

Long-term Storage of Oragene®•DNA Samples

Unpurified Oragene•DNA/saliva samples	Purified DNA from Oragene•DNA samples
1. Unpurified samples may be stored at room temperature for up to 5 years.	1. Store purified DNA in 1× TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0 or greater).
2. Freezing samples at -20°C is best for indefinite long-term storage because it minimizes evaporation of the liquid sample.	2. Store DNA frozen at -20°C.
3. To reduce storage space, samples may be split into aliquots and stored in microcentrifuge tubes. To ensure sample homogeneity, heat the entire sample for 2 hours at 50°C in an air incubator before aliquoting (ref. 1).	3. Minimize the number of freeze-thaw cycles by splitting the DNA into multiple aliquots.

Table 1. Oragene•DNA long-term storage recommendations

Introduction

The preservation and storage of DNA samples is an important consideration for molecular epidemiology and population studies. Table 1 summarizes recommendations for the long-term storage of purified and unpurified saliva samples collected with the Oragene•DNA Self-Collection Kit. This bulletin also discusses the rationale for these recommendations.

Storage of unpurified Oragene•DNA/saliva samples

Storage at room temperature

DNA from saliva is stable in the Oragene•DNA collection kit for up to 5 years (ref. 2). This stability is achieved with proprietary reagents that inactivate bacteria and nucleases in saliva and minimize chemical hydrolysis of DNA.

Frozen storage

Oragene•DNA/saliva samples may also be stored at -20°C or -80°C. Samples may undergo at least three freeze-thaw cycles with no evidence of DNA degradation. Although the Oragene•DNA collection tube is designed to ensure a tight seal, frozen storage may further reduce evaporation of the liquid medium during long-term storage.

Aliquots in microcentrifuge tubes

The Oragene•DNA collection tube is designed for user-friendly saliva collection from donors. However, in the lab, storage in more compact tubes may be preferable. To reduce storage space, the Oragene•DNA/saliva sample may be split into 4 aliquots of 1 mL each and stored in 1.5 mL screw-top microcentrifuge tubes. It is advisable to use tubes with O-rings to ensure a tight seal and minimize long-term evaporative loss.

References

1. Laboratory protocol for manual purification of DNA from 0.5 mL of Oragene•DNA/saliva. PD-PR-006.
2. Birnboim, H.C., Long-Term Stability of DNA from Saliva Samples Stored in Oragene•DNA. *DNA Genotek*. PD-WP-005.
3. O'Brien, D. (2002). High-throughput DNA purification. *Modern Drug Discovery*. 5(3), 25–26.
4. Kasper, Y. and Lenz, C. (2004). Stable 8-year storage of DNA purified with the QIAamp DNA Blood Mini Kit. *QIAGEN News*. 2004 e10.
5. Biobanks for health: Optimising the use of European biobanks and health registries for research relevant to public health and combating disease. *Report and recommendations from an EU workshop held at Voksenåsen Hotel, Oslo. January 28-31, 2003*.
6. Sample handling and storage: Subgroup protocol and recommendations. Version 1.0. *UK Biobank*. July 7, 2004.

Storage of purified DNA from Oragene•DNA samples

Preventing DNA degradation

There are three major causes of DNA degradation in a purified sample (ref. 3). Samples may be accidentally contaminated by bacteria, but storage at -20°C or lower will minimize bacterial metabolism and the release of nucleases. DNases may be inadvertently introduced from the skin, but this can be minimized by wearing gloves when handling samples. Repeated cycles of freezing and thawing may also contribute to DNA degradation. This may be minimized by splitting the purified DNA into multiple aliquots and thawing one at a time.

Comparison of TE and water

Kasper and Lenz (2004) performed an 8-year study of DNA stored in water or Buffer AE (10 mM TrisHCl; 0.5 mM EDTA, pH 9.0). DNA stored in Buffer AE at -20°C or 2-8°C showed no degradation by gel electrophoresis and amplified well in a PCR assay. DNA in water remained intact when stored at -20°C but samples were degraded when stored at 2-8°C and performed poorly in a PCR assay. Pure water lacks buffering capacity and an acidic pH may lead to DNA hydrolysis.

Biobank recommendations

An EU workshop on Biobanks (ref. 5) recommends freezing DNA samples to prevent bacterial contamination and to minimize evaporation of the sample. Tris-EDTA (TE) buffer contains sufficient buffering capacity to prevent acid hydrolysis of DNA. Similarly, the UK Biobank (ref. 6) recommends the storage of DNA in a nuclease-inhibiting environment at a temperature of -20°C, since no significant increase in stability is observed at temperatures below -20°C.