Stabilization of purified human genomic DNA using DNAstable® *Plus*, at 60°C for 3 years

INTRODUCTION

DNAstable® *Plus* (Cat. # 53091-016) has been shown to preserve purified DNA samples during shipping and storage at ambient or elevated temperatures in both liquid and dry formats. Specifically, previous data has shown DNA stabilization in DNAstable® *Plus* for 18 months at 60°C or 6 months at 85°C, corresponding to at least 20 years of ambient temperature storage based on accelerated stability studies[1]. In addition, DNA protected by DNAstable® *Plus* has been shown to be compatible with downstream applications, such as the PCR, qPCR, long range PCR and next generation sequencing[2]. Here we present additional data showing up to 3 years stability of genomic DNA stored in DNAstable® *Plus* at 60°C, which is equivalent to at least 40 years at room temperature based on accelerated stability studies. The data below demonstrates that, upon storage in DNAstable® *Plus,* recovered DNA retains similar integrity to DNA samples preserved by cold storage, with similar performance in long-range PCR and qPCR applications. This data confirms the effectiveness of DNAstable® *Plus* in preserving genomic DNA at ambient and elevated temperatures, thus providing a viable alternative to cold storage for long term biobanking of precious DNA samples without the costs and risks associated with cold chain shipping and storage.

MATERIALS AND METHODS

Sample Preparation and Storage

20µL samples of genomic DNA purified from human blood were suspended in 5µL DNAstable® *Plus* or Tris-EDTA (TE) buffer at varying concentrations (0.05 ng/µL, 0.5 ng/µL, 5 ng/µL, and 50 ng/µL), dried onto SampleGard® plates using a vacuum concentrator, and stored at 60°C for 3 years. Control samples were prepared in water and stored at 4°C for 3 years.

DNA Rehydration and Analysis

DNA samples were rehydrated in 25 µL water for 15 minutes at room temperature, with shaking at 600 rpm. Rehydrated DNA samples were analyzed by the following methods:

1. Agarose Gel Electrophoresis: 100ng of rehydrated DNA was visualized on a 1% agarose gel stained with ethidium bromide.
2. Long-range PCR: A 7.5 kb fragment of the human β-globin gene was amplified using the KOD Xtreme™ Hot Start PCR kit (Novagen). Primers specific to the human β-globin gene were synthesized by Integrated DNA Technologies Inc. (Table 1).

Primer	Sequence
β-globin Forward	5'-CTGCTGAAAGAGATGCGGTGG-3'
β-globin Reverse	5'-GCACTGGCTTAGGAGTTGGACT-3'

Table 1: *Primer sequences*

KOD Xtreme™ Hot Start PCR kit	Final Concentration
Buffer	1x
DNTP	0.4 mM
Forward Primer	0.3 µM
Reverse Primer	0.3 µM
Enzyme mix	1 U
Template DNA	100 ng

Table 2: *Final PCR reagent concentrations*

   Long-range PCR reactions of 50µL were amplified using the GeneAmp® PCR System 9700 (Applied Biosystems®) and 20% of the reaction products were visualized on a 1% agarose gel stained with ethidium bromide.
3. qPCR: rehydrated DNA samples were amplified on a CFX96 Real-Time PCR Instrument (Bio-Rad), using TaqMan® Universal PCR Master Mix (Life Technologies) with RNase P primers and probe synthesized by Integrated DNA Technologies Inc. (Table 3).

Primer	Sequence
Forward	5'-CTGCTGAAAGAGATGCGGTGG-3'
Reverse	5'-GCACTGGCTTAGGAGTTGGACT-3'
Probe	5'/56FAM/TTCTGACCTGAAGGCTCTGCGCG/3BHQ_1/-3'

Table 3: *RNase P primer and probe sequences*

RESULTS

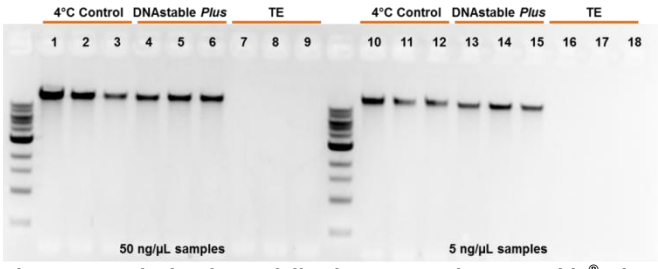

Figure 1: Analysis of DNA following storage in DNAstable® *Plus*. Samples 1-3 and 10-12 represent DNA stored in TE buffer for 3 years at 4°C in liquid format (positive control). The remaining samples were stored dry at 60°C for 3 years. Samples 4-9 represent 50 ng/µL DNA stored in either DNAstable® *Plus* (4-6) or TE (7-9). Samples 13-18 represent 5 ng/µL DNA stored in either DNAstable® *Plus* (13-15) or TE (16-18). 100 ng of the triplicate samples per condition were run on a 1% agarose gel stained with ethidium bromide.

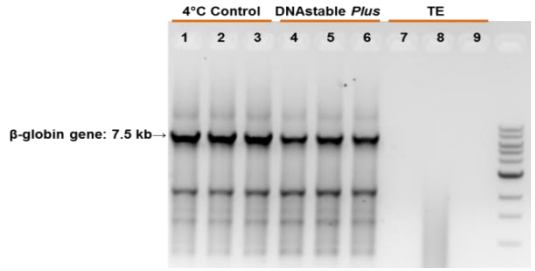

Figure 2: Long-range PCR assays using DNA samples stored in DNAstable® *Plus*. Samples 1-3 represent DNA stored for 3 years in TE buffer at 4°C in liquid format while the rest of the samples were stored dry at 60°C for 3 years at 50 ng/µL. Samples 4-6 represent DNA stored in DNAstable® *Plus*, while samples 7-9 represent DNA stored in TE buffer. 100 ng of rehydrated DNA from triplicate samples were amplified using KOD Xtreme™ Hot Start PCR kit (Novagen) with 7.5 kb human β-globin primers.

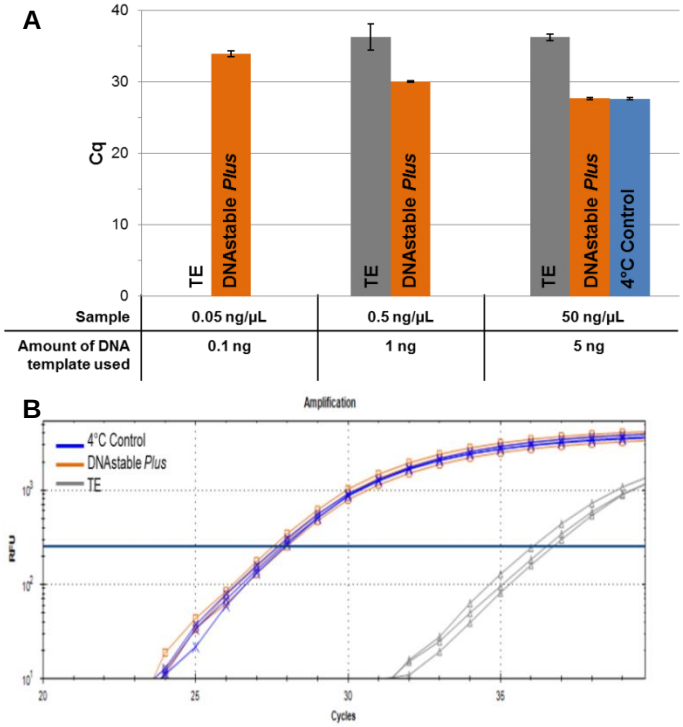

Figure 3: qPCR assays using DNA samples stored in DNAstable® *Plus*. Quantification cycles from all samples are shown in Figure 3A with error bars indicating standard deviation. Representative traces of 50 ng/µL DNA samples are shown in Figure B. Varying amounts of template DNA (shown in Figure 3A) from each condition were amplified using TaqMan® Universal PCR Master Mix with RNase P primers and probe in two technical qPCR replicates from triplicate samples per condition.

SUMMARY

In this study, we evaluated the stability of DNA following three years of storage in DNAstable® *Plus* at 60°C using agarose gel electrophoresis, long-range PCR, and qPCR. The data presented here supports the hypothesis that DNAstable® *Plus* protects human genomic DNA stored in dry format, even at 60°C for 3 years. DNAstable® *Plus* preserved high molecular weight DNA, which was completely degraded in DNA samples stored in TE buffer (Figure 1). DNA stored in DNAstable® *Plus* produced the expected 7.5 kb amplicon from the human β-globin gene, with equivalent performance to that of control DNA stored at 4°C, whereas the unprotected DNA stored dry in TE buffer failed to amplify during PCR due to shearing of the DNA template (Figure 2). Therefore, our data suggests that DNAstable® *Plus* protected the β-globin DNA template from shearing and maintained its integrity for use in long-range PCR applications. DNA stabilized in DNAstable® *Plus* also showed similar performance to control samples stored at 4°C in qPCR Taqman® assays. Our findings indicate that DNA stored dry in DNAstable® *Plus* at different concentrations, ranging from 0.05 ng/µL to 50 ng/µL, amplified with similar efficiency (and similar Cq) to control samples, while the DNA stored unprotected in TE buffer showed poor performance (failure to amplify or higher Cq). All the data presented here demonstrates the DNA stabilizing capabilities of DNAstable® *Plus* under heat stress conditions. This data, collected after storage at 60°C for 3 years, corresponds to more than 40 years of storage at 22°C based on our accelerated stability calculations[1]. Overall, our data indicates that DNAstable® *Plus* can be used as a long term solution for storage of precious DNA samples for research and biobanking applications.

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References

[1]Plambeck, J., Chemical Sciences: The Arrhenius Rate Law.
[2]Liberal, V., Stassinopoulos, A. and Muller, R. Performance of DNAstable *Plus*® with genomic DNA storage under high stress conditions, in both liquid and dry format.