

Bacterial samples harboring plasmid DNA (non-purified DNA) storage in SampleMatrix™

Introduction

Millions of nucleic acid samples are constantly being processed, distributed and stored for research projects worldwide. Currently, samples are stored under refrigerated conditions at -80°C, -20°C or 4°C. Annually millions of nucleic acid samples are transported between laboratories on ice or in bacterial vectors as frozen glycerol stocks. Biomātrica, Inc. has utilized synthetic polymer chemistry and the basic principles of anhydrobiosis (molecular stabilization mechanisms used by extremophiles that survive extreme drought conditions) found in nature and developed SampleMatrix™, a proprietary storage medium. The SampleMatrix™ allows the long term storage of air dried nucleic acids, eliminating the need for freezers and cold-rooms. Samples stored dry in SampleMatrix™ can be transported over long distances with fluctuating temperatures without undergoing degradation or loss of viability. The data presented demonstrates that room temperature storage of dried *Escherichia coli* (*E. coli*) harboring plasmid DNA (pDNA) in SampleMatrix™ results in fully intact pDNA that is functional for downstream applications such as transformation and PCR without prior purification of the pDNA.

Materials and Methods

Project 1: Spotting and storage of *E. coli* on SampleMatrix™: Glycerol stocks stored at -80°C of DH5α harboring pUC18 (2.7kb) and Stb12 harboring a Feline Immunodeficiency Virus (pFIV) clone in pUC119 (13kb; kind gift of Dr. J. Elder, The Scripps Research Institute, La Jolla, CA) were scraped into tubes containing LB with ampicillin and grown overnight. The cultures were used to prepare mixtures of 1 μl, 2 μl, or 5 μl of *E. coli* with 10 μl liquid SampleMatrix™. The mix was spotted into a 96 well SampleGard™ plate and allowed to dry overnight in a laminar flow hood. The plates were then sealed and placed at room temperature. **PCR analysis:** After 3 months, wells were hydrated with 25 μl water for 15 min and complemented with 2.5 U Taq polymerase (NEB), 3 μl 10x thermopol reaction buffer (NEB), 0.5 μl dNTPs (10 μM each), and pUC18 primers (DH5α) or pFIV primers (Stb12) in a final volume of 30 μl for PCR analysis. Cycling parameters were initial denaturation at 95°C for 5 min followed by 30 cycles of 95°C for 15 sec, 55°C for 30 sec and 72°C for 30 sec. 10 μl of PCR reactions were run on a 0.8% agarose/ethidium bromide gel.

Project 2: Spotting and storage of *E. coli* on SampleMatrix™: Glycerol stocks stored at -80°C of DH5α harboring pUC18 (2.7kb) were scraped into tubes containing LB with ampicillin and grown overnight. 20 μl of the overnight growth were spotted on dried SampleMatrix™ in SampleGard™ plates and allowed to dry in a laminar flow hood. Plates were then sealed and stored at 50°C for 2.5 months (accelerated aging condition).

Transformation: SampleMatrix™ stored dried *E. coli* containing pUC18 were hydrated in 10 μl water for 15 min on the bench top. The samples were added to 100 μl of competent DH5α *E. coli* and placed on ice for 20 min. The bacteria were heat-shocked at 42°C for 30 sec and placed on ice for 2 min. 900 μl LB broth were added to each tube and the samples were placed on a shaker at 37°C for 40 min. 100 μl of transformed cells were plated on LB plates containing 10mg/ml ampicillin and incubated at 37°C overnight. Colonies were counted on three separate plates per condition. **Miniprep analysis:** Three colonies were picked from each plate and 3 ml LB/amp were inoculated for overnight growth. Plasmid DNA was extracted by conventional alkaline lysis. The extracted pUC18 was then restricted for 30 min in EcoRI and analyzed by gel electrophoresis.

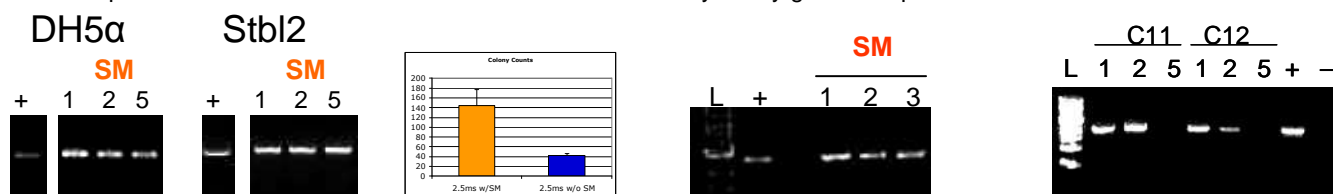


Figure 1: Different amounts (1 μl, 2 μl, 5 μl) of anhydrous, SampleMatrix™ (SM) stored *E. coli* (DH5α containing pUC18 or Stb12 containing pFIV) were hydrated and analyzed by PCR amplification using pUC18 (DH5α) or pFIV (Stb12) primers. Positive controls were amplified simultaneously (+) for either set.

Figure 2: DH5α containing pUC18 (10 μl) dry stored in SampleMatrix™ (SM) for 2.5 months were hydrated with 10 μl water for 15 min and transformed into 100 μl competent cells. Colonies were counted after 18 h incubation.

Figure 3: Three colonies (1,2,3) were picked from the transformation (Figure 2) using SampleMatrix™ (SM) pUC18 for overnight growth. Plasmid DNA was extracted and restricted using EcoRI and compared to freezer stored pUC18 (+). L: ladder.

Figure 4: Two wells (C11, C12) containing *E. coli* stored for **2 years** were hydrated and 1, 2 or 5 μl used for PCR analysis. +: positive control. -: no template control. L: ladder. Note: The larger volume (5 μl) of *E. coli* contains higher amounts of bacterial debris which can be inhibitory to PCR.

Results and Discussion

The protective properties of the SampleMatrix™ preserve pDNA within *E. coli* vectors and under increased heat stress. *E. coli* cultures harboring pDNA were grown and premixed with or spotted on SampleMatrix™ for dry storage either at room temperature or at 50°C. At various time points the dried samples were used for PCR analysis, transformation and pDNA analysis by alkaline extraction. The SampleMatrix™ consistently showed preservation of the pDNA and allowed for easy PCR analysis or extraction of pDNA from the dried *E. coli* for downstream applications.