Forensic Science International: Genetics 8 (2014) 170–178

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Evaluation of DNAstable™ for DNA storage at ambient temperature

Susanne E. Howlett [a,c], Hilda S. Castillo [a,c], Lora J. Gioeni [b], James M. Robertson [a], Joseph Donfack [a,*]

[a] FBI Laboratory Division, Counterterrorism and Forensic Science Research Unit, 2501 Investigation Parkway, Quantico, VA 22135, United States
[b] FBI Laboratory Division, Chemical Biological Radiological Nuclear Science Unit, 2501 Investigation Parkway, Quantico, VA 22135, United States
[c] Oak Ridge Institute for Science and Education, Oak Ridge, TN, United States

ARTICLE INFO

Article history:
Received 5 June 2013
Received in revised form 17 August 2013
Accepted 10 September 2013

Keywords:
Forensic science
Long-term DNA storage
DNAstable™
Room-temperature storage
qPCR
STRs

ABSTRACT

Preserving DNA is important for validation of prospective and retrospective analyses, requiring many expensive types of equipment (e.g., freezers and back-up generators) and energy. While freezing is the most common method for storing extracted DNA evidence or well-characterized DNA samples for validation studies, DNAstable™ (Biomatrica®), a commercially available medium for room temperature storage of DNA extracts was evaluated in this study. Two groups of samples consisting of different DNA quantities were investigated, one ranging from 20 to 400 ng (group 1) and the other one ranging from 1.4 to 20 ng (group 2). The DNA samples with and without DNAstable™ were stored at four different temperatures [~25 °C (room temperature), −20 °C, 37 °C or 50 °C]. DNA degradation over several months was monitored by SYBR® Green-based qPCR assays and by PCR amplification of the core CODIS STR markers for group 1 and 2 DNA samples, respectively. For the time points tested in this study (up to 365 days), the findings indicate that the −20 °C controls and the DNAstable™ protected samples at room temperature provided similar DNA recoveries that were higher compared to the unprotected controls kept at RT, 37 °C or 50 °C. These results suggest that DNAstable™ can protect DNA samples with effectiveness similar to that of the traditional −20 °C freezing method. In addition, extrapolations from accelerated aging experiments conducted at high temperatures support that DNAstable™ is an effective technology for preserving purified DNA at room temperature with a larger protective impact on DNA samples of low quantity (<20 ng).

1. Introduction

Long-term storage of DNA is of vital importance in the forensic and clinical research worlds. Preserving high quality DNA is recommended by many forensic laboratory protocols in order to facilitate prospective and retrospective analyses [1–4]. The adoption of automation by several clinical and forensic laboratories has increased the speed and effectiveness of DNA extraction and quantification. As a result, growing numbers of extracted stock DNA samples need to be properly stored and maintained. Additionally, dilutions of stock extracts that are made for DNA profiling assays may need to be maintained, substantially increasing the number of samples requiring medium- to long-term storage.

Currently, freezing is the most common method used for storing DNA extracts [2–5]. However, this method can be non-practical and expensive, requiring a large number of freezers and back-up generators. If samples need to be transported frozen, it may be difficult to maintain them in that state. According to FedEx shipping guidelines, packages can be exposed to temperatures as high as 60 °C during shipping, depending on the time of the year [6]. Additionally, it has been reported that loss of sample integrity can occur due to repeated freeze and thaw cycles or microorganism activity [7,8].

In recent years, new technologies for the stabilization and storage of biological samples at room temperature have been developed [9–11]. While these technologies differ in their implementation, the overall paradigm remains the same, to provide long-term stabilization and storage of biological samples. Biomatrica® is one of the developers of a commercially available alternative to low temperature storage of samples, and their product may provide an environmentally friendly and cost effective solution for storing extracts of DNA samples. DNAstable™ is a synthetic polymer that mimics the natural molecular principles of anhydrobiosis by forming a thermo-stable barrier around the biological sample to protect it from degradation. Anhydrobiosis is a strategy used by a variety of organisms including bacteria, yeast, fungi, plants, and insects to survive in extreme dry states [12]. During this process, cells retain minimal amounts of water and no metabolic activity occurs [13]. Anhydrobiotic organisms protect their cellular structures (i.e., DNA, RNA, proteins, and membranes) in this dry state for long-term survival and are later

* Corresponding author. Tel.: +1 703 632 4593; fax: +1 703 632 4500.
E-mail address: Joseph.Donfack@ic.fbi.gov (J. Donfack).

1872-4973/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fsigen.2013.09.003

revived by simple rehydration. This method of sample storage is widely used in academic institutions and is gaining acceptance by forensic laboratories [10,14,15].

There are several types of DNA damage that occur in biological samples due to both environmental and cellular sources [16–20]. These types of damage vary depending on sample storage or physiological conditions and include base modifications, mispairs, cross-linked nucleotides and double-stranded DNA breaks. Fragmentation of longer DNA regions into shorter ones accounts for the majority of physical property changes that occur during DNA degradation and can be monitored via gel electrophoresis or PCR-based methods. Previous studies testing the efficacy of DNAstable™ have primarily utilized standard COmbined DNA Index System (CODIS) Short Tandem Repeat polymorphisms (STR) kits [14,15,21–30]. The original commercial kits for CODIS STR markers amplify amplicons ranging from 97 to 464 base pairs (bp) in size, and current kits target even smaller amplicons (<300 bp) [30]. However, because longer DNA fragments (i.e., 500–1000 bp) are more susceptible to degradation, we hypothesized that monitoring for a decrease in the number of longer DNA fragments would be more informative in detecting moderate DNA degradation compared to shorter DNA fragments. To test this hypothesis, DNA degradation was induced by storing DNA extracts with quantities ranging from 20 to 400 ng (group 1 samples) at 37 °C and 50 °C. Degradation was monitored by SYBR® Green-based qPCR utilizing four primer sets designed to amplify amplicons of 92, 250, 508 and 970 bp in size. Since degradation is easily detected in low quantity DNA samples (e.g., below 20 ng), a sensitivity study was also carried out to determine whether the protective benefits of DNAstable™ are more pronounced as the amount of DNA to be preserved decreases. To achieve this goal, DNA extracts were serially diluted from 1.4 to 20 ng (group 2 samples) and stored at 37 °C and 50 °C. The sensitivity limits of the four primer sets used in the SYBR® Green-based qPCR assays described above varied from 0.020 ng (for the 92 bp amplicon) to 2 ng (for the 970 bp amplicon) of DNA. The input amounts of the DNA samples tested in the sensitivity study were below the sensitivity limit of the least robust primer set (2 ng). As a result, DNA degradation for these samples (group 2) was monitored by the progressive loss of CODIS STR alleles using the AmpFlSTR® Identifiler® PCR Amplification Kit (Applied Biosystems, Foster City, CA).

2. Materials and methods

2.1. Blood collection and DNA extraction

Peripheral blood was collected in purple top tubes (BD Vacutainer tubes coated with EDTA) from three human subjects, according to the policies of the FBI Institutional Review Board. Samples were processed immediately for genomic DNA extraction utilizing the Gentra® Puregene® Blood Kit (Qiagen, Valencia, CA), according to the manufacturer's recommendations. Each DNA extract dissolved in DNA hydration buffer (Qiagen, Valencia, CA) formed the pristine stocks of DNA samples, which were quantified using a NanoDrop™ 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE). The instrument's detection limit is 2 ng/μL (User's Manual). The concentrations of the pristine stocks of DNA as determined by the NanoDrop™ were used to prepare two groups of DNA dilutions using nuclease-free water (Applied Biosystems); 20–400 ng (group 1 samples) and 1.4–20 ng (group 2 samples).

2.2. SYBR® green-based qPCR detection of DNA degradation

2.2.1. PCR primers optimization

Fourteen custom primer sets were designed using NCBI/Primer BLAST [31] and synthesized by Applied Biosystems (Foster City, CA). Optimization for each primer set was carried out as recommended by the manufacturer (Applied Biosystems [32]). Real-time qPCR was performed in duplicate per sample using 25 ng of input DNA in 25 μL reactions on an Applied Biosystems 7500 RT-PCR System. The cycling conditions were as follows: 10 min hold at 95 °C, followed by 40 denaturing and annealing/extension cycles of 15 s at 95 °C and 1 min hold at 60 °C. A dissociation stage, consisting of 15 s at 95 °C, 1 min at 60 °C and another 15 s at 95 °C, was added to the suggested thermal cycling parameters. Of the 14 PCR primer sets tested, four resulted in a single desired amplicon and no amplicon for the "no DNA template control" sample, and therefore were considered suitable and chosen for use in detecting DNA degradation (Table 1).

2.2.2. Sample setup

Twenty microlitres of pristine DNA were added into each well of a 96-well plate pre-coated with DNAstable™ (Biomatrica®, San Diego, CA) in a dried state. The quantities of the DNA samples (group 1) were as follows; 20 ng for room temperature storage (about 25 °C) and 20, 100, and 400 ng for higher temperatures (37 °C and 50 °C). All DNA samples mixed with DNAstable™ herein are referred to as "experimental" samples. The plates were dried, sealed and stored as recommended by the manufacturer. "Control" samples were also prepared by adding 20 μL of the DNA described above (group 1) into DNAstable™ -free micro-tubes (TempAssure), with storage at the corresponding temperatures. Overall, four replicates of each sample were used to detect DNA degradation during each harvest (DNA recovery) time point. To account for possible false positive or negative results, two

Table 1
Primer sets used in the SYBR® Green qPCR Assays.

Primer name	Primer sequence	Amplicon size (bp)	Master mix F:R ratio	R^2_a	R^2_b
D13S317	**F:** AAC AGC AAA ACT GGT GGC TGC AAA **R:** ACT GCC TCA CAG TGG ATT TGG AAA C	250	3:1	0.99	0.95
D7S820	**F:** GGT GCT TCC TTG CTC AGC CTG TC **R:** GTG CTT TGC TCC CTC ATA GTG GTT T	508	1:1	0.87	1
CCR5	**F:** TCA CCA AAC ATC TGA TGG TCT TGC C **R:** TGC GGG AAG CCT GCA CTC TT	92	1:1	0.89	0.97
ACTB	**F:** CCT CAG GGA GGT CAG GGA GCC **R:** GGG TGG TGC CCA CGT CAC CT	970	9:1	0.92	0.91

Master mix ratios are defined by Applied Biosystems' SYBR® Green protocol as containing 0.25 μL (denoted by "1"), 0.75 μL (denoted by "3") or 2.25 μL (denoted by "9") of the forward or reverse primer. The ratios (Forward:Reverse) tested were 1:1, 1:3, 1:9, 3:1, 3:3, 3:9, 9:1, 9:3, and 9:9. R^2a is the coefficient of determination between the average Cts and DNA concentrations (serial dilution of intact DNA). R^2b is the coefficient of determination between the average Cts and degradation times (progressive degradation of intact DNA). In addition to choosing the adequate forward and reverse primer ratios (i.e., resulting in a single desired amplicon and no amplicon for the "no DNA template control" sample), R^2a and R^2b were additional variables used for the final selection of the suitable primer sets (shown in this table) to be used for detecting DNA degradation.