

Evaluation of Next Generation Sequencing Technology to DNA Protected by DNASTABLE® Plus

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Introduction

We have previously shown that storage of genomic DNA in DNASTABLE® Plus (Cat No. 53091-016) protected DNA integrity under high stress conditions, such as extreme temperatures. DNA stored in DNASTABLE Plus remains high quality for at least one year in liquid format and decades in dry format at ambient temperature; whereas non-protected DNA degrades. In addition, we have demonstrated that DNA protected by DNASTABLE Plus can be applied in multiple downstream applications, such as Agilent Bioanalyzer, PCR, qPCR, long range PCR, and SNP genotyping. Here we further show that human genomic DNA stored in DNASTABLE Plus, under extreme conditions, can be used for next generation sequencing (NGS), with similar performance with the DNA kept at cold temperature.

Materials and Methods

Liquid DNA storage: Triplicate samples of human genomic DNA were stored in DNASTABLE Plus (125µg/0.5mL) at 60°C for 4 weeks. DNA controls in both DNASTABLE Plus and water (125µg/0.5mL) were stored at 4°C for the same period of time. All DNA samples were visualized on a 1% agarose gel stained with ethidium bromide after 4-week storage.

Dry DNA storage and rehydration: Triplicate samples of human genomic DNA were prepared in DNASTABLE Plus at the concentration of 500ng/µL. 100µL (50µg) were applied into each well of a 96-well Samplegard® plate. The plate was dried overnight in a tissue culture hood and stored at 85°C for 4 weeks. DNA controls at same concentrations were prepared in water and stored at 4°C for the same period of time. Each dried sample was rehydrated by adding 100µL of water and transferring the DNA into a vial, and then additional 100µL of water was added into the same well and transferred to the same vial. Each vial was incubated at room temperature on an orbital shaker for 10 minutes. The DNA samples were visualized on a 1% agarose gel stained with ethidium bromide after rehydration.

Samples for next generation sequencing: For each sample to be analyzed by NGS, 5µg genomic DNA (100µL at 100ng/µL) were prepared in either water (controls) or DNASTABLE Plus and shipped to the Genomics Core Facility at University of Chicago. The libraries were generated by either Illumina or Life Technologies platforms. Next generation sequencing experiments were performed using Life Technologies' SOLID platform.

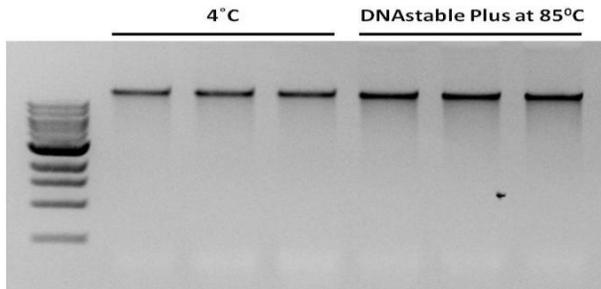


Figure 1: Stability of human genomic DNA from blood samples. The samples were stored in DNASTABLE Plus (**dry format**) at 85°C for 1 month. Control samples were stored at 4°C. 100ng of each sample are visualized on a 1% agarose gel stained with ethidium bromide.

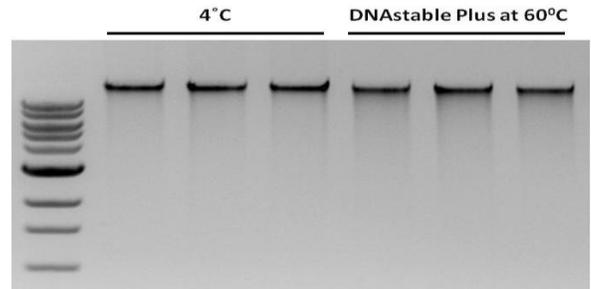
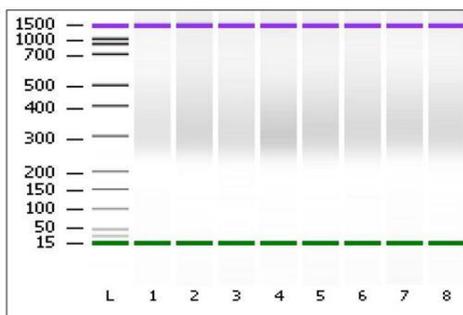


Figure 2: Stability of human genomic DNA from blood samples. The samples were stored in DNASTABLE Plus (**liquid format**) at 60°C for 1 month. Control samples were stored at 4°C. 100ng of each sample were visualized on a 1% agarose gel stained with ethidium bromide.

A: Illumina Library



B: SOLID Library

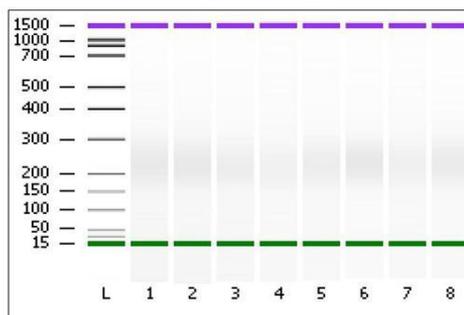


Figure 3: Generation of DNA libraries using next generation sequencing platforms from Illumina (**A**) or Life Technologies (**B**). Agilent Bioanalyzer profiles obtained from libraries generated with genomic DNA samples stored in DNASTABLE Plus under stress conditions, in both liquid (lanes 3, 4) or dry (lanes 5, 6) formats, are identical to those obtained from control samples stored at 4°C in both H₂O (lanes 1, 2) and DNASTABLE Plus (lanes 7, 8).

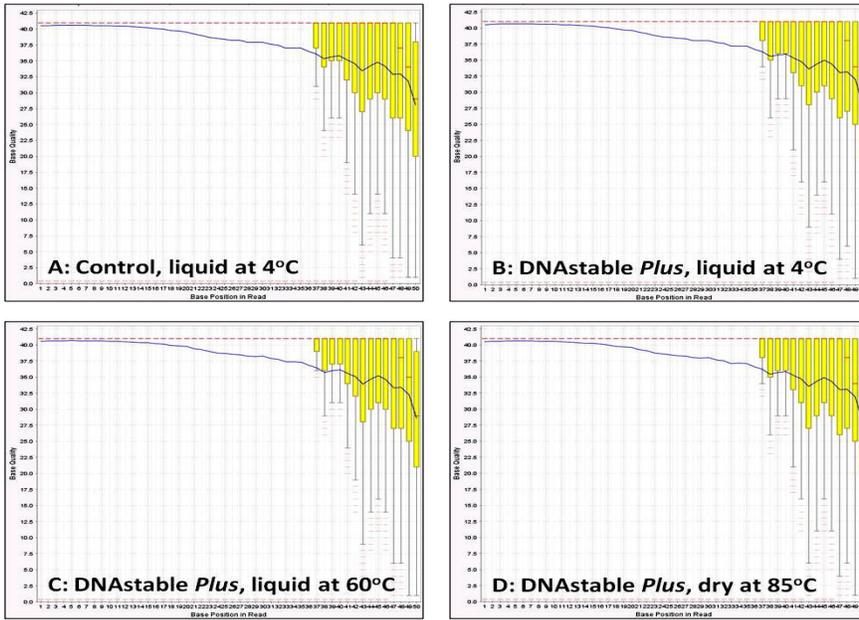


Figure 4: Qualities of sequencing reads using SOLID platform (Life Technologies). Base qualities from the samples sequenced by next generation sequencing technology show that genomic DNA stored in DNASTable Plus at 4°C (B), high stress conditions in liquid format (C), and dry format (D) are similar to the control samples stored at 4°C (A).

Sample	Total number of reads	Average coverage over all chromosomes	Average coverage over whole exome	BWA mapping of fastq reads files	
Control-1	16337019	0.25	0.25	56.29%	
Control-2	13365453	0.2	0.2	58.88%	
DNASTable Plus	Liquid, 4°C-1	12137308	0.19	0.18	58.10%
	Liquid, 4°C-2	15265718	0.23	0.23	58.24%
	Liquid, 60°C-1	11165313	0.17	0.17	56.38%
	Liquid, 60°C-2	12934088	0.2	0.19	59.20%
	Dry, 85°C-1	17509052	0.27	0.26	58.61%
	Dry, 85°C-2	13737151	0.21	0.2	57.89%

Table 1: Overview of next generation sequencing of human genomic DNA using SOLID platform (Life Technologies). Overall coverage statistics, such as total number of sequencing reads, average coverage of the human genome and exome, as well as percentage of mapped sequencing reads, show no difference between controls and samples stored in DNASTable Plus, even under extreme conditions.

Results and Discussion

The protective properties of DNASTable Plus prevent degradation of genomic DNA that are stored under high stress conditions such as high temperatures, in both liquid and dry formats. We have previously shown the great performance of DNA samples protected by DNASTable Plus in downstream applications such as Agilent Bioanalyzer, PCR, qPCR, long range PCR, and SNP genotyping. In this study, we further demonstrated the high qualities of genomic DNA stored in DNASTable Plus under high stress conditions by next generation sequencing technology. Genomic DNA remains high qualities in DNASTable Plus at elevated temperatures for one month (Figure 1, 2). Using NGS technologies from two of the leading providers, Illumina and Life Technologies, we show that DNA stored in DNASTable Plus in either liquid or dry format generate identical profiles as control samples in cold storage (Figure 3, A and B). In addition, figure 4 shows the identical base qualities by sequencing reads for all samples stored in DNASTable Plus at various conditions (B, C, and D) verse control samples stored at 4°C (A). Furthermore, by examining the total number of sequencing reads, average coverage of the human genome or exome, and percentage of mapped sequencing reads, the overall coverage statistics show no difference between controls and samples stored in DNASTable Plus, even under extreme conditions. In summary, human genomic DNA protected by DNASTable Plus can be used for next generation sequencing.

Note: Please read all instructions for the [DNASTable® Plus](#) prior to using this protocol.

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