DNA genotek

Long-term stability of DNA from saliva samples stored in the Oragene® self-collection kit†

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Storage of specimens by refrigeration or freezing can significantly increase costs. The Oragene® kit eliminates these costs by allowing saliva specimens to be stored for years at room temperature without DNA degradation. This document presents evidence that Oragene/saliva samples stored at room temperature maintain high molecular weight DNA for at least 5 years.

Introduction

Large population-based studies, involving thousands of subjects, are increasingly being used to investigate the genetic determinants of complex diseases. Saliva is a convenient source of genomic DNA because it can be collected in a painless and non-invasive manner. For logistical reasons, samples often need to be stored prior to the extraction of DNA. Common storage methods, such as refrigeration and freezing, can add significant costs and inconvenience to a large genetic study.

Ideally, a kit that would allow saliva samples to be stored at room temperature for long periods of time with no significant degradation of the DNA would be highly desirable. The Oragene kit is specifically designed for collecting and preserving DNA in saliva. This technical bulletin provides evidence that the Oragene kit can preserve the integrity of DNA in saliva at room temperature for many years, as well as maintain stability at temperatures up to 50°C for 187 days.

Materials and methods

Oragene/saliva samples were collected and stored at either room temperature (24°C), 37°C or 50°C for periods of time up to 187 days. In addition, saliva samples from 7 donors collected in Oragene solution and stored at room temperature for 5 years were analyzed. Aliquots of the various samples were removed and processed using the prepIT™•L2P (DNA Genotek) purification protocol[1]. Approximately 200 ng of DNA from each sample was analyzed by agarose gel electrophoresis and ethidium bromide staining. The size of the extracted DNA was determined by comparison with a Lambda-Hind III digest ladder.

DNA yield was determined by the highly specific Fluorescence/DNase method[2]. The F/D method quantifies DNA using SYBR Green I™ dye (Molecular Probes, Inc.), with or without DNase treatment.

Results

Samples stored at 24°C, 37°C or 50°C for up to 187 days

DNA from Oragene/saliva samples stored at 24°C and 37°C had a molecular weight > 23,000 bp and showed no evidence of degradation at the indicated time points (Figure 1). Samples stored at 50°C showed only slight degradation at 187 days. There was no change in the yield of DNA in any of the samples, regardless of storage temperature, as determined by the F/D method.

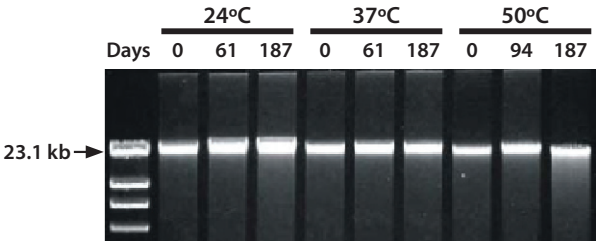

***Figure 1**: Agarose gel electrophoresis of DNA extracted from Oragene/saliva samples. A Lambda-Hind III digest was used as the marker in Lane 1.*

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

Samples stored at 24°C for 5 years

Saliva samples were collected in Oragene kits and stored undisturbed at room temperature for 5 years. DNA was extracted and analyzed by agarose gel electrophoresis. The results shown in Figure 2 indicate that high molecular weight DNA can be extracted from 5 year old samples that had been stored at 24°C.

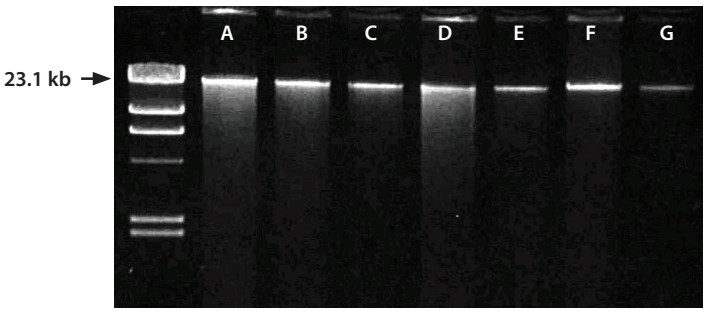

***Figure 2**: Agarose gel electrophoresis of DNA extracted from Oragene/saliva samples stored at room temperature for 5 years.*

Discussion and conclusions

Accelerated-aging studies of Oragene/saliva samples stored at 50°C for 187 days indicated that DNA would remain stable for at least 30 months at room temperature (Figure 1). This was calculated using the Arrhenius equation that is based upon the assumption that the rate of a chemical reaction typically decreases by half for every 10°C decrease in temperature. Therefore the rate of chemical degradation of DNA at 24°C was expected to be 5-fold slower than the rate of degradation at 50°C.

Oragene/saliva samples stored at room temperature for 5 years were analyzed. In agreement with the prediction from the accelerated-aging experiments, we have found that the DNA was stable at room temperature; the majority of the DNA remained > 23 kb in size after 5 years (Figure 2).

In summary, Oragene/saliva samples in tightly closed containers are expected to maintain the integrity of DNA at room temperature for at least 5 years.

Long-term storage recommendations

The evidence presented here indicates that Oragene/saliva samples may be stored in tightly capped containers at room temperature for many years with the expectation that high molecular weight DNA will still remain present. For longer-term storage, it is recommended that unpurified Oragene/saliva samples be distributed in aliquots and that these aliquots be stored at -20°C or lower temperatures[3].

References

1 Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.
2 DNA quantification using the Fluorescence/DNase (F/D) assay. Replaced by DNA quantification using SYBR Green I dye and a micro-plate reader. DNA Genotek. PD-PR-075.
3 Long-term storage of Oragene®/saliva samples. DNA Genotek. PD-PR-012.

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