A simplified workflow for direct DNA elution from silica columns using DNAstable® *Plus*

INTRODUCTION

DNAstable® *Plus* (Cat #53091-016) has been shown to preserve purified DNA samples during shipping and storage at ambient and elevated temperatures, both in liquid and dry formats[1]. In addition, DNA protected by DNAstable® *Plus* has been shown to be compatible with downstream applications, such as the Agilent Bioanalyzer, PCR, qPCR, and long range PCR[1].In the following study, DNAstable® *Plus* was used during the DNA purification process as a DNA eluent, following binding of the DNA to silica columns. The data presented below demonstrates that DNAstable® *Plus* is compatible as an eluent with QIAamp® Mini Spin Columns, and retains its ability to protect purified DNA for >80 months based on accelerated stability studies.

MATERIALS AND METHODS

DNA Purification: Human genomic DNA was purified from 20 µL or 200 µL blood samples using the QIAamp DNA Mini Kit (Qiagen Cat #51304) according to the manufacturer's purification protocol for blood, with the exception of using two different methods for eluting the DNA. Half of the samples were eluted in 100 µL of Buffer AE, as stated in the manufacturer's protocol, and the rest of the samples were eluted in 100 µL of 5-fold diluted DNAstable® *Plus* in Buffer AE (20 µL of DNAstable® *Plus* to 80 µL of Buffer AE), to achieve the final DNAstable® *Plus* recommended concentration for sample storage.

DNA Analysis: DNA quantification and A_{260}/A_{280} ratio were obtained by UV absorbance using a Take3 plate with a BioTek® Synergy 3 reader. Also, 10% of the eluted DNA (~10 µL per sample) was visualized on a 1% agarose gel stained with ethidium bromide.

Dry DNA Storage and Rehydration: A total of 100 ng of DNA purified from each sample was dried down in 0.2 mL PCR tubes using a vacuum concentrator and was stored at 85°C in sealed moisture-barrier silver foil bags with desiccant packs. Control samples included 100 ng of DNA eluted in buffer AE with DNAstable® *Plus* added after elution, and 100ng of DNA eluted in buffer AE stored at -20°C. After the indicated times, samples were rehydrated in 15 µL water for 5-10 minutes and the entire amount of rehydrated DNA was visualized on a 1% agarose gel stained with ethidium bromide.

RESULTS

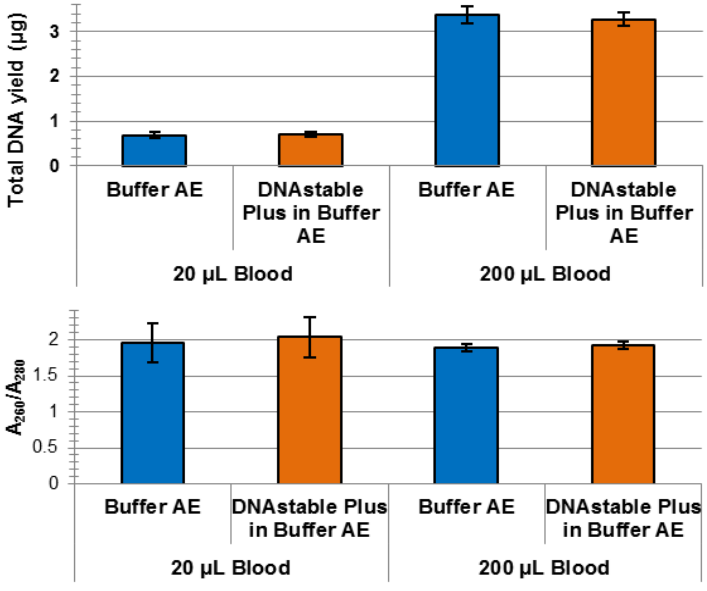

Figure 1. DNA yield and quality is maintained when eluted with DNAstable *Plus*. UV absorbances of total genomic DNA eluted in buffer AE (blue bars) or DNAstable *Plus* diluted 5 fold in buffer AE (orange bars) were measured. **A)** Total DNA yield in mg from either 20 µL or 200 µL of blood using each eluent. **B)** Average A_{260}/A_{280} ratio of DNA prepared from either 20 µL or 200 µL of blood using each eluent. Three replicates for each condition were tested. Error bars indicate the standard deviation.

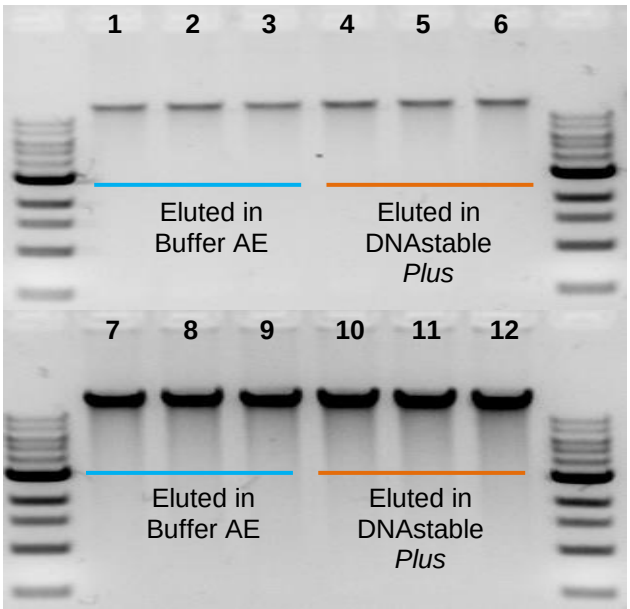

Figure 2. DNA quality is unchanged when eluted with DNAstable *Plus*. Total genomic DNA from either 20 µL (Samples 1-6) or 200 µL (Samples 7-12) of whole blood was eluted in 100 µL of either buffer AE (Samples 1-3, 7-9) or DNAstable *Plus* diluted 5 fold with buffer AE (Samples 4-6, 10-12). Ten percent of the triplicate eluates per condition were run on a 1% agarose gel stained with ethidium bromide.

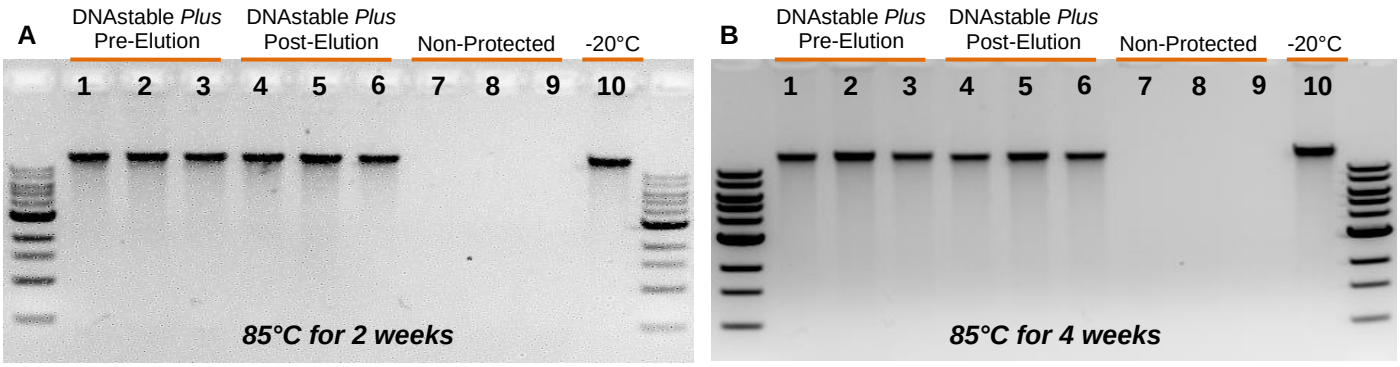

Figure 3. Stability of genomic DNA eluted in DNAstable *Plus* at 85˚C for 2 weeks (A) and 4 weeks (B). 100ng of total DNA from each sample was dried in a vacuum concentrator and stored at 85˚C for the indicated times. Samples 1-3 were eluted and dried in 5-fold diluted DNAstable *Plus*, Samples 4-6 were eluted in buffer AE and dried with 20% DNAstable *Plus* (final concentration of DNAstable *Plus* in DNA is the same as Samples 1-3), and Samples 7-9 were eluted and dried in buffer AE. Sample 10 was stored in liquid format at -20˚C as a control.

SUMMARY

In this study, we evaluated the performance of DNAstable® *Plus* as an eluent during QIAamp DNA Mini column purification in parallel to using DNAstable® *Plus* according to our standard protocol. In Figures 1 and 2, genomic DNA purified from either 20 µL or 200 µL of blood shows equivalent yield and quality for both control (AE buffer) and alternative (DNAstable *Plus*) elutions. Figure 3 shows that DNAstable® *Plus* maintains its DNA-protecting performance after 4 weeks of sample storage under high stress conditions at 85°C when used to elute DNA from spin columns (DNAstable® *Plus* Pre-Elution). As positive controls, the standard protocol of adding DNAstable® *Plus* to purified DNA (DNAstable® *Plus* Post-Elution) or traditional freezer storage method are shown. Storage for this duration and temperature is equivalent to more than 80 months of storage at ambient laboratory temperatures, based on accelerated aging[2]. Our results indicate that DNAstable® *Plus* can be efficiently used as a DNA eluent from silica columns while maintaining its functionality. We therefore recommend incorporating DNAstable® *Plus* during the DNA isolation, contributing to the simplification of the sample processing workflow, which is especially important for high throughput sample processing and automated workflows.

References

[1] 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.
[2]The Arrhenius Rate Law. James A. Plambeck. Instuite Science, 1996.