ApplicationNote

Stable 16-year storage of DNA purified with the QIAamp® DNA Blood Mini Kit

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In this application note, we describe the success of the QIAamp DNA Blood Mini Kit in the preparation of highly stable DNA, as evidenced by 16-year storage data. We also report the best storage conditions for maximal protection against degradation.

Introduction

Most nucleic-acid–based technologies, such as PCR, rely on purified DNA. Storing purified DNA instead of the original sample material saves storage space and avoids having to purify DNA each time it is required for analysis. Very often, DNA needs to be stored before or between analyses. For reliable and reproducible analyses, purified DNA must be stored under conditions that ensure stability and prevent degradation. In our ongoing stability study, we show that DNA purified with the QIAamp DNA Blood Mini Kit is stable for at least 16 years. Our data indicate however, that DNA stability is dependent upon storage conditions.

Materials and methods

DNA was purified from 24 human peripheral blood samples using the QIAamp DNA Blood Mini Kit, according to the protocol entitled "DNA Purification from Blood or Body Fluids (Spin Protocol)", outlined in the *QIAamp DNA Mini and Blood Mini Handbook*, April 2010. DNA was eluted either in Buffer AE (10 mM Tris·Cl; 0.5 mM EDTA, pH 9.0; supplied with the kit) or in water. The purified DNA was stored at either

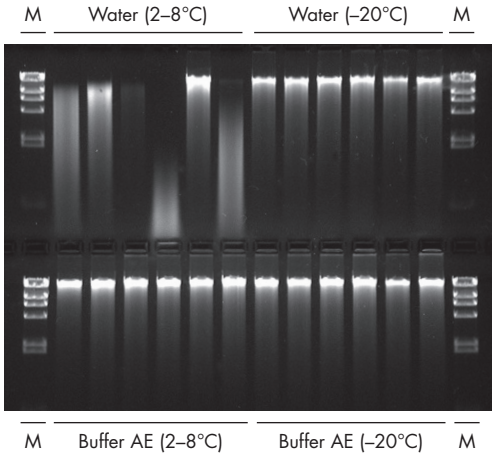

Figure 1. Agarose gel analysis of genomic DNA stored for 8 years. Agarose gel analysis (0.8% agarose gel, 1x TBE buffer, 90 V, 75 minutes) of approximately 500 ng DNA from 24 samples was performed. DNA was eluted in Buffer AE or water, and stored for 8 years at 2–8°C or –20°C. M: Marker (λ *Hind*III).

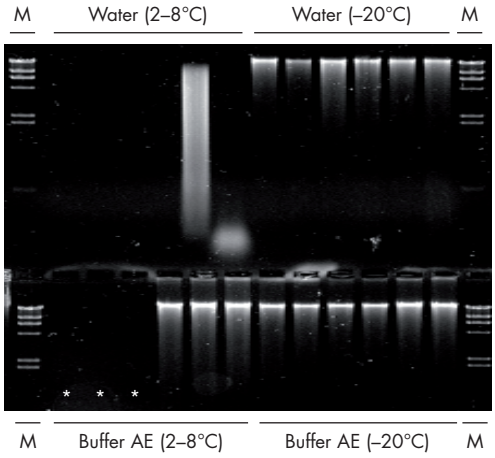

Figure 2. Agarose gel analysis of genomic DNA stored for 16 years. Agarose gel analysis (0.8% agarose gel, 1x TBE buffer, 90 V, 75 minutes) of approximately 500 ng DNA from 24 samples was performed. DNA was eluted in Buffer AE or water, and stored for 16 years at 2–8°C or –20°C. M: Marker (λ *Hind*III).

** No sample loaded in these lanes.*

2–8°C or –20°C for 16 years, and analyzed at regular intervals by agarose gel electrophoresis and PCR amplification of a 1 kb fragment from the human, single-copy Hugl gene. The purified DNA stored at –20°C was thawed and refrozen only at test time points.

Results

The results of this stability study indicate that both storage buffer and temperature may influence DNA stability. The results correlate well with our 8-year study where we concluded that DNA samples remained intact when stored in Buffer AE at 2–8°C or –20°C (Figure 1, lower panel), or in water at –20°C, but were degraded to varying degrees when stored in water at 2–8°C (Figure 1, upper panel). Our 16-year stability study further underscored these observations. Analysis by agarose gel electrophoresis showed that DNA eluted and stored in Buffer AE was stable for at least 16 years at either 2–8°C or –20°C (Figure 2, lower panel). DNA stored in water for 16 years at –20°C remained intact, but showed varying degrees of degradation when stored at 2–8°C (Figure 2, upper panel).

These results are confirmed by the data obtained from PCR amplification (Figures 3–4). The expected 1 kb fragment was amplified from DNA samples stored in Buffer AE at 2–8°C or –20°C, and from DNA samples stored in water at –20°C. For the DNA samples stored in water at 2–8°C, the amount of PCR product varied between samples and correlated with the extent of degradation observed by agarose gel electrophoresis (Figures 1–2). For samples with severe DNA degradation, there was no amplification of the 1 kb fragment.

The degradation of the DNA samples stored in water at 2–8°C may have been due to acid hydrolysis (at pH 5–6), since water is unbuffered and can be slightly acidic. Buffer AE is intended to protect DNA during storage, with the Tris buffering against low pH and the EDTA inhibiting nucleases.

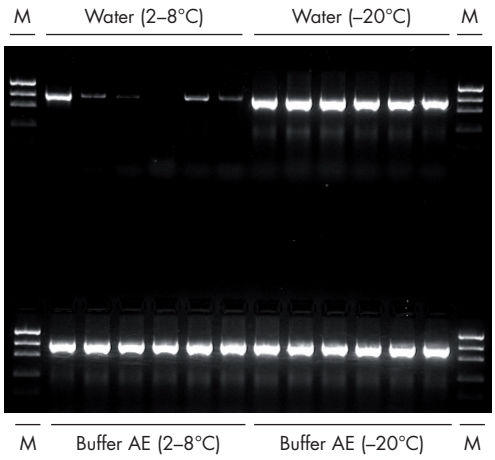

Figure 3. PCR amplification using genomic DNA stored for 8 years. PCR amplification of a 1 kb fragment of the singlecopy gene HugI is shown.
M: Marker (Low DNA Mass Ladder, Life Technologies).

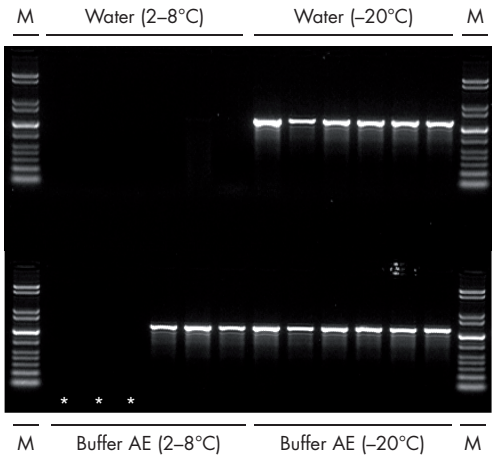

Figure 4. PCR amplification using genomic DNA stored for 16 years. PCR amplification of a 1 kb fragment of the singlecopy gene HugI is shown.
M: Marker GelPilot® 1 kb Plus Ladder.
** No sample loaded in these lanes.*

Conclusions

- DNA purified with the QIAamp DNA Blood Mini Kit is stable for at least 16 years.
- Buffer AE is a superior alternative to water for long-term storage for DNA at 2–8°C. If DNA is to be archived, Buffer AE should be used for elution to protect against degradation.
- DNA eluted in Buffer AE can be stored at either 2–8°C or –20°C.
- DNA stored at –20°C is not affected by repeated thawing and refreezing.
- DNA eluted in water must be stored at –20°C. DNA stored in water at 2–8°C can degrade after prolonged storage.

Ordering Information

Kit name	Contents	Cat. no.
QIAamp DNA Blood Mini Kit (50)	For 50 DNA minipreps: 50 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51104
QIAamp DNA Blood Mini Kit (250)	For 250 DNA minipreps: 250 QIAamp Mini Spin Columns, QIAGEN Protease, Reagents, Buffers, Collection Tubes (2 ml)	51106

Visit **www.qiagen.com/blood-DNA-storage** for more information!