

DNAstable[®] protects plasmid DNA stressed at high temperature

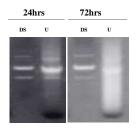
Introduction

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. DNAstable[®], developed by Biomatrica[®] 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 DNAstable 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

<u>Stress test</u>: Plasmid pUC18 DNA (1 μ g) in ddH₂O was aliquoted into microfuge tubes containing dried DNAstable or <u>no</u> SampleMatrix (unprotected DNA control). Application of the plasmid DNA in liquid form (TE or water) rehydrates the DNAstable, 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 DNAstable[®] 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 μ l water for 15 min on the bench top before analysis in a 0.8% agarose gel containing ethidium bromide.

<u>*Transformation:*</u> Heat stressed samples stored with or without DNAstable were rehydrated in 10 μ l water for 15 min at room temperature. The entire sample was then added to 50 μ l competent DH5 α 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 μ l) was added to each tube and the samples were placed on a shaker at 37°C for 40 min. Transformed cells (50 μ l) were plated on LB plates containing 10 mg/ml ampicillin and grown at 37°C overnight.



Colony Counts

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

Figure 2: Heat stressed plasmid DNA samples stored in DNAstable (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 DNAstable inhibit degradation of DNA even under extreme conditions, such as elevated temperatures. We heated plasmid DNA samples maintained dry in DNAstable or without the protection (unprotected control) for 24 or 72 h at 70°C. The samples were rehydrated and analyzed by gel electrophoresis. DNAstable clearly protects plasmid DNA even at high temperatures over time (Figure 1, DS at 24 or 72h), compared to the unprotected control that was completely degraded. Without the protective effect of DNAstable[®], 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 DNAstable[®]. DNAstable 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 DNAstable allow storage and transport of plasmid DNA at ambient temperatures. Samples stored dry in DNAstable are protected from degradation even at extreme temperatures such as 70°C. Use of DNAstable for sample storage can protect precious samples from degradation and provides an attractive alternative to cold sample storage and shipment.