

DNAstable[®] protects genomic DNA from degradation under high pressure and extreme temperatures above 120°C

Introduction

DNAstable[®] is a novel, proprietary medium which allows the storage of complex biological molecules at ambient temperatures without degradation, thus eliminating the need for costly freezer units and shipment under cold conditions. The most common storage technique for biologically sensitive materials, such as nucleic acids, is cold storage at various temperatures from minus 80°C to 4°C. This technique is a time tested yet inefficient storage method. Large refrigeration units use up enormous and valuable lab space, as well as incur substantial energy costs. Machines are prone to failure, power fluctuations can cause inconsistent temperatures, and replacement of high-pressure refrigerant gas is very costly. As an alternative to conventional cold storage technologies, we have developed DNAstable, a stabilization technology that is based on the molecular principles of anhydrobiosis (organisms that can survive extreme drought conditions for hundreds of years in a dried state) and synthetic chemistry that allows the long-term storage of biomolecules at room temperatures. The data presented shows that storage of DNA under extreme conditions (e.g. autoclaving) in DNAstable maintains DNA integrity.

Materials and Methods

Stress test and PCR analysis: Various amounts of human genomic DNA (500 ng, 200 ng, 100 ng, 50 ng and 4 ng) were applied to DNAstable or into empty microfuge tubes (unprotected sample) and allowed to dry in a laminar flow hood. Samples were then placed in an Erlenmeyer flask covered with foil and subjected to a dry cycle autoclave run. Autoclaving occurred at 250°F/121°C for 15 min at 15 lb/in² and dried for 30 min at 150°F/66°C. Samples were re moved from the autoclave and allowed to cool. The DNA was re-hydrated in 10 µl water for 15 min at room temperature. Samples were used to amplify the human β-actin (hβactin) gene using 2.5 U Taq DNA polymerase (NEB), 3 µl 10x Thermopol reaction buffer (NEB), 0.5 µl dNTPs (10 µM each nucleotide), hβactin forward (5' CTACCTCATGAAGATCCTCACC 3') and hβactin reverse (5' GTACTTGGCGCTCAGGAGGAGC3') in a final volume of 30 µl. Cycling parameters were initial denaturation at 94°C for 5 min, followed by 40 cycles of 94°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 gel containing ethidium bromide.



Figure 1: 500 ng gDNA were amplified using h βactin primers after storage of unprotected DNA in the freezer (U) or DNAstable (DS) protected DNA stored at room temperature.

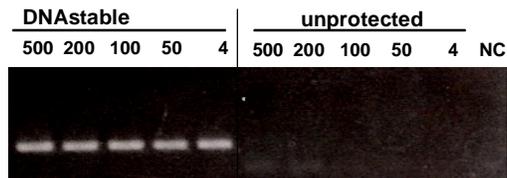


Figure 2: Various amounts (500 ng; 200 ng; 100 ng; 50 ng; 4 ng) of gDNA were dried with DNAstable or without (unprotected) and subjected to autoclaving. Hydrated DNA was PCR analyzed using hβactin primers. NC: no template control.

Results and Discussion

DNAstable was assayed first under PCR conditions resulting in no noticeable difference in amplification of gDNA stored in DNAstable as compared to freezer stored material (Figure 1). Various amounts of gDNA were then subjected to autoclaving conditions. The gDNA was either protected in DNAstable or left unprotected and autoclaved in a dry cycle. The absence of DNAstable resulted in complete degradation of gDNA and no visible product was amplified, whereas DNAstable fully protected the gDNA during the autoclave cycle; even minute amounts of gDNA (4 ng) remained viable and could be amplified. DNAstable protects DNA even at extreme conditions such as high pressure and extreme heat for prolonged periods without reducing its performance in amplification reactions.

DNAstable storage product is a novel technology for the long-term storage and cataloging of precious DNA samples. The exceptional protective property of DNAstable allows the long-term storage and transport of genomic DNA at room temperatures without fear of sample degradation. Samples can be stored with minimal effort at ambient temperature, thus greatly reducing the need for costly freezer storage and high energy consumption. DNAstable can be extremely useful for the transport and shipment of DNA samples. The fluctuating and inconsistent temperatures during shipment will not affect the DNA protected dry in DNAstable. Individual tubes or plates can be simply slipped into mailing envelopes and sent, thus eliminating the need for bulky and costly shipments of Styrofoam[®] packaging and dry ice.