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THE BIOSTABILITY COMPANY

Long-term storage of oligonucleotides in DNAstable®

Introduction

Nucleic acid samples are currently being processed, distributed and stored for research projects in continually rising numbers worldwide. Currently, samples are stored at -80℃, -20° C or 4℃ using conventional cold-storage methods and shi pped using cold-packs or dry ice. Biomatrica®, Inc. has utilized the basic natural principles of anhydrobiosis and developed DNAstable®, an innovative room temperature storage medium as an alternative to conventional cold-storage of biological samples. DNAstable allows the dry storage of nucleic acids for long time periods, eliminating the need for freezers and cold shipments. Samples stored dry in DNAstable can be transported over extended distances and time, even with fluctuating temperatures, without fear of sample loss due to degradation. **Long-term stability studies performed under accelerated aging conditions indicate equivalence of greater than 30 years of room temperature storage.** The data presented demonstrates that storage of oligonucleotides in DNAstable at increased temperatures results in fully intact DNA that is functional in downstream applications without the need for further purification.

Materials and Methods

Storage, rehydration and analysis of oligonucleotides in DNAstable: Aliquots of 10 µM single-stranded forward (5'TTCGGTGATGACGGTGAAAA3') or reverse (5'TCCGCTTACAGACAAGCTGTGA3') oligonucleotides used for the amplification of pUC19 plasmid were applied to DNAstable or into empty wells (unprotected control) and allowed to dry overnight in a laminar flow hood before sealing and storage. Long-term stability of DNA was assessed at room temperature and under accelerated aging conditions at 60°C. Samples were analyzed after 26 months. Wells containing oligonucleotides stored dry in DNAstable or unprotected were re-hydrated with 10 µl water for 15 min at room temperature before analysis. The entire re-hydrated sample was used in each PCR reaction. Identical samples stored at –20°C served as positive controls.

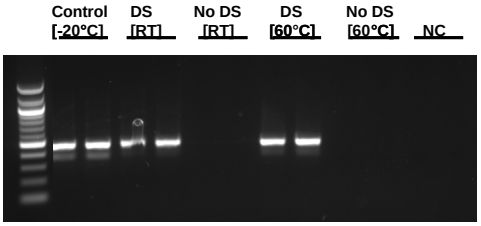

Figure 1: Control pUC19 primers stored at –20°C were used in PCR amplification analysis as positive controls. Duplicate wells of 10 µM oligonucleotide samples stored dry in DNAstable (DS) or unprotected (no DS) for 26 months at ambient room temperature (RT) or at 60˚C under accelerated aging conditions (equivalent to 30 years of room temperature storage), were re-hydrated and used in PCR analysis using pUC19 plasmid DNA as templates. NC: negative oligonucleotide control.

Results and Discussion

The protective properties of DNAstable significantly inhibit degradation of single-stranded DNA primers (20mers) when exposed to increased temperatures. Aliquots of pUC19 specific primers were stored dry in DNAstable 96-well plates and stored for 26 months at either room temperature or 60°C. Storage for 26 months under accelerated aging conditions of 60°C is equivalent to 30 years of room temperature storage. Duplicate wells were re-hydrated and used for PCR analysis using identical oligonucleotide samples stored at conventional –20°C. Samples protected in DNAstable were fully functional in PCR analysis, while the unprotected oligonucleotides were degraded and did not amplify pUC19 template plasmid DNA (Figure 1; DS compared to no DS).

DNAstable technology is a novel innovation for the safe and long-term storage of single-stranded oligonucleotide samples. Primers of varying lengths can be stored dry at ambient temperatures using DNAstable, significantly reducing the need for costly and precious freezer space. Ambient temperature storage also allows for easier cataloguing and retrieval of samples instantly. Additionally, primer samples can be transported between laboratories with great ease by simply using envelope shipment, thus reducing shipping costs.