

Performance of DNASTable Plus® with genomic DNA storage under high stress conditions, in both liquid and dry format

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Introduction

Scientific discoveries are at an all-time high resulting in an ever-increasing need to store and ship molecules such as DNA, RNA and proteins. Most molecular laboratories are dependent on cold rooms and freezers for the storage of these basic molecules. Freezers and cold rooms use up valuable lab space and result in high-energy costs. The ground-breaking SampleMatrix™ Technology has been developed using the natural principles of anhydrobiosis, alleviates the need of cold rooms and freezers, allowing storage of molecules at ambient lab temperatures. The data presented here show that storage of genomic DNA in DNASTable Plus protects DNA integrity even under high stress conditions, such as extreme temperatures. While DNA stored non-protected degrades, DNA stored in DNASTable Plus remains high quality for at least one year of storage in liquid format and decades in dry down format (based on accelerated aging). Storage in DNASTable Plus allows for complete elimination of the cold chain for genomic DNA storage, and protects DNA even under extreme temperatures.

Materials and Methods

Liquid DNA storage: Human genomic DNA samples were suspended in either DNASTable Plus or TE at diverse concentrations, and stored under stress conditions at high temperatures (45°C or 60°C) for the amount of time specified in each experiment. Control samples were prepared in water and stored at 4°C. Samples were visualized on a 1% agarose gel stained with ethidium bromide.

Dry DNA storage and rehydration: Human genomic DNA samples were suspended as above, dried onto Samplegard® plates in a laminar flow hood or under a vacuum concentrator, and stored under stress at high temperatures (60°C or 85°C). Samples were also prepared in water, at the same concentrations, and stored at 4°C. After storage for the time specified in each experiment, samples were rehydrated in water for 10-15 minutes and visualized on a 1% agarose gel stained with ethidium bromide.

qPCR: 10µl of DNA samples (0.5ng/µl) were dried in a laminar hood and stored non-protected or in DNASTable Plus. Samples were rehydrated in 10µl of water and added directly to a qPCR reaction (iQ™ SYBR® Green Supermix-Biorad), constituting 30% of the total qPCR reaction, and run on an ABI 7200 Real Time PCR machine. DNA recovery was compared to recovery from samples stored at 4°C, relative to standard concentrations of freshly prepared DNA samples.

Long range PCR: 4µL of 50ng/µL DNA samples stored at high temperatures in liquid format, non-protected or in DNASTable Plus, were added directly to a 50µL Long Range PCR reaction (KOD Xtreme Hot Start PCR, Novagen) and run on an ABI GeneAmp PCR System, 9700. A 7.5Kb fragment of the human β-Globin gene was amplified, and compared to amplification from 4°C DNA samples.

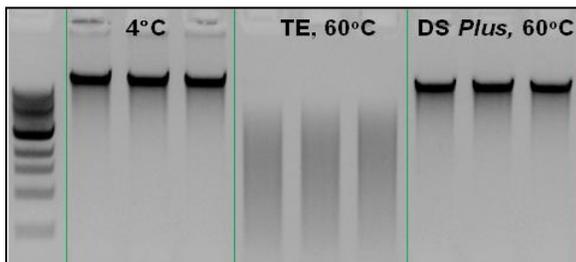


Figure 1: Stability of human genomic DNA extracted from blood, after liquid format storage in DNASTable Plus (DS Plus) or without protection (TE), at 60°C for 1 month. Control samples were stored at 4°C at the same concentration (100ng/µl). 100ng of each sample were visualized on a 1% agarose gel stained with ethidium bromide.

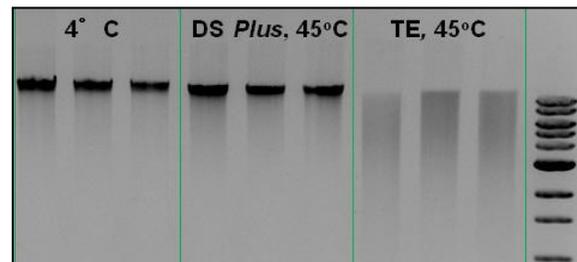


Figure 2: Stability of human genomic DNA extracted from blood, after liquid format storage in DNASTable Plus (DS Plus) or without protection (TE), at 45°C for 6 months. Control samples were stored at 4°C, at the same concentration (50ng/µl). 100ng of each sample were visualized on a 1% agarose gel stained with ethidium bromide.

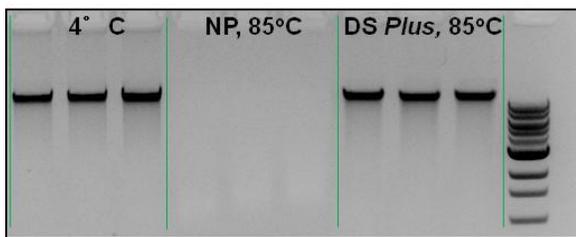


Figure 3: Stability of human genomic DNA extracted from blood after dry format storage in DNASTable Plus (DS Plus) or without protection (NP), at 85°C for 6 months. Control samples were stored at 4°C at the same concentration (50ng/µl). DNA samples were rehydrated and 100ng visualized on a 1% agarose gel stained with ethidium bromide.

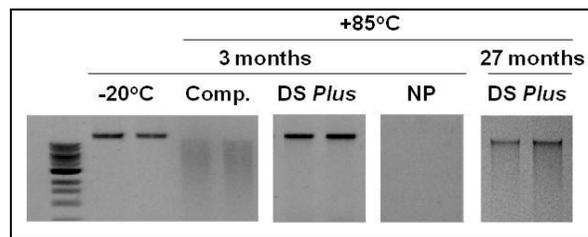


Figure 4: Stability of genomic DNA extracted from HEK293 cells after dry format storage in DNASTable Plus (DS Plus), a competitor (Comp.), or without protection (NP), at 85°C for the specified time. Control samples were stored at -20°C. DNA samples were rehydrated and 100ng visualized on a 1% agarose gel stained with ethidium bromide.

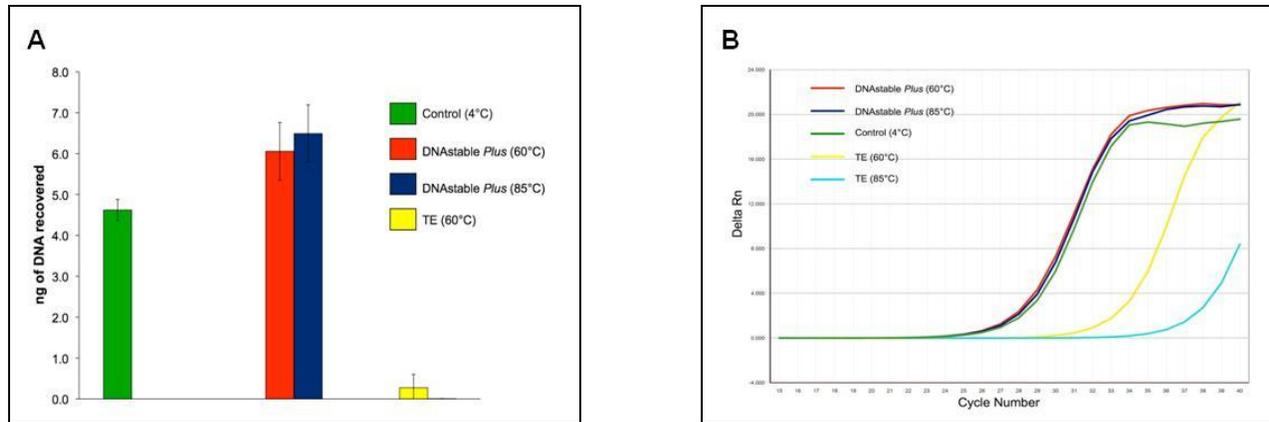


Figure 5: Stability of human genomic DNA extracted from blood after **dry format** storage in DNastable Plus (DS Plus) or without protection (TE), at 60°C and 85°C for 6 months. Control samples were stored at 4°C at the same concentration (0.5ng/ul). DNA samples were rehydrated into 10µl of water and the total sample (5ng) was used as template for qPCR amplification of a RNase P amplicon, using the iQ™ SYBR® Green Supermix (Biorad) according to the manufacturer's instructions. DNA/stabilizer constituted 30% of the total qPCR reaction volume.

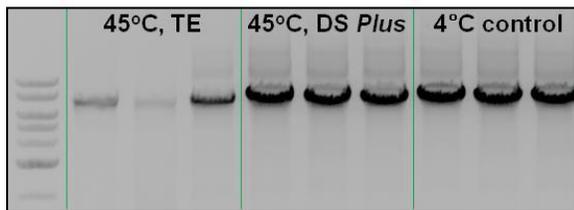


Figure 6: Quality of human genomic DNA extracted from blood samples, after storage at high temperatures in **liquid format** DNastable Plus (DS Plus). Samples were stored in DNastable Plus or without protection (TE), for 1 month at 45°C. Control samples were stored at 4°C at the same concentration (50ng/µl). An equal amount (200ng, 4ul) of all DNA samples was used as template for Long Range PCR (LR-PCR) amplification of a 7.5Kb fragment of the human β-Globin agene. 20% of the LR-PCR reaction product was visualized on a 1% agarose gel stained with ethidium bromide.

Results and Discussion

The protective properties of the novel DNastable Plus inhibit degradation of genomic DNA even when samples are stored under high stress conditions such as high temperatures, in both liquid and dry down formats. The integrity of DNA samples was analyzed by gel electrophoresis, qPCR and long range PCR. DNastable Plus clearly protects genomic DNA stored for one month under extreme stress, at 60°C in liquid format (Figure 1). Without the protective effect of the DNastable Plus, genomic DNA stored unprotected in TE gets degraded, as shown by the DNA smear and the absence of the high molecular weight band. When DNA samples are stored in liquid format at 45°C, DNastable Plus is able to preserve DNA integrity for at least 6 months, while non-protected samples are degraded by this time (Figure 2). This data shows the great stabilizing properties of DNastable Plus for short to medium term storage of genomic DNA samples in liquid format, without any workflow changes.

For long term storage of genomic DNA samples, we recommend the use of DNastable Plus in a liquid to dry format, which reduces the hydrolysis rate of the DNA. To test the stabilization capabilities of DNastable Plus under extreme conditions in a dry down format, genomic DNA samples were prepared in DNastable Plus or under non-protected conditions (NP), dried and stored at 85°C. Samples stored in a competitor formulation (C) were prepared following the manufacturer's instructions, and stored at the same conditions. Samples stored non-protected at 85°C get degraded extremely fast, while samples stored for 6 months in DNastable Plus show similar integrity to control samples stored at -20°C (Figure 3). Data accumulated during product development of the DNastable Plus (Figure 4) shows that DNA samples stored non-protected or using a competitor stabilizer cannot withstand these extreme temperatures, and are degraded after 3 months of storage. Demonstrating the remarkable stabilizing capabilities of DNastable Plus, samples stored dry in DNastable Plus remain of high quality even after 27 months of storage at 85°C, which translates into protection of DNA samples stored at room temperature for decades, based on accelerated aging.

In order to confirm the stabilizing capabilities of DNastable Plus and it's compatibility with downstream applications, DNA samples stored in dry format for 6 months at elevated temperatures were rehydrated and DNA recovery quantified by qPCR (Figure 5). While DNA stored in TE is degraded and therefore cannot be recovered from these samples, DNA stored in DNastable Plus remains of high quality, and can be completely recovered from these samples, with similarly to DNA from samples stored at 4°C. Similar data was also obtained from samples stored in liquid format at elevated temperatures (data not shown).

The integrity of genomic DNA samples stored in DNastable Plus was further confirmed by long range PCR amplification of a 7.5Kb fragment of the human β-Globin amplicon, using as template genomic DNA samples stored in liquid format under elevated temperatures (Figure 6). After one month of storage at 45°C, amplification from DNA stored non-protected in TE is clearly reduced, while amplification from DNA stored in DNastable Plus is comparable to amplification from control samples stored at 4°C, showing the high DNA protection conferred by DNastable Plus, even when samples are stored under high stress conditions.

All the data presented here demonstrates the outstanding DNA stabilizing capabilities of DNastable Plus in both liquid and dry format, as well as the easy workflow and compatibility with downstream applications. DNastable Plus is a great alternative to cold storage for short to medium term storage of genomic DNA samples in liquid format, without any necessary changes to sample, processing workflows. For longer term storage, the use of DNastable Plus in dry down format, protects samples stored for decades at room temperature, and confers protection even when samples are challenged with high stress conditions.