QIAGEN News 2004 e10

Stable 8-year storage of DNA purified with the QIAamp DNA Blood Mini Kit

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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. Quite often, DNA needs to be stored before or between analyses. For reliable and reproducible analyses, purified DNA must be stored under conditions in which it is stable and does not degrade. In our on-going stability study, we show that DNA purified with the QIA amp DNA Blood Mini Kit is stable for at least 8 years. Our data indicate how ever, that DNA stability is dependent upon storage conditions.

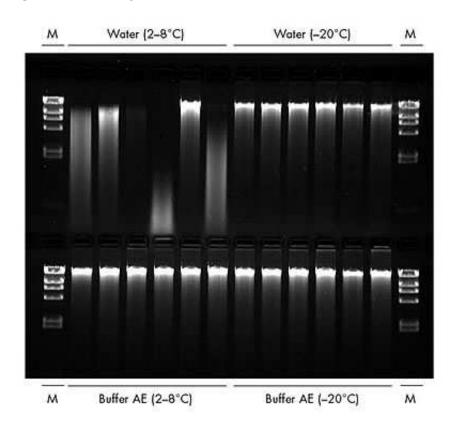
Materials and methods

DNA was purified from 24 human peripheral blood samples using the QIAamp DNA Blood Mini Kit according to the <u>Blood and Body Fluid Spin Protocol</u>. 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 2–8°C or –20°C for 8 years, and analyzed annually 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 thaw ed and refrozen only once each year.

Results and discussion

The results of this stability study indicate that both storage buffer and temperature may influence DNA stability. Analysis by agarose gel electrophoresis show ed that DNA eluted and stored in Buffer AE w as stable for at least 8 years at either –20°C or 2–8°C (see figure "Agarose Gel Analysis of Stored Genomic DNA", low er panel). DNA stored in w ater for 8 years show ed different results. The DNA samples remained intact w hen stored at –20°C, but w ere degraded to varying degrees w hen stored at 2–8°C (see figure "Agarose Gel Analysis of Stored Genomic DNA", upper panel).

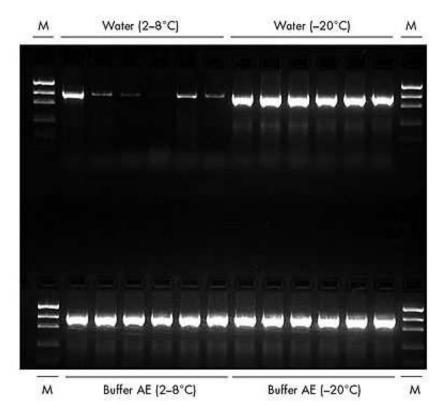
Agarose Gel Analysis of Stored Genomic DNA



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

These results are confirmed by the data obtained from PCR amplification (see figure "PCR Amplification from Stored Genomic DNA"). 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. However, for 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. For samples with severe DNA degradation, there was no amplification of the 1 kb fragment.

PCR Amplification from Stored Genomic DNA



PCR amplification of a 1 kb fragment of the single-copy gene Hugl from the DNA samples shown in figure "Agarose Gel Analysis of Stored Genomic DNA". **M**: marker (Low DNA Mass Ladder, Invitrogen).

The degradation of the DNA 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. Alternatively, the DNA may have been eluted with water from a water source contaminated with minor traces of nucleases, or the DNA may have been contaminated with nucleases during the annual opening of the sample tubes. Buffer AE is intended to protect DNA during storage, with the Tris buffering against low pH and the EDTA inhibiting nucleases.

Conclusions

- DNA purified with the QIAamp DNA Blood Mini Kit is stable for at least 8 years.
- 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 annual thaw ing 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.

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