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Efficiency of a novel forensic room-temperature DNA storage medium

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ABSTRACT

The success of forensic genetics has led to considerable numbers of DNA samples that must be stored. Thus, the ability to preserve the integrity of forensic samples is essential. The possibility of retesting these samples after many years should be guaranteed. DNA storage typically requires the use of freezers. Recently, a new method that enables DNA to be stored at room temperature was developed. This technology is based on the principles of anhydrobiosis and thus permits room-temperature storage of DNA. This study evaluates the ability of this technology to preserve DNA samples mimicking true mixture casework samples for long periods of time. Mixed human DNA from 2 or 3 persons and at low concentrations was dried and stored for a period ranging from 6 months to 2 years in the presence of a desiccant. The quality of the stored DNA was evaluated based on quantitative peak height results from Short Tandem Repeat (STR) genotyping and the number of observed alleles. Furthermore, we determined whether this matrix has a potential inhibitory or enhancing effect on the PCR genotyping reactions. In our previous work, we demonstrated the considerable potential of this new technology. The present study complements our previous work. Our results show that after 2 years of aging at room temperature, there is a decrease in the number of observed alleles and in the peak height of these alleles.

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1. Introduction

The success of forensic genetics has highlighted the importance of effective storage methods for extracted DNA.

Numerous forensic samples recovered from crime scenes are degraded or damaged, leading to a reduced likelihood of obtaining meaningful results. This observation has led to the design of new amplification methods for low quantities of low quality DNA by targeting mini STRs. It is also obvious that such DNA evidence should be preserved in a way that reduces the potential for damage to occur. Poor storage methods may compromise samples and negatively impact results, as testing is not always performed immediately. Moreover, retesting is a vital component of forensic science. Such casework DNA is typically frozen at −20 °C or −80 °C; however, with increasing numbers of samples, freezing becomes costly and not without risk of failure. Although DNA is routinely frozen, little is known about potential DNA degradation following freezing/thawing cycles. Several studies have suggested that repeated freezing/thawing impairs the quality of the DNA [1–3], while others do not report such freezing/thawing degradation of DNA [2,4]. Moreover, some authors claim that degradation can even be observed during storage at −20 °C [2].

Bonnet et al. investigated dry storage of solid-state DNA [5,6]. Their studies showed that both humidity and oxygen have detrimental effects on DNA. The authors also suggested that in absence of water and oxygen, DNA has a very long lifetime. Many sample storage studies have considered dry storage to be a solution [7], although DNA damage can occur even at low moisture levels [8]. Nevertheless, the absence of water and oxygen is rarely attained without the use of costly equipment.

To address these problems, Morin and co-worker tested the use of trehalose as a method for storing DNA in an easy and safe manner at room temperature [9]. Trehalose is one of the major compounds that accumulates during anhydrobiosis [10,11]. Trehalose, in addition to other compounds, replaces water and interacts with macromolecules during dehydration. The work of Smith and Morin showed that drying DNA in the presence of trehalose is an acceptable alternative to freezing [9]. DNA could be stored and remain amplifiable for up to 1 year [9]. Molina and Anchordoquy, however, investigated the storage of plasmid DNA lyophilized in trehalose [12] and detected single-strand breaks after 2 weeks at 20 °C. This observation was confirmed by Colotte et al. [6].

Trehalose is also known to retain the activities of dry enzymes for days [13] and has been used to preserve various biological materials, including vaccines [14].

Biomatrica Inc. has developed a sample matrix, based on anhydrobiosis, to address the need to stabilize and prevent the degradation of DNA. The samples are dried in a synthetic matrix

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and can be stored at room temperature. This matrix, also called Qiasafe, is a synthetic polymer that mimics the anhydrobiosis process. The protective effect appears to be based on its ability to form glass through minor groove interactions, thus stabilizing DNA [15]. GenVault produced a similar product called GenTegra.

Biomatrica Inc. has performed internal studies using their matrix and accelerated aging studies by heating DNA samples. Heating of DNA is known to induce damage [16]. This strategy allows samples to undergo virtual years of aging in only a few weeks. According to Biomatrica Inc., the matrix can protect DNA for approximately 30 accelerated years [17]; however, this artificial aging does not perfectly mimic natural aging.

An early study underlined the high potential of this technology. Using the Qiagen (Qiasafe) conservation matrix, DNA was stored for up to 3 weeks at room temperature without obvious degradation or loss [17].

Our previous study proved that medium to low quantities (150 pg/μL) of DNA can be safely stored for up to 6 months at room temperature using this method [18]. We also showed that multiple cycles of dehydration/hydration had no detrimental effects on the DNA. We observed that the conservation efficiency appeared to be dependent on the extraction method. DNA extracted with phenol/chloroform could be stored in the GenTegra matrix for more than 6 months without any degradation. By contrast, DNA extracted using magnetic beads could not be safely stored over the same period of time [18]. Lee et al. evaluated the storage of low DNA quantities for 1 year at room temperature [15]. This second study confirmed that samples stored in a matrix permitted a much higher recovery compared with samples stored in a freezer [18]. Lee et al., however, observed a loss of DNA stored both at −20 °C and in the storage matrix, but this loss was lower in the latter case. This group concluded that storage in the Qiagen matrix is better than freezing at −20 °C.

These results demonstrate the potential of this technology, but the effects of long-term studies and true aging should also be studied to confirm its efficiency. Additionally, a great portion of the samples treated in forensic laboratories is obtained from skin contact. The quantities of DNA obtained from such skin samples are low. Moreover, the DNA obtained is often a mixture of DNA from several persons.

Here, we studied the potential boosting effect of the Qiasafe matrix on PCR amplification.

Finally, we stored low quantities of DNA mimicking casework samples, obtained from a mixture of two or three persons, for 6 months, followed by rehydration and dehydration for a supplemental storage period to reach a conservation period of 2 years in the matrix at room temperature (natural aging).

2. Materials and methods

2.1. Samples

Samples mimicking casework mixtures were obtained from proficiency tests.

One table, which includes the type of sample, the origin of the sample and the storage condition used is presented in the supplemental data section (Table S1).

2.2. DNA extraction

DNA was extracted using a standard organic extraction protocol with phenol/chloroform/isoamyl alcohol, followed by ethanol precipitation.

First, cells were lysed for 6 h at 56 °C in 1 vol. of HOMO PK (0.1 M NaCl, 0.01 M EDTA, 0.5% SDS, 0.01 M Tris–HCl (pH 8)), to which 0.2 mg of Proteinase K (Promega, Madison, USA) and 0.02 vol. of β-mercaptoethanol were added. After 6 h, 0.2 mg of Protease K was added for a second incubation of 2 h at 56 °C.

One volume of phenol/chloroform/isoamyl alcohol was added to the lysate and gently mixed for 10 min, followed by centrifugation. The aqueous phase was preserved. Another volume of phenol/chloroform/isoamyl alcohol was added to the aqueous phase, gently mixed for 10 min and centrifuged. The aqueous phase was again preserved, and two volumes of ether were added to the upper layer, followed by gentle mixing and centrifugation. The organic phase was removed. The remaining ether in the lower phase was removed by evaporation at 37 °C for 1 h 30 min. The DNA was precipitated using ethanol and resuspended in $T_{10}E_{0.1}$.

Epithelial or semen fractions (Table S1) were prepared by differential lysis using Proteinase K, followed by a standard organic extraction with phenol/chloroform and an ethanol precipitation.

2.3. DNA quantification

The nuclear DNA in the extracts was quantified using the Quantifiler system (Applied Biosystems, Carlsbad, USA) and an Applied Biosystems 7500 Real-Time thermal cycler, according to the manufacturer's specifications. Each quantification was performed in duplicate. The average concentration was considered for the experiments.

2.4. PCR amplification with the ESI kit

The ESI kit (Promega, Madison, USA) was used according to the manufacturer's recommendations. Ten μL of sample per reaction, in a total reaction volume of 25 μL, were amplified on a C1000 Thermal Cycler (Bio-Rad, Nazareth, Belgium). Each sample was amplified once per experiment. Samples were analyzed on a 3500xL Genetic Analyzer (Applied Biosystems, USA) using a 36 cm capillary array (Applied Biosystems, USA), POP-4 polymer (Applied Biosystems, USA) and 1× genetic analysis buffer with EDTA (Applied Biosystems, Carlsbad, USA). Injection conditions were as follows: 15 kV and 8 s or 12 s. For the PCR-enhancing study, the samples were analyzed on a 3130xL Genetic Analyzer (Applied Biosystems, USA) using a 36 cm capillary array (Applied Biosystems, USA), POP-4 polymer (Applied Biosystems, USA), 1× genetic analysis buffer with EDTA (Applied Biosystems, USA), a 5 s injection and 15 kV electrophoresis. Two μL of each PCR product were loaded with 10 μL of formamide (Applied Biosystems, USA). The Genemapper IDx 1.2 software (Applied Biosystems, USA) was used for analysis. The detection threshold was fixed at 50 RFU for the samples analyzed on the 3130xL Genetic Analyzer. For the 3500xL Genetic Analyzer, the detection threshold was calculated based on the following formula: (average background + 10 STD) and was dependent on the color, as follows: 70 RFU for green; 60 RFU for yellow; 50 RFU for blue and 60 RFU for red.

A stochastic threshold of 135 RFU was calculated for analysis on the 3500xL Genetic Analyzer. The following formula was used: ((average background + 3 STD) × 3 + (average background + 3STD) × ΔPHR)). ΔPHR (the peak height ratio between two alleles of a heterozygous system) was fixed at 0.6.

2.5. Application of DNA to the conservation matrix

The Qiasafe (Qiagen) conservation matrix was used according to the manufacturer's specifications. Twenty microliters of diluted DNA extract were applied to a tube coated with the matrix, mixed gently by pipetting and allowed to dry overnight (16 h) under a chemical flow hood with a constant air humidity of 18%. The humidity was measured using a hygrometer (Oregon Scientific, Portland, USA). Then, tubes were closed and placed in the aluminum bags in which the Qiasafe tubes were delivered. These

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