidence in cells and the DNA extracts from them is particularly important since testing is generally not performed immediately following collection. Furthermore, retesting of stored DNA samples may be needed in casework for replicate testing, confirmation of results, and to accommodate future testing with new technologies.

A novel room temperature DNA storage medium, SampleMatrix™ (SM; Biomatrica, Inc., San Diego, CA), was evaluated for stabilizing and protecting samples. Human genomic DNA samples at varying amounts (0.0625–200 ng) were stored dry in SM for 1 day to 1 year under varying conditions that included a typical ambient laboratory environment and also through successive freeze–thaw cycles (3 cycles). In addition, spiking of 1–4× SM into samples prior to analysis was performed to determine any inhibitory effects of SM. Quantification of recovered DNA following storage was determined by quantitative PCR or by agarose gel electrophoresis, and evaluation of quantitative peak height results from multiplex short tandem repeat (STR) analyses were performed to assess the efficacy of SM for preserving DNA.

Results indicate no substantial differences between the quality of samples stored frozen in liquid and those samples maintained dry at ambient temperatures protected in SM. For long-term storage and the storage of low concentration samples, SM provided a significant advantage over freezer storage through higher DNA recovery. No detectable inhibition of amplification was observed at the recommended SM concentration and complete profiles were obtained from genomic DNA samples even in the presence of higher than recommended concentrations of the SM storage medium. The ability to stabilize and protect DNA from degradation at ambient temperatures for extended time periods could have tremendous impact in simplifying and improving sample storage conditions and requirements. The current work focuses on forensics analysis; however this technology is applicable to all endeavors requiring storage of DNA.

© 2011 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Storage of DNA evidence either in the original sample or derived in the form of an extract is an important issue in forensic DNA analysis. Environmentally challenged, degraded or damaged samples [1–3] and/or those with low DNA concentrations [4,5] may reduce the chance of obtaining informative results. Cold storage (i.e. +4 °C, −20 °C, −80 °C or liquid nitrogen) in polypropylene tubes may lead to further loss through repeated freeze-thawing [6,7], retention to the tubes [8], evaporation and/or denaturation [9]. DNA samples may be the only remaining probative item of evidence and it is imperative that they be protected from storage damage.

* Corresponding author. Tel.: +00 1 408 924 2948; fax: +00 1 408 924 2953.
E-mail address: sblee999@gmail.com (S.B. Lee).
1 Current address: Applied Genetics Group, National Institute of Standards and Technology, Gaithersburg, MD 20899, United States.

1872-4973/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.fsigen.2011.01.008

Poor sample handling and storage may lead to further loss or degradation of samples and could impact obtaining useful results. Additional factors that may compromise sample integrity include high humidity, temperature, persistence of nucleases and other chemical agents as well as other sub-optimal conditions that may occur not only during transport, but also within storage facilities [10]. Typically, cold conditions (i.e. liquid nitrogen, −80 °C, −20 °C) are required for long term storage. Such conditions are costly; the equipment is subject to mechanical failure and there are logistical problems for transport. Often, forensic biological samples are dried to minimize degradation. Such a requirement has not always been practical, especially for some types of evidentiary samples and particularly for DNA extracts. Thus, there is a need to develop effective alternative strategies for storage procedures [10–12].

Dry storage of nucleic acids has been recommended to eliminate the need for cold storage based on the assumption that nucleic acids are stable when dry. However there are numerous examples where degradation occurs during storage, in the cold or at ambient conditions, that can irreversibly damage samples in solution or even those that are dehydrated [13]. Although DNA can be dried without serious damage in the short term, it is nevertheless imperative to prevent chemical degradation and aggregation for optimal recovery of samples.

Several methods are commonly used to dehydrate DNA samples, such as spray drying, spray freeze drying, air drying or lyophilization [13]. The purified DNA can be dried without additives ("naked") or in the presence of additives such as trehalose, a disaccharide found in high concentration in organisms that undergo periods of desiccation as part of their life cycle [14,15]. Since proteins and membranes could be stabilized in a dry state in the presence of trehalose [16], the compound has been used to stabilize a variety of biological materials ranging from vaccines and liposomes to hypothermic storage of human organs [15]. Trehalose has recently been examined for use in stabilizing highly diluted genomic DNA samples compared with the performance of samples stored in Tris EDTA (TE) buffer and lambda DNA [17]. Best results occurred for DNA in the presence of trehalose stored dried at room temperature or at −80 °C, although significant quality loss was detected with −20 °C and +4 °C storage. Studies with trehalose as an additive for dried room temperature plasmid DNA storage indicates initial protection from degradation and light-induced damage; but after 8 weeks degradation was observed [18]. Plasmids are in general more stable than genomic DNA samples and even after only 2 weeks at −20 °C using highly purified trehalose, DNA showed signs of degradation [13]. Other sugars such as sucrose, glucose and lactose have been studied for their ability to stabilize purified dried DNA, however trehalose had the highest stability for all sugar based stabilizers [18].

To address the need to stabilize and prevent degradation of biological materials in a dried state, a novel storage medium, SampleMatrix™ (SM; Biomatrica, Inc., San Diego, CA), was developed to protect samples dry at ambient temperatures that should be amenable to sample collection, transport, storage and analytical practices. SM technology is based on the principles of anhydrobiosis, a biological mechanism employed by some multicellular organisms that enables their survival while dry for >100 years [14]. The synthetic matrix is predicted to act with DNA molecules through the minor groove by hydrogen bonding, similar to trehalose and other sugars. While in the dry state, the matrix components form a thermo-stable barrier around the DNA protecting the sample from further damage and degradation. The matrix completely dissolves following rehydration. Once hydrated, the sample is ready for immediate use in downstream applications, thus eliminating the need for further purification and any associated sample loss due to manipulation.

Studies were conducted to evaluate the use of the medium for the dry storage of DNA samples. The studies included an elevated temperature storage study, a sensitivity study, a freeze–thaw stress study and a study to assess any inhibitory effects of the medium. Advances in technologies for convenient ambient temperature dry storage of DNA that eliminate some of the detrimental variables associated with sample collection, transport and storage will have useful applications for forensics analysis, as well as other fields of nucleic acids research and analysis.

2. Materials and methods

2.1. Storage study

For experiments comparing storage of DNA at room temperature to −20 °C storage, multiwell plates containing the storage medium or individual tubes with the storage medium (SampleMatrix™ (SM); distributed as QIAsafe™ DNA by Qiagen, Valencia, CA) were used following the manufacturer's guidelines. 200 ng aliquots of human genomic DNA (10 μl of a 20 ng/μl DNA sample-Novagen, Gibbstown, NJ) were applied into SM and dried overnight in a laminar flow hood with a sterile kimwipe over the plates to reduce contamination. Non-protected samples were prepared by drying identical aliquots into empty tubes or wells not containing SM. Dried samples were then stored at room temperature on the bench top in ambient light conditions or 50 °C for various times. Identical aliquots of reference samples were stored at −20 °C for the same duration. There were a total of 6 samples that were tested (3 for each environmental treatment), 1 with SM (SM), 1 control held at −20 °C (+) and 1 not protected (NP). At various time points, samples were rehydrated with 10 μl of deionized water for 10 min at room temperature and used immediately without further purification in downstream applications to assess sample integrity and stability. Agarose gel electrophoresis was used to assess the integrity and quality of DNA stored [19] at room temperature, 50 °C and −20 °C with and without SM. Rehydrated samples were run on a 0.8% agarose gel containing 1 μg/ml ethidium bromide (Gibco BRL, Carlsbad, CA) and detected using UV transillumination [19].

2.2. Sensitivity studies

In this study, the SM 96-well plates were evaluated against the current storage methods (−20 °C) for 6 concentrations of DNA at seven time points ranging from 1 day to 1 year. Controls were prepared as liquid DNA extracts which are routinely stored in the freezer (−20 °C) and then thawed for analysis at the six time points. The SM 96-well plates were also evaluated at each time point with respect to two different room temperature storage conditions – in the presence (SM+D) or absence (SM-D) of desiccant. Dried samples were stored in identical storage cabinets, one containing desiccant (D) in order to maintain a constant relative humidity environment <50% (SM+D), as is recommended by the manufacturer, and the other without desiccant (SM-D). These plexiglass clear cabinets were kept at room temperature, consist of a clear door that permits ambient light in and were kept inside a standard HVAC-controlled laboratory environment, albeit in a hot and humid climate zone (Florida) with an average relative humidity of 60%.

In this sensitivity study, 2 replicates of DNA extracted from bucccal cells of 2 individuals (one male and one female) using the Promega DNA IQ system (Promega Madison WI) at 7 different amounts (0.0625, 0.125, 0.25, 0.5, 1.0, 2.0 and 4 ng), were stored under 3 different conditions and sampled 7 times over the course of 1 year for a total of 588 data points. The Beckman Coulter BioMek NXP was used to aliquot two 20 μl volumes of each sample from a stock tube into SM 96-well plates and SM individual tubes

Please cite this article in press as: S.B. Lee, et al., Assessing a novel room temperature DNA storage medium for forensic biological samples, Forensic Sci. Int. Genet. (2011), doi:10.1016/j.fsigen.2011.01.008

Table 1
Percentages of complete STR Loci allele calls for samples stored over 1 year.

	Control	Stnd dev	SM+D	Stnd dev	SM−D	Stnd dev
0.625 ng						
6 weeks	25.000	13.502	23.438	10.674	12.500	0.000
8 weeks	4.688	3.125	15.625	11.968	17.188	13.858
12 weeks	3.125	3.608	21.875	3.608	10.938	5.984
52 weeks	9.375	6.250	9.375	8.069	4.688	5.984
0.125 ng						
6 weeks	71.875	14.878	60.938	9.375	56.250	14.434
8 weeks	48.438	34.752	62.500	8.839	68.750	14.434
12 weeks	54.688	20.650	62.500	8.839	47.917	9.547
52 weeks	39.063	30.778	48.438	18.663	40.625	10.825
0.25 ng						
6 weeks	95.313	5.984	96.875	3.608	96.875	3.608
8 weeks	96.875	3.608	90.625	8.069	96.875	3.608
12 weeks	93.750	8.839	100.000	0.000	89.063	5.984
52 weeks	92.188	9.375	89.063	9.375	60.938	20.650

Results of multiplex STR analysis using replicate DNA samples that were stored dry over a 1 year period in SM at room temperature with (SM+D) or without (SM−D) desiccant present. Control liquid samples were stored at −20 °C for the identical time period. SM+D: DNA dried in SM stored with desiccant present at room temperature; SM−D: DNA dried in SM stored without desiccant present at room temperature; control: liquid samples stored at −20 °C.

for each time point and condition. The NXP simultaneously created replicate −20 °C liquid DNA freezer control samples of 20 μl aliquots of each sample from the same stock into dolphin tubes. Control samples were stored in a −20 °C freezer, while the SM samples were dried overnight in a laminar flow hood and stored in their respective conditions at room temperature.

SM samples were rehydrated with 20 μl of autoclaved water for sample recovery. All recovered DNA samples were quantified using Applied Biosystems Quantifiler™ Human DNA Quantification kit on the ABI 7000 (as per manufacturer recommendations; ABI, Foster City, CA) and compared to determine if DNA stored on the SM was recovered at the same, lower, or higher concentrations than those in the −20 °C freezer condition. Optimal quantification was achieved using more than 250 pg or more of genomic DNA. Both the SM samples and the −20 °C freezer samples were also compared to a baseline created of the original DNA stock tube at the time samples were plated. The remaining sample was amplified using Promega's multiplex STR PowerPlex™ 16 system. Once amplified, the samples were run on Applied Biosystems 3130xl Genetic Analyzer (ABI) and results were analyzed with GeneMapper ID software using a 75 rfu threshold for allele designation to evaluate the integrity of the DNA after storage on the SM.

The integrity of the sensitivity samples was determined by observing % of allele calls and comparing the average relative florescence unit (RFU) values of each allele at each locus. Complete loci calls were determined by overlapping the replicate electropherograms for each sample. Percentages of complete allele calls from 6 weeks to 1 year are shown in Table 1 and Fig. 2.

Average relative recovery of control −20 °C freezer condition versus those stored dried in SM at room temperature with dessicant were calculated for 4, 2, 1 and 0.5 ng samples stored over 1 year (Fig. 3A), for 4 ng stored over 1 day, 1 week, 1 month, 3 months and 1 year (Fig. 3B) and for 0.5 ng stored over 1 day, 1 week, 1 month, 3 months and 1 year (Fig. 3C). Average calculated recovery from replicate samples ($n = 4$) stored under 3 conditions for all seven amounts after 1 year are shown in Fig. 3D. The calculated average from the replicate samples ($n = 4$) are also shown in Table 3 with their respective standard deviation.

2.3. Stress study

DNA from a buccal swab was extracted using the QIAamp DNA Mini Kit (Qiagen). The final volume of 100 μl was separated into six aliquots: 2 controls of 5 μl each in SM tubes stored at −20 °C for the entire duration of the study (SM control); 2 aliquots of 20 μl each air-dried into SM tubes and stored at room temperature during the duration of the long-term experiment; and 2 aliquots of 20 μl each into regular polypropylene tubes subjected to multiple

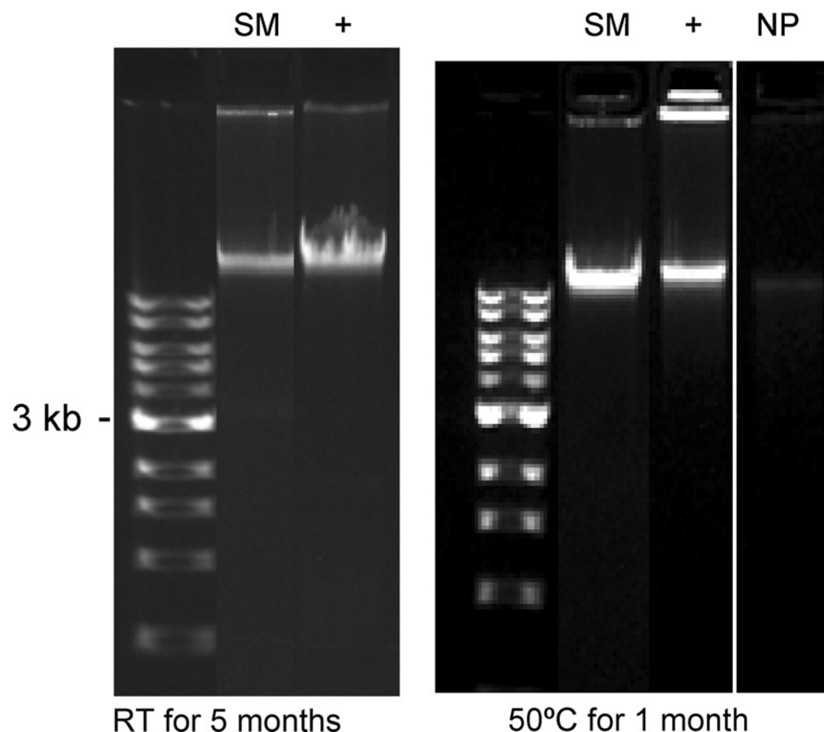

Fig. 1. Integrity of DNA following dry storage in SM at room temperature for 5 months and 50 °C, for 1 month. Genomic DNA (200 ng) stored dry in SM was protected from degradation during long-term storage at room temperature (left) or at elevated temperatures for up to 1 month (right). SM: SampleMatrix; +: reference sample stored at −20 °C; NP: non-protected dried DNA.

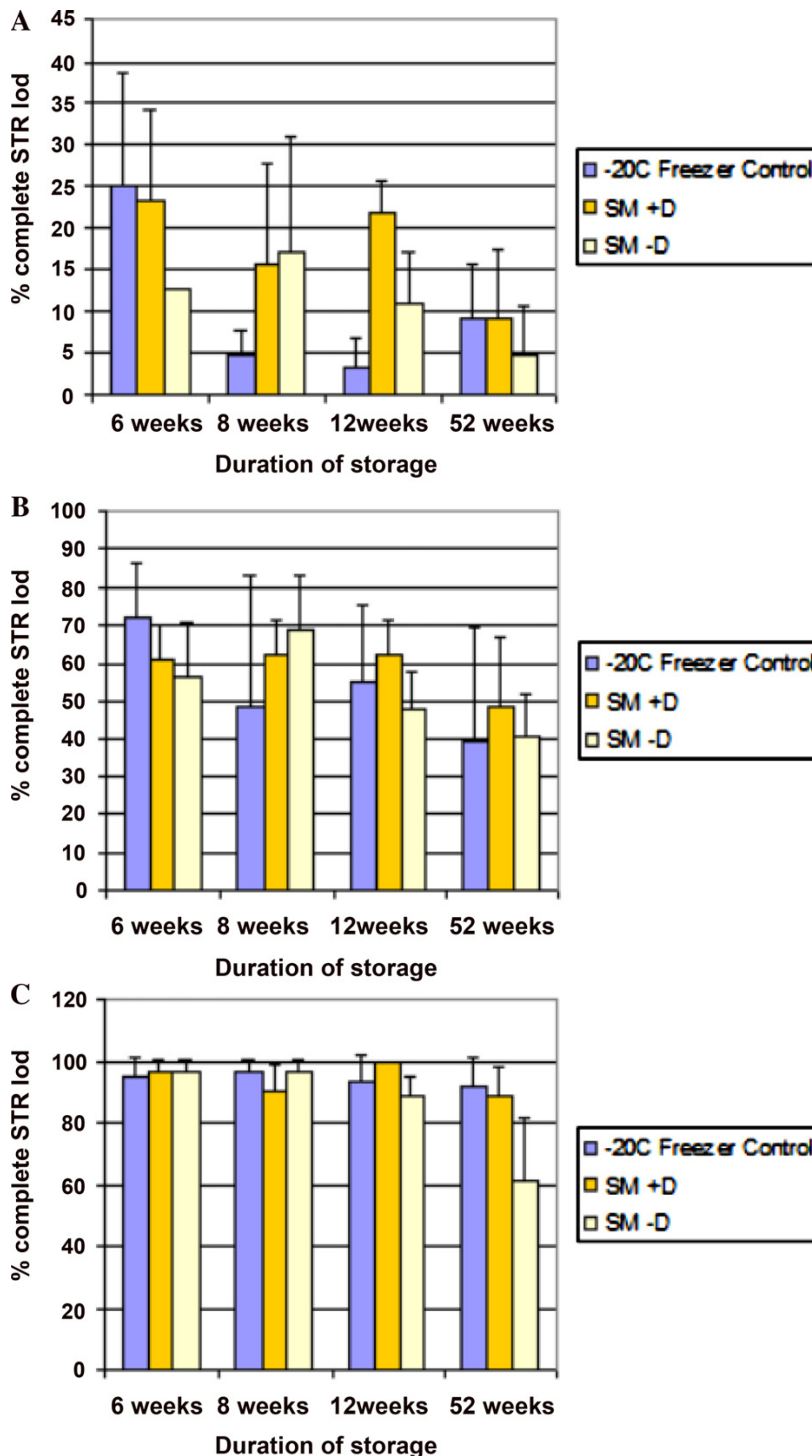

Fig. 2. Average percentages of complete STR allele calls from (A) 0.625 ng, (B) 0.125 ng and (C) 0.25 ng replicate samples stored 6 weeks to 52 weeks. (In (A), only one sample was analyzed for the 0.625ng sample at 6 weeks as the other replicate was below the threshold of allele designation.)

rounds of freeze–thaw (non-protected (NP) samples). The SM tubes were dried overnight in a fume hood and kept at room temperature. After one week, the frozen NP samples were allowed to thaw, 5 μl aliquots were removed and placed into SM tubes, dried and then returned to the freezer until ready for use; NP samples were returned to −20 °C and allowed to refreeze. The two SM aliquots stored dry at room temperature were resuspended to 20 μl with water and a 5 μl aliquot was removed from each tube and placed into SM tubes, dried overnight on the bench top and then stored at room temperature for later amplification. Each

Table 2
Average recovery of DNA (ng) from replicate samples stored over 1 year ($n = 4$)[a].

Control	Stnd Dev	SM+D	Stnd Dev	SM−D	Stnd Dev
0.004	0.000	0.006	0.002	0.002	0.001
0.007	0.005	0.013	0.003	0.005	0.002
0.008	0.001	0.026	0.009	0.008	0.004
0.005	0.004	0.046	0.006	0.030	0.006
0.023	0.007	0.095	0.020	0.047	0.014
0.094	0.049	0.173	0.020	0.103	0.022
0.148	0.025	0.252	0.067	0.158	0.035

[a] It should be noted that in the analysis of these samples at 1 year, the standard used for quantification was systematically off by a factor of 10 resulting in the lower apparent recovery for all samples including the control.

week, the same steps were repeated until there were three complete cycles of freeze–thaw or dehydration–rehydration. At the end of the test period, the 14 SM tubes were resuspended in 5 μl of water, allowed to incubate at room temperature, and amplified with both the PowerPlex 16™Kit (Promega Corporation, Madison, WI) and the Quantifiler™ Human DNA Quantification Kit (ABI). Amplified samples were run on Applied Biosystems 3130xl Genetic Analyzer™ (ABI) and results were analyzed with GeneMapper ID software using a 75 rfu threshold for allele designation to evaluate the integrity of the DNA following stress conditions.

2.4. Assessing inhibition from SM during amplification

To assess any effects of SM remaining in samples, DNA samples extracted from blood using a standard organic extraction or the QIAamp DNA Mini Kit were prepared with increasing concentrations of SM (1–4× concentration) and then used in multiplex amplification reactions for STR analysis utilizing the Powerplex 16 System Kit (Promega, Madison, WI). Preparation of the duplicate sample sets with no SM (0×), and one, two, three and four concentrations of SM (1×, 2×, 3× and 4×, respectively) were prepared for both 200 pg and 500 pg total DNA in each reaction. For the 0× samples, 9947A control DNA was diluted to 100 pg/μl DNA stock solution and 2 μl was used directly in amplification reactions with the PowerPlex16™ System kit (Promega). The 1× sample was prepared by aliquoting 15 μl of the same DNA stock solution into a tube containing dried SM and allowing the mixture to incubate for 15 min at room temperature prior to amplification. The 2×, 3× and 4× samples were prepared by serially rehydrating two, three and four tubes, respectively, of SM with the 15 μl of the stock DNA solution. Samples were then run on a 3130xl Genetic Analyzer (ABI) and results were analyzed with GeneMapper ID™ software using a 75 rfu threshold for allele designation to evaluate the integrity the effects of SM present in multiplex amplification reactions.

3. Results

Samples protected in SM were subjected to a range of environments including storage at elevated temperatures to create accelerated aging conditions [20], multiple rounds of rehydration–dehydration and also storage at ambient laboratory conditions both with and without desiccant present.

3.1. Storage study

Dried down genomic DNA samples were stored in SM at room temperature for 5 months and 50 °C for 1 month. The samples containing SM were stored either at room temperature or under elevated temperature. Fig. 1 shows recovery of DNA following dry storage in SM at room temperature for 5 months and at 50 °C for 1 month. Storing samples under conditions of extreme environmental stress (i.e. heat) can be used to correlate accelerated sample stability, and in this case, storage for 1 month at 50 °C is equivalent to sample stability for 6 months at room temperature [20]. Results indicate that DNA is protected from degradation during dry storage in SM, as compared to non-protected samples with no detectable degradation as compared to the non protected sample at 50 °C (Fig. 1).

3.2. Sensitivity studies

Percentages of complete profiles for samples stored under the three conditions are shown in Fig. 2A–C and Table 1. Full profiles

Table 3
Average recovery and standard deviation of DNA (ng) from replicate samples stored over 1 year ($n = 4$) for all time points[a].

Time	0.063		0.125		0.250		0.500		1.000		2.000		4.000	
	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD	Avg	SD
Control														
1 day	0.101	0.029	0.134	0.075	0.170	0.088	0.438	0.164	1.207	0.284	2.295	0.118	4.815	0.345
1 week	0.166	0.000	0.236	0.184	0.574	0.143	0.931	0.243	1.815	0.465	3.085	0.246	5.300	0.502
2 weeks	0.079	0.060	0.131	0.081	0.291	0.135	0.582	0.114	1.304	0.052	1.926	0.262	4.450	0.654
1 months	0.119	0.000	0.359	0.075	0.454	0.238	0.945	0.186	1.355	0.300	3.075	0.304	5.375	0.742
2 months	0.053	0.020	0.065	0.038	0.197	0.129	0.302	0.114	0.770	0.191	1.441	0.384	3.150	0.171
3 months	0.003	0.000	0.002	0.001	0.008	0.002	0.016	0.006	0.027	0.003	0.060	0.010	0.122	0.006
12 months	0.004	0.000	0.007	0.005	0.008	0.001	0.005	0.004	0.023	0.007	0.094	0.049	0.148	0.025
SM+D														
1 day	0.077	0.066	0.173	0.110	0.302	0.231	0.695	0.232	1.112	0.090	2.480	0.208	4.800	0.526
1 week	0.188	0.097	0.296	0.161	0.449	0.194	1.369	0.193	2.076	0.404	4.235	0.201	8.010	0.826
2 weeks	0.050	0.009	0.214	0.117	0.304	0.105	0.900	0.205	1.548	0.305	3.250	0.157	5.675	0.681
1 months	0.313	0.018	0.403	0.196	0.857	0.487	1.478	0.254	2.274	0.588	5.225	0.359	8.290	1.493
2 months	0.068	0.005	0.132	0.049	0.286	0.031	0.695	0.287	1.493	0.493	3.160	0.329	5.945	1.614
3 months	0.003	0.001	0.006	0.003	0.008	0.002	0.034	0.009	0.055	0.006	0.103	0.011	0.146	0.034
12 months	0.006	0.002	0.013	0.003	0.026	0.009	0.046	0.006	0.095	0.020	0.173	0.020	0.252	0.067
SM−D														
1 day	0.050	0.007	0.096	0.021	0.237	0.071	0.546	0.221	1.107	0.095	2.329	0.439	4.235	0.525
1 week	0.223	0.129	0.232	0.096	0.505	0.203	1.368	0.264	2.054	0.415	4.120	0.372	7.775	1.144
2 weeks	0.083	0.013	0.219	0.035	0.327	0.156	0.627	0.139	1.145	0.103	2.615	0.264	4.645	0.177
1 months	0.202	0.030	0.379	0.156	0.710	0.284	1.375	0.115	2.320	0.554	4.395	0.597	8.120	1.478
2 months	0.132	0.180	0.255	0.240	0.493	0.230	1.240	0.276	2.163	0.525	3.960	0.128	6.555	1.083
3 months	0.003	0.000	0.005	0.004	0.008	0.003	0.031	0.013	0.047	0.017	0.092	0.012	0.159	0.025
12 months	0.002	0.001	0.005	0.002	0.008	0.004	0.030	0.006	0.047	0.014	0.103	0.022	0.158	0.035

[a] It should be noted that in the analysis of these samples at 3 months and 1-year, the standard used for quantification was systematically off by a factor of 10 resulting in the lower apparent recovery for all samples including the control.

Table 4
Quantification of DNA after 3 cycles of freeze–thaw versus three cycles of drying and rehydration in SM.

Cycles	DNA recovered (ng/μl)	
	Rehydration–dehydration	Freeze–thaw
0	9.67 ± 0.13	6.30 ± 1.19
1	7.11 ± 0.29	9.03 ± 1.53
2	9.14 ± 1.15	9.80 ± 0.01
3	9.67 ± 0.13	8.52 ± 0.80

One set of DNA samples was stored using traditional freezer storage, unprotected (NP) and subjected to three cycles of freezing and thawing. A second set was stored in SM dried. These samples were rehydrated, quantified and then re-dried three times.

were observed for SM+D and frozen control samples stored for up to one year from as low as 0.25 ng DNA in 20 μl. The lower end of the sample concentrations (0.0625 ng) exhibited allele drop out in both SM+D and −20 °C freezer control samples throughout the time points (Table 1) likely due to stochastic effects at this low concentration.

Based on quantification values, following 1 year of storage, a 2 to nearly 10-fold increase (for the 0.5 ng samples) in recovery of DNA from all samples stored dried in SM at room temperature in the presence of a desiccant (SM+D) was observed as compared to non-protected liquid control samples stored frozen at (−20 °C) in standard microfuge tubes (Table 3 and Fig. 3). The quantification results for the 1 day samples were unexpectedly lower than those at 1 week and were due in part to differences in standards utilized. Total recovery for the 3 months and 1 year samples displayed what appears to be a significant drop in all samples (including controls). It should be noted that in the analysis of these samples at 3 months and 1 year, the standard used for quantification was systematically off by a factor of 10 resulting in the lower apparent recovery for all samples including the control.

All RFU values of SM+D samples were comparable to those of the control samples; As expected, the dried SM samples stored at room temperature without desiccant present did not perform as well as samples stored in the presence of a desiccant, but still resulted in higher recovery than freezer stored material (Tables 2 and 3, Figs. 2 and 3D).

Overall, the data supports that the integrity of single source samples was not compromised when stored dry in SM in the presence of desiccant over a one year time period under ambient laboratory conditions, as is recommended by the manufacturer. In fact in most instances, recoverability of DNA samples stored in SM significantly exceeded that of freezer storage by more than 2-fold, especially in low concentration aliquots of <1 ng after 12 months

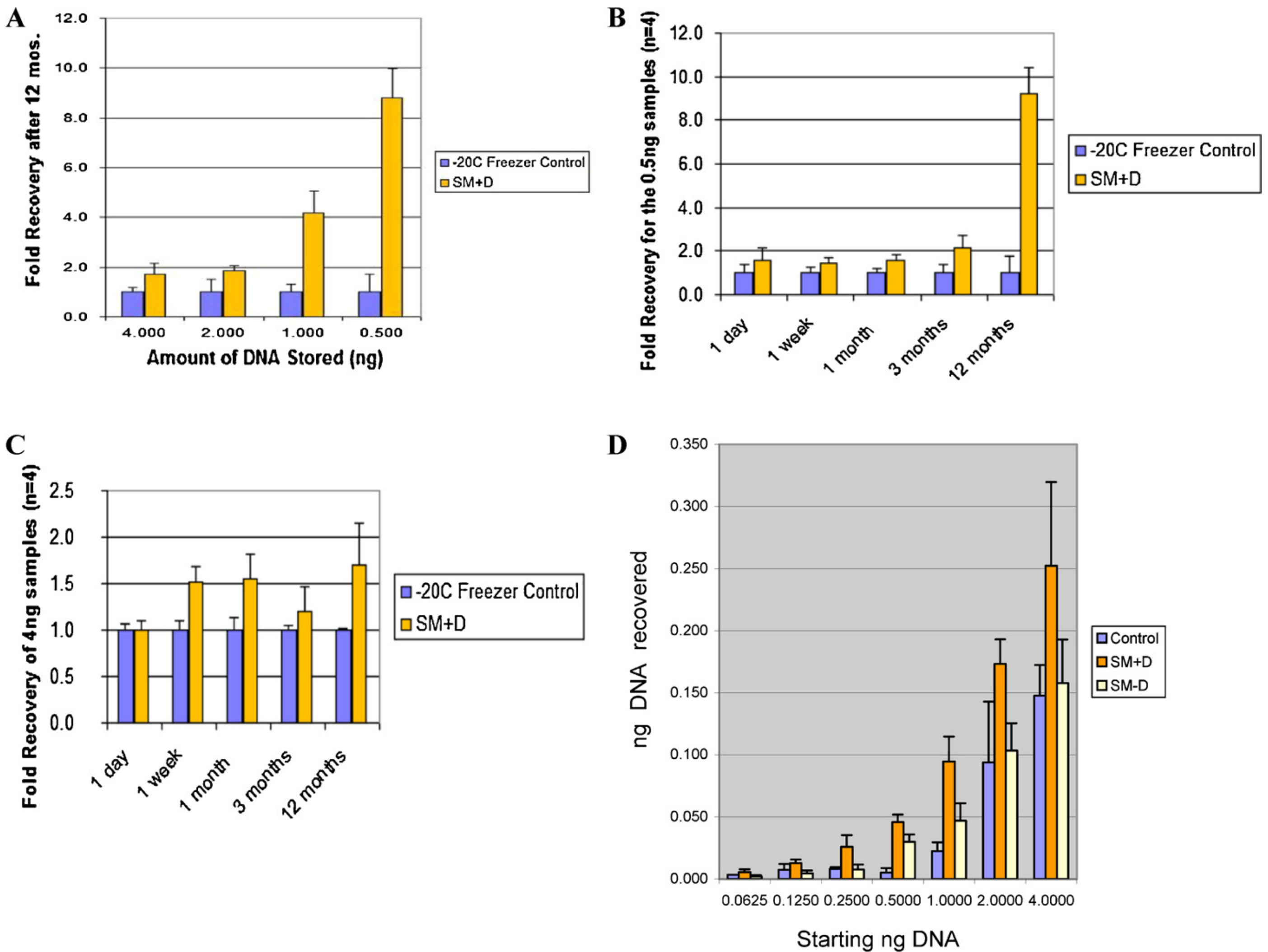

Fig. 3. Quantification of DNA after 1 year of storage. (The quantification results for the 1-day samples were unexpectedly lower than those at 1 week and were due in part to differences in standards utilized.) (A) Fold recovery after 1 year compared to −20 °C freezer controls. (B) Fold recovery for 4 ng DNA replicates. (C) Fold recovery for the 0.5 ng replicates. (D) Average recovery in ng from replicate samples stored for 1 year. (It should be noted that in the analysis of these samples at 3 months and 1 year, the standard used for quantification was systematically off by a factor of 10 resulting in the lower apparent recovery for all samples including the control.)

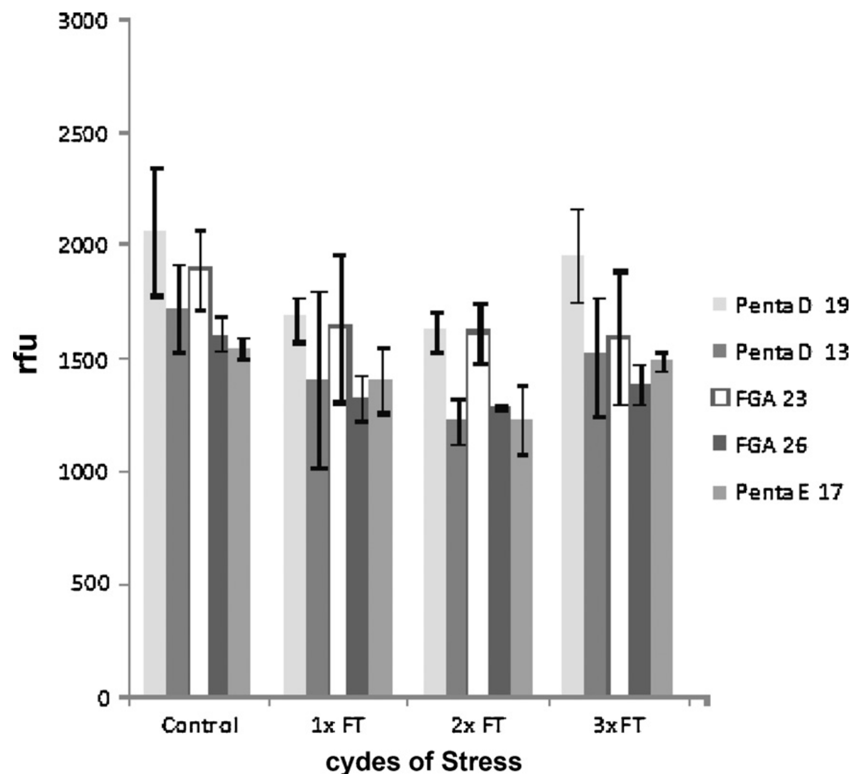

Fig. 4. Average STR peak heights for freeze-thawed (FT) stressed samples. Control samples were held frozen at −20 °C. 1xFT, 2x FT and 3xFT were subjected to 1, 2, or 3 rounds of freeze thaw respectively. Replicate DNA samples (n = 3) were dried for storage in the storage medium (SM) and subjected to three successive cycles (1–3×) of rehydration–dehydration over a 4-week period prior to multiplex STR analysis. Average peak heights and standard deviation for selected loci of Powerplex 16 are shown as compared to samples stored frozen at −20 °C and subjected to identical rounds of freeze–thaw stress (FT) over the same time period. Control samples were stored as frozen liquids and thawed immediately prior to use.

of storage more than 4-fold higher recovery was obtained (Tables 2 and 3 and Figs. 2 and 3).

3.3. Stress study

Replicate samples stored in SM were subject to multiple rounds of dehydration and hydration and control samples were subject to multiple rounds of freeze–thaw. Multiplex STR analysis indicate that no significant changes in peak heights were detected in the relative fluorescence units (RFUs) between control DNA samples stored frozen in SM for the entire 4 weeks and samples subjected to freeze–thaw cycles (Table 4 and Fig. 4). Average DNA recovery for the frozen samples after 3 dehydration and rehydration cycles were 9.14 ng + 1.15 and for samples from the 3× freeze thaw were 9.80 ng + 0.01 (Table 4).

3.4. Evaluation of inhibition from SM

The effect of SM present during amplification for multiplex STR analysis was evaluated by increasing the concentration of SM present in the reaction from one- to four-times the recommended SM concentration followed by amplification using Powerplex 16. A subset of the 16 loci with the average representative peak heights and standard deviation (n = 2) for samples without and with additional SM are shown in Fig. 5.

A complete profile was obtained for all samples tested and the RFUs for each locus were comparable at 2 concentrations. Fig. 5a contains results from the 200 pg samples in the 0×SM, 1×SM, 2×SM, 3×SM and 4×SM and Fig. 5b contains results for the 500 pg at 0×SM, 1×SM, 2×SM, 3×SM and 4×SM. Results indicate that increasing amounts of SM up to two times the recommended concentration does not inhibit amplification reactions in the concentrations tested, and there is no need to purify rehydrated samples prior to addition into reactions used for STR analysis. Similar results were observed for the 200 pg (Fig. 5a) and the 500 pg (Fig. 5b) in that for both template amounts, 0×, 1× and 2× resulted in no significant differences in peak heights between samples without versus with SM. Additional amounts of 3–4× concentrated SM may result in a negative effect suggesting that samples be rehydrated to the same volume as the original stock.

4. Discussion

DNA sample storage is of paramount importance in forensic, epidemiological, clinical and genetic laboratories. There is always the possibility that cases or studies may be re-opened and the stored DNA may need to be re-tested. Moreover, the integrity of the DNA should be maintained to be as high as is possible when first stored. Thus, the results from an initial test and a subsequent test will be similar and comparable. This need to maintain samples and their integrity is especially important with limited DNA from materials such as hairs, bones, teeth and other degraded samples [1]. In addition to sample limitations, manipulations, such as freeze thaw and even long-term storage, can lead to loss of DNA. Utilization of the most efficient storage method for sample stability should be sought. Better storage methods of DNA extracts also should consider cost, ease of handling, and amenability to downstream analysis.

SM is a polymer that when added to DNA allows for dry storage of the material at ambient temperatures; thus there is no need for expensive refrigeration systems that will eventually fail. SM may protect DNA by forming a protective sheath around DNA, forming a barrier to degradation and loss. There have been a number of dry-down approaches, but to date, all have experienced some degree of

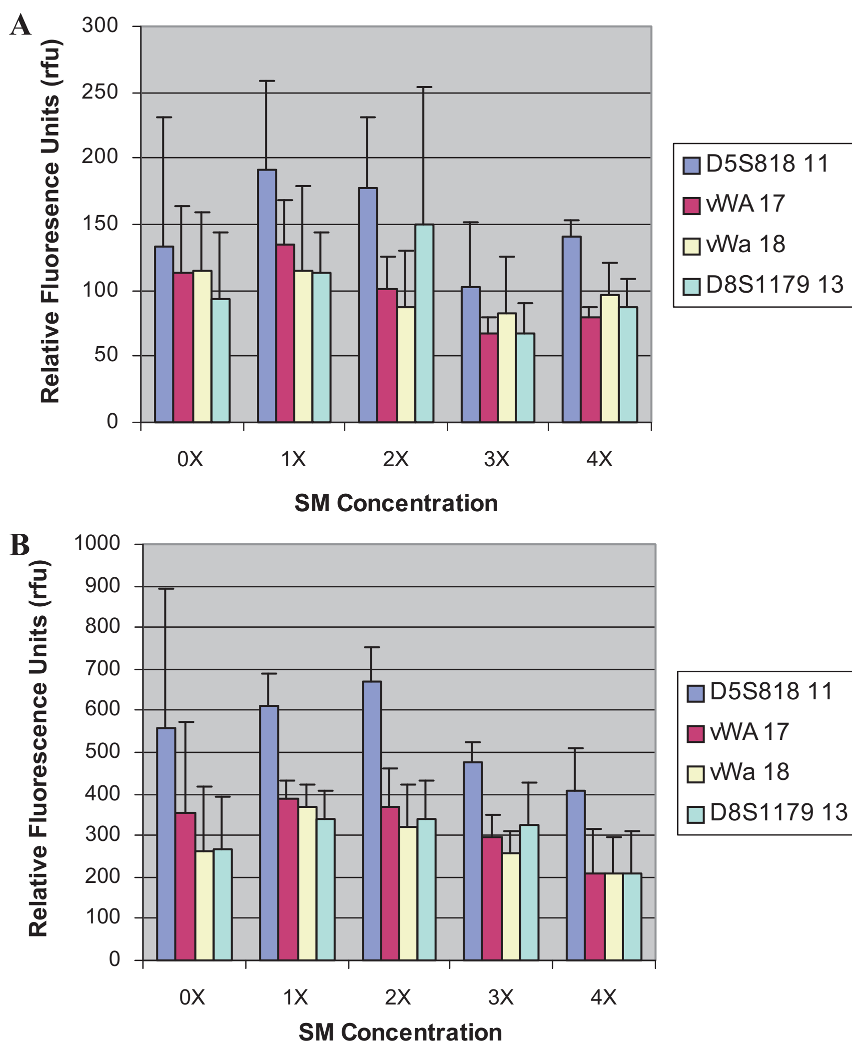

Fig. 5. Average peak heights and standard deviation for replicate samples at 200 or 500 pg with varying amounts of SM. Control samples were frozen at −20 °C. 1×, 2×, 3× and 4× samples contained 200 pg (a) or 500 pg (b) with 1, 2, 3 or 4 times the recommended amount of SM.

sample instability [21]. The studies reported herein, however, have found SM-treated DNA samples are stable for long periods of time (up to one year) without degradation or loss of integrity. The DNA in these studies was derived from forensically relevant materials such as blood and buccal cells. Once extracted, the DNA was subjected to stress conditions that would simulate typical and atypical storage conditions. The integrity of DNA under storage and storage manipulations was equal to or better with SM-treated samples compared with dried and refrigerated/frozen samples. 2–10-fold better recovery was observed for DNA in SampleMatrix with dessicant over −20 °C freezer controls following 1 year of storage. Further studies to assess SM storage have been performed including a shipping study and an accelerated environmental abuse, and storage of DNA extracted from bone [12,22].

The studies described herein might be considered preliminary. However, chemistry theory predicted such an outcome and testing to date further supports the utility of SM treatment as an effective way to maintain the integrity of a sample (once treated) under short and long term storage. This is noteworthy because the DNA extracts were liquids and these are notoriously unstable even to some degree when maintained frozen. Additionally, in a recently reported study, a consortium of DNA biodiversity laboratories also conducted research on DNA storage that focused on new and suitable protective substances, storage at higher temperatures, rehydration of lyophilized DNA, and the usage of special cryotubes [21]. They concluded that samples stored in SampleMatrix provided comparable results to the theoretical best practice of samples storage in liquid nitrogen [21]. Thus, there is strong evidence that SM will be an invaluable material for sample storage, particularly for critical forensic samples. We believe the data are sufficiently impressive to warrant further investigation by the forensic science community and consideration as a beneficial and effective means for extracted DNA storage. There are a greater range of samples that could be tested including those resulting from alternate extraction methods such as Chelex extracted DNA [23] or the new Prepfiler extraction method [24]. Storage studies utilizing DNA from additional extraction methods are underway; however, it is likely the outcome will be similar regardless of the condition of the sample, because truly dried samples are unlikely to degrade [10]. Additional testing on a wider range of samples is recommended to establish a sound basis for using SM. These samples include the DNA from: sexual assault samples, low copy DNA samples, substrates that might impart inhibitors to the process (such as clothing with dyes and sizing treatments, soils with humic acids and wood products) and polymers that may arise in nature that could compete in the dry-down process. Dry-down with SM or other storage media should be tested on intact samples (i.e., prior to DNA extraction). Not all forensic evidence (in some

cases) is extracted initially. Maintaining forensic biological evidence prior to extraction would also be useful especially in cases where immediate transport to controlled conditions is not possible.

Finally, forensic methods that are based on RNA, require optimizing RNA storage as well as DNA. Differential expression may assist in determining the origin of biological evidence based on the relative abundance of messenger RNA [25–30]. In addition, estimating the age of a bloodstain was reported using analysis of mRNA:rRNA ratios [31]. This type of assay may provide information relevant to the time evidence was deposited and assist in determination of when a crime was committed. These RNA-based assays require sensitivity, quantitative results, underscoring the need to optimize and stabilize DNA and RNA storage. RNA storage for gene expression analysis out to 11 days [32] and for microarray expression analysis out to 4 weeks [33] has recently been reported.

5. Conclusion

We evaluated the feasibility of using a novel synthetic storage medium SampleMatrix (SM) for dry storage of forensics source DNA samples at ambient temperature. DNA samples stabilized in SM are sufficiently protected from degradation during dry storage at room temperature, unlike equivalent samples stored in the freezer. SM stored samples allowed between a 2 and 10-fold higher recovery over samples stored in the freezer. This can be extremely important for low concentration and touch samples. We did not experimentally evaluate if this phenomenon is caused by the protective properties of SM, by interfering with surface adsorption of DNA to the tube material, or a combination of both, but it is evident that prolonged freezer storage results in a gradual sample loss and that sample loss is accelerated at lower DNA concentrations. Even under high humidity conditions of 60% relative humidity, DNA samples were stable and showed in most cases higher recovery than freezer stored controls. DNA samples after rehydration and in the presence of SM were successfully used in a variety of downstream applications, such as quantitative real-time PCR, multiplex STR analysis and agarose gel electrophoresis. Rehydrated samples were used directly without further purification; with no interference or inhibition detected with up to 2× concentrated SM during STR amplification. Experiments to further evaluate stabilization of degraded DNA samples in SM and additional studies on forensic DNA samples from mixtures, bone and teeth have been initiated.

Overall, this study suggests that not only is SM a viable format to store low-concentration forensic samples, but it has compelling advantages in maintaining excellent stability and recoverability of samples, especially when compared to traditional freezer storage. The same storage media as well as new sample collection technologies [34] have additional applications in sample and tissue collection and may be extremely valuable for situations where remote collection sites require storage of the collected samples without refrigeration [34].

Acknowledgements

The authors would like to dedicate this paper in loving memory of Jeane Marie Crouse. The authors would like to thank Amy McGuckian and Julie Conover-Sikorsky of PBSO and Linda Le of SJSU for their technical help during this project. This work was supported by a California State University Program for Education and Research in Biotechnology Joint Venture Grant to Steven Lee at SJSU and a National Science Foundation Research Experiences for Undergraduates grant [DBI-0647160 to Julio Soto, Cleber Ouverney and Steven Lee at SJSU and a California Association of Criminalists A. Reed and V. McGlaughlin Scholarship to Kimberly Clabaugh and Brie Silva. The authors also acknowledge support for this project provided by Dr. Rolf Muller and Dr. Judy Muller-Cohn and thank Dr. Omo Clement of Biomatrica and Ines Iglesias-Lee of Oakland Police Department Crime Laboratory for their careful reviews of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fsigen.2011.01.008.

References

[1] B. Budowle, A.J. Eisenberg, A. van Daal, Validity of low copy number typing and applications to forensic science, Croat. Med. J. 50 (2009) 207–217.
[2] B. Budowle, A. van Daal, Extracting evidence from forensic DNA analyses: future molecular biology directions, Biotechniques 46 (2009) 339–350.
[3] M.D. Coble, J.M. Butler, Characterization of new miniSTR loci to aid analysis of degraded DNA, J. Forensic Sci. 50 (2005) 43–53.
[4] P. Gill, Application of low copy number DNA profiling, Croat. Med. J. 42 (2001) 229–232.
[5] J.A. Irwin, M.D. Leney, O. Loreille, S.M. Barritt, A.F. Christensen, T.D. Holland, B.C. Smith, T.J. Parsons, Application of low copy number STR typing to the identification of aged, degraded skeletal remains, J. Forensic Sci. 52 (2007) 1322–1327.
[6] D.L. Davis, E.P. O'Brien, C.M. Bentzley, Analysis of the degradation of oligonucleotide strands during the freezing/thawing processes using MALDI-MS, Anal. Chem. 72 (2000) 5092–5096.
[7] K. Shikama, Effect of freezing and thawing on the stability of double helix of DNA, Nature 207 (1965) 529–530.
[8] C. Gaillard, F. Strauss, Avoiding adsorption of DNA to polypropylene tubes and denaturation of short DNA fragments, Elsevier Technical Tips on-line, 1998, downloaded from http://www.frstrauss.free.fr/reprints/gaillard_TTO98.pdf, accessed July 03, 2004.
[9] C. Gaillard, F. Strauss, Eliminating DNA Loss and Denaturation during Storage in Plastic microtubes, International Biotechnology Laboratory, 2000, 6, downloaded from http://www.frstrauss.free.fr/reprints/gaillard_IBL00.pdf, accessed February 14, 2010.
[10] J. Bonnet, M. Colotte, D. Coudy, V. Couallier, J. Portier, B. Morin, S. Tuffet, Chain and conformation stability of solid-state DNA: Implications for room temperature storage, Nucleic Acids Res. 38 (2010) 1531–1546.
[11] T. Ahmad, R.W. Miller, A.B. McGuckian, J. Conover-Sikorsky, C.A. Crouse, Biomatrica DNA SampleMatrix?: A new prospect for forensic DNA sample storage, in: Poster presentation at the American Academy of Forensic Science Meeting, Denver, CO, February, 2009.
[12] K. Clabaugh, B. Silva, K. Odigie, R. Fourney, J. Stevens, G. Carmody, M.D. Coble, O. Loreille, M. Scheible, M. Kline, T.J. Parsons, A. Pozder, A. Eisenberg, B. Budowle, S.B. Lee, Storage of DNA samples at ambient temperature using DNA-SampleMatrix, in: Poster presentation at the 18th Annual Meeting of International Symposium on Human Identification, Hollywood, CA, October, 2007.
[13] T.J. Anchordoquy, M.C. Molina, Preservation of DNA, Cell Preserv. Technol. 5 (2007) 180–188.
[14] J.H. Crowe, J.F. Carpenter, L.M. Crowe, The role of vitrification in anhydrobiosis, Annu. Rev. Physiol. 60 (1998) 73–103.
[15] J.H. Crowe, Trehalose as a "chemical chaperone": fact and fantasy, Advanced Experimental Medical Biology 594 (2007) 143–158.
[16] J.H. Crowe, L.M. Crowe, S.A. Jackson, Preservation and functional activity in lyophilized sarcoplasmic reticulum, Arch. Biochem. Biophys. 220 (1983) 477–484.
[17] S. Smith, P.A. Morin, Optimal storage conditions for highly dilute DNA samples: a role for trehalose as a preserving agent, J. Forensic Sci. 50 (2005) 1101–1108.
[18] B. Shirkey, N.J. McMaster, S.C. Smith, D.J. Wright, H. Rodriguez, P. Jaruga, M. Birincioglu, R.F. Helm, M. Potts, Genomic DNA of Nostoc commune (Cyanobacteria) becomes covalently modified during long-term (decades) desiccation but is protected from oxidative damage and degradation, Nucleic Acids Res. 31 (2003) 2995–3005.
[19] B. Budowle (Ed.), DNA Typing Protocols: Molecular Biology and Forensic Analysis, Eaton Publishing Company/Biotechniques Books, Westborough, MA, 2000.
[20] K.J. Hemmerich, Accelerated Aging, Medical Plastics and Biomaterials Magazine, 1998, p. 16.
[21] DNA Bank Network, Long Term DNA Storage Workshop Proceedings, downloaded from http://www.dnabank-network.org/publications/Workshop_Long-term_DNA_storage-Summary_and_Abstracts.pdf, accessed February 15, 2010.
[22] S.B. Lee, C.A. Crouse, M.C. Kline, Optimizing storage and handling of DNA extracts, Forensic Sci. Rev. 22 (2010) 131–144.
[23] P.S. Walsh, D.A. Metzger, R. Higuchi, Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material, Biotechniques 10 (1991) 506–513.
[24] M.G. Brevnov, H.S. Pawar, J. Mundt, L.M. Calandro, M.R. Furtado, J.G. Shewale, Developmental validation of the PrepFiler™ forensic DNA extraction kit for extraction of genomic DNA from biological samples, J. Forensic Sci. 54 (2009) 599–607.
[25] M. Bauer, RNA in forensic science, Forensic Sci. Int. Genet. 1 (2007) 69–74.

[26] J. Juusola, J. Ballantyne, Messenger RNA profiling: a prototype method to supplant conventional methods for body fluid identification, Forensic Sci. Int. 135 (2) (2003) 85–96.
[27] J. Juusola, J. Ballantyne, mRNA profiling for body fluid identification by multiplex quantitative rt-PCR, J. Forensic Sci. 52 (6) (2007) 1252–1262.
[28] J. Juusola, J. Ballantyne, Multiplex mRNA profiling for the identification of body fluids, Forensic Sci. Int. 152 (1) (2005) 1–12.
[29] C. Nussbaumer, E. Gharehbaghi-Schnell, I. Korschineck, Messenger RNA profiling: a novel method for body fluid identification by real-time PCR, Forensic Sci. Int. 157 (2–3) (2006) 181–186.
[30] D. Zubakov, E. Hanekamp, W. van Ijken, M. Kayser, Stable RNA markers for blood and saliva identification revealed from whole genome expression analysis of time-wise degraded stains, Int. J. Legal Med. 122 (2008) 135–142.
[31] S. Anderson, B. Howard, G.R. Hobbs, C.P. Bishop, A method for determining the age of a bloodstain, Forensic Sci. Int. 148 (1) (2005) 37–45.
[32] E. Wan, M. Akana, J. Pons, J. Chen, S. Musone, P.Y. Kwok, W. Liao, Green technologies for room temperature nucleic acid storage, Curr. Issues Mol. Biol. 12 (3) (2009) 135–142.
[33] G.E. Hernandez, T.S. Mondala, S.R. Head, Assessing a novel room-temperature RNA storage medium for compatibility in microarray gene expression analysis, Biotechniques 47 (2) (2009) 667–668.
[34] S.P. Wilkinson, A. Stassinopoulos, L. Shireen, A. Berner, M. Kostovic, F. Black, R. Cohen, et al., Impact of collection, stabilization and isolation of bovine ear punches on high and low density genotyping arrays, Int. Soc. Anim. Genet. (2010) downloaded from http://www.biomatrica.com/media/dnagard/poster_dnagard_isag_2010.pdf , accessed on 08/28/10.