

Long-term storage of DNA in SampleMatrix®

Introduction

Millions of nucleic acid samples are currently being processed, distributed and stored worldwide. Currently, samples are stored under refrigerated conditions at either -80°C, -20°C or 4°C. Annually millions of nucleic acid samples are transported between laboratories on ice. Biomātrica, Inc. has utilized the basic principles of anhydrobiosis found in nature and developed SampleMatrix®, a proprietary storage medium. The SampleMatrix® allows storage of nucleic acids in dried form for long periods, eliminating the need for freezers, cold-rooms and shipping on ice. Samples stored dry in SampleMatrix® can be transported over long distances with fluctuating temperatures without undergoing degradation or loss of viability. **Long-term stability studies have been performed under accelerated aging conditions that are equivalent to 11 years of room temperature storage.** The data presented demonstrates that storage of DNA in SampleMatrix® at ambient lab temperatures results in fully intact DNA and is functional for downstream applications without purification.

Materials and Methods

Spotting, storage and rehydration of plasmid or genomic DNA in SampleMatrix®: One µg or 50ng of plasmid DNA (pDNA: puc18) or 20ng of human genomic DNA (gDNA) were spotted into SampleMatrix® or empty wells and allowed to dry overnight in a laminar flow hood. After drying each well was sealed. Long-term stability of pDNA and gDNA was assessed at ambient temperature on the laboratory bench and accelerated aging studied at increased temperatures at 50°C or 60°C. The plasmid samples were analyzed after 1.5 years after room temperature storage using transformation assays. Samples stored under accelerated aging conditions (plasmid 50°C; genomic 60°C) after 10 months. The wells containing DNA in dried SampleMatrix® were hydrated with 10 µl water for 15 min on the bench top before analysis. Identical DNA samples stored at 4°C or -20°C were used as controls. **PCR analysis:** The 50ng puc18 samples were used for PCR analysis using puc18 primers. The 20ng gDNA was amplified using human beta actin primers. qPCR was performed using 1/20th of the starting material with a theoretical total concentration of 1ng per reaction in triplicates using beta actin primers. **Transformation:** Rehydrated pDNA stored in SampleMatrix® and control cold stored DNA were transformed into 100 µl competent DH5α *E. coli*. Transformed cells were plated on LB plates containing ampicillin and grown at 37°C overnight. Colonies were counted the next day.

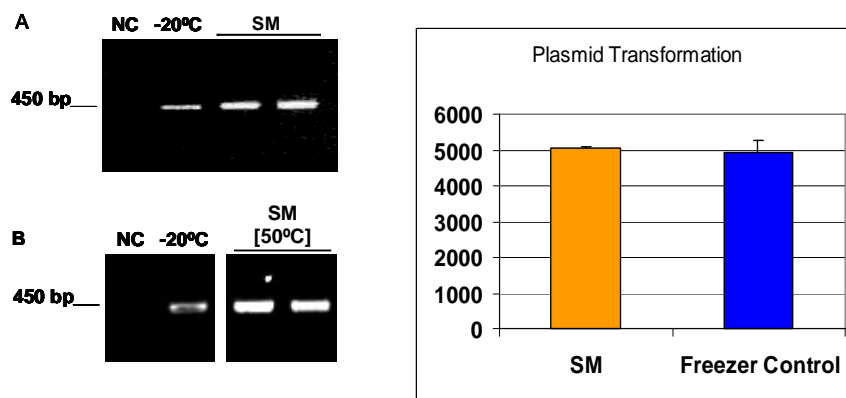


Figure 1: 50ng of pDNA, stored in SampleMatrix® for (A) 2 years at ambient temperature or (B) 10 months at 50°C under accelerated aging conditions (equivalent to 5 years of room temperature storage), were analyzed by PCR amplification using puc 18 primers. 50ng of control DNA was stored in a -20°C freezer, NC: negative control.

Figure 2: Colony counts of bacteria transformed with pDNA stored for 1.5 years at room temperature in SampleMatrix® (SM) compared to Freezer stored control pDNA.

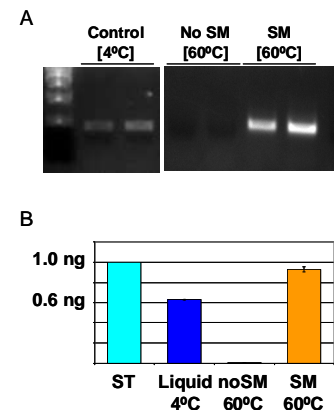


Figure 3: 20ng of Human genomic DNA were stored in SampleMatrix® (SM) or left unprotected (No SM) and exposed to 60°C for 10 months under accelerated aging conditions (equivalent to 11 years of room temperature storage). 20ng of identical control DNA was stored at 4°C for 10 months. (A) PCR; (B) quantitative PCR: 1ng fraction of stored material; fresh DNA standard (ST = 1ng)

Results and Discussion

The protective properties of SampleMatrix® inhibit degradation of DNA and allow the retrieval of viable plasmid or genomic DNA even after prolonged storage under dry and fluctuating room temperature conditions. Plasmid DNA stored in SampleMatrix® for 2 years at ambient temperature was fully viable and could easily be amplified resulting in a strong amplification product (Figure 1A). Plasmid DNA stored in SampleMatrix® and exposed to increased temperatures also maintained viability and produced heightened amplification (Figure 1B). Longterm storage (1.5 years) of pDNA was used for transformations and resulted in colony counts that are comparable to cold stored pDNA controls (Figure 2). Human gDNA was protected by SampleMatrix® under accelerated aging conditions for 10 months at 60°C that is equivalent to 11 years of DNA storage at room temperature.

The novel, proprietary SampleMatrix® allows safe storage and transport of DNA in dry form at ambient temperatures. This groundbreaking new storage medium will allow significant reductions in energy costs and lab space, liberating resources for scientists to conduct seminal research important to biological discovery and diagnostics.