

DNA SampleMatrix[®] protects plasmid DNA stressed at high temperature

Introduction

Enormous advances in scientific research and innovation are resulting in an ever-increasing need to store and ship biological samples, such as DNA, RNA and proteins. Most molecular laboratories are dependent on cold rooms and freezers to protect their samples from degradation for storage. Freezer based storage is inconvenient, expensive, uses up valuable lab space and result in high-energy costs. DNA SampleMatrix[®], developed by Biomātrica using the natural principles of anhydrobiosis (molecular principles of organisms that can survive extreme drought conditions for hundreds of years) and synthetic chemistry, alleviates the need of cold rooms and freezers, allowing storage of molecules dry at ambient lab temperatures. The data presented show that plasmid DNA stabilized in DNA SampleMatrix[®] is protected from degradation, even at increased temperatures, as unprotected DNA is completely degraded at these high temperatures. These results demonstrate DNA stability under accelerated aging conditions.

Materials and Methods

Stress test: Plasmid pUC18 DNA (1 µg) in ddH₂O was aliquoted into microfuge tubes containing dried DNA SampleMatrix or no SampleMatrix (unprotected DNA control). Application of the plasmid DNA in liquid form (TE or water) rehydrates the DNA SampleMatrix, establishing a mixture of plasmid and the protective matrix. Samples were allowed to air dry in a laminar flow hood. Tubes containing either DNA protected in SampleMatrix[®] or the unprotected plasmid were then closed and placed in a heating block maintained at 70°C for storage. Samples were removed from heat after 24 or 72 h. The DNA was rehydrated in 10 µl water for 15 min on the bench top before analysis in a 0.8% agarose gel containing ethidium bromide.

Transformation: Heat stressed samples stored with or without DNA SampleMatrix were rehydrated in 10 µl water for 15 min at room temperature. The entire sample was then added to 50 µl 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. LB media (450 µl) was added to each tube and the samples were placed on a shaker at 37°C for 40 min. Transformed cells (50 µl) were plated on LB plates containing 10 mg/ml ampicillin and grown at 37°C overnight.

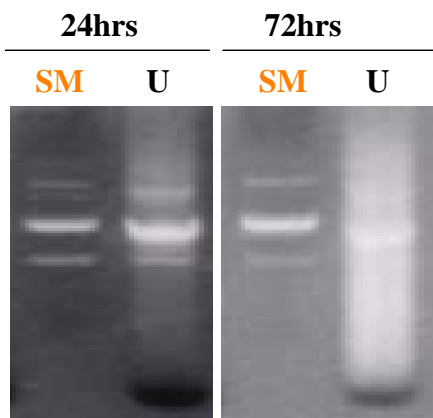


Figure 1: puc18 plasmid DNA (1 µg) stored dry in DNA SampleMatrix (SM) or unprotected (U) were heated at 70°C for 24 or 72 h. Samples were rehydrated in 10 µl of ddH₂O and run on a 0.8% agarose gel containing ethidium bromide.

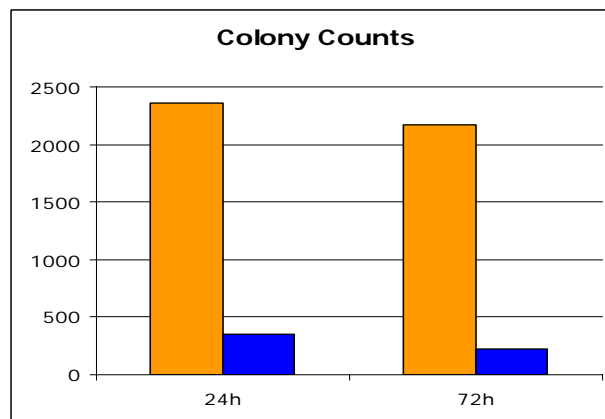


Figure 2: Heat stressed plasmid DNA samples stored in DNA SampleMatrix (orange) or unprotected (blue) were heated for 24 or 72h before rehydration and used in bacterial transformations. Colonies were counted after overnight incubation.

Results and Discussion

The protective stabilization properties of DNA SampleMatrix inhibit degradation of DNA even under extreme conditions, such as elevated temperatures. We heated plasmid DNA samples maintained dry in DNA SampleMatrix[®] or without the protection (unprotected control) for 24 or 72 h at 70°C. The samples were rehydrated and analyzed by gel electrophoresis. DNA SampleMatrix[®] clearly protects plasmid DNA even at high temperatures over time (Figure 1, SM at 24 or 72h), compared to the unprotected control that was completely degraded. Without the protective effect of DNA SampleMatrix[®], plasmid DNA begins to denature by 24 h and is fully degraded after 72 h at 70°C (Figure 1, U at 24 or 72 h). We then tested the transformation ability of plasmid DNA maintained at high temperatures when protected in DNA SampleMatrix[®]. DNA SampleMatrix maintains the integrity of the plasmid and results in elevated colony counts after transformation (Figure 2, orange bars), while unprotected samples show severe degradation leading to low colony counts (Figure 2, blue bars).

The unique stabilizing properties of DNA SampleMatrix allow storage and transport of plasmid DNA at ambient temperatures. Samples stored dry in DNA SampleMatrix are protected from degradation even at extreme temperatures such as 70°C. Use of DNA SampleMatrix for sample storage can protect precious samples from degradation and provides an attractive alternative to cold sample storage and shipment.