Application Note

GenTegra

Room Temperature Storage of Purified Plasmids in GenTegra®-DNA Tubes

H. McMahon[1], D. Wong[1], K. McWeeny[1] and R. Nuñez[1]
[1]GenTegra LLC, Pleasanton, CA

Abstract

GenTegra-DNA tubes are a novel technology that allow storage of purified plasmid DNA at room temperature in a manner that preserves DNA integrity, quality and purity. Recovering plasmid DNA from GenTegra-DNA tubes is a simple process that does not require further purification prior to transformation or sequencing.

Introduction

GenTegra LLC has developed GenTegra-DNA, a novel technology which provides a dry state, room temperature environment for DNA storage. GenTegra-DNA tubes contain an inert chemical matrix that allows storage of DNA in a "bone dry", water-free environment, which protects DNA samples from hydrolysis, oxidation and microbial growth. The GenTegra-DNA matrix ensures integrity, stability,
and quantitative recovery of DNA samples. The purpose of this study was to evaluate the integrity, quality and purity of plasmid DNA stored in GenTegra-DNA tubes and its use in downstream applications including transformation and DNA sequencing.

Materials and Methods

Plasmids

pCMV-sport plasmid (Invitrogen) containing a 1.3kb EST insert was used for all experiments.

Application, Storage and Recovery of DNA

Plasmid DNA (1µg aliquots) was applied to cluster tubes with or without GenTegra-DNA matrix and dried overnight according to the manufacturer's instructions. After drying, the GenTegra tubes were stored at room temperature (25°C), 37°C, 56°C, and 76°C for 18 days. Eighteen days of storage at accelerated temperature corresponds to 36 days, 144 days, and 1.6 years of room temperature storage time for the 37°C, 56°C, and 76°C temperatures, respectively[1]. An aliquot of plasmid DNA was also stored at -20°C as a control. DNA was recovered from cluster tubes according to the manufacturer's instructions and stored at 4°C thereafter.

Gel Analysis

The molecular weight of the recovered DNA and frozen control was examined by running 200ng of unamplified DNA directly on a 1.2% agarose gel stained with ethidium bromide.

Transformation

100pg of DNA recovered from cluster tubes stored at 76°C with or without GenTegra-DNA matrix, and control DNA stored at -20°C was used for transformation of One Shot® Mach1™ T1 Phage-Resistant Chemically Competent E.coli (Invitrogen) according to the manufacturer's instructions. A single colony was cultured from each transformation and purified using the Plasmid Mini Kit (Qiagen) prior to sequencing.

Sequencing

DNA sequencing of 600ng of DNA was performed by Genewiz using SP6 primers (5'-d(GAT TTA GGT GAC ACT ATA G)-3'). CLC DNA Workbench 5 was used for sequence alignment.

Results

The molecular weight of the recovered DNA was examined by gel electrophoresis (Figure 1). Samples stored in the presence of GenTegra-DNA matrix at room temperature, 37°C, 56°C, and 76°C for 18 days were indistinguishable from control samples stored at -20°. Conversely, samples stored at 56°C and 76°C without GenTegra-DNA matrix exhibited degradation.

All samples, regardless of storage condition, were transformed successfully, with similar transformation efficiencies (Figure 2). A single colony from each transformation was selected for amplification and sequencing.

No differences in the DNA sequence was detected in samples stored at 76°C with or without GenTegra-DNA matrix and controls stored at -20°C. A partial sequence alignment is shown in Figure 3.

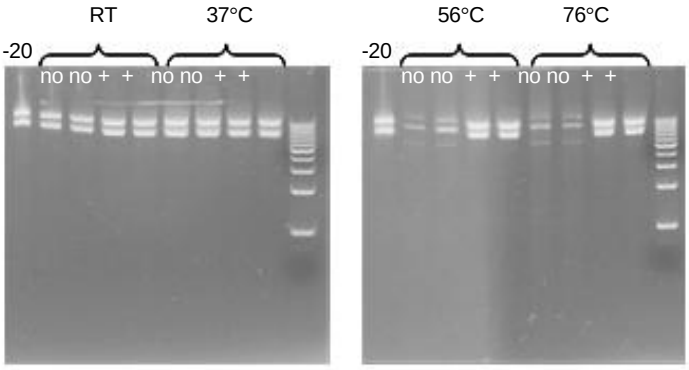

Figure 1. Gel analysis. 200ng of each sample was examined on a 1.2% agarose gel stained with ethidium bromide. -20 - samples stored at -20°C; RT - room temperature; no - samples stored without GenTegra-DNA matrix; + -samples stored with GenTegra-DNA matrix.

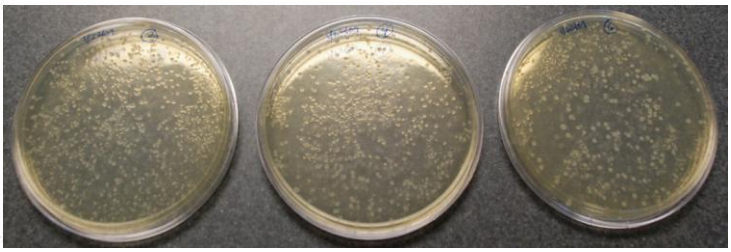

Figure 2. 100pg of each sample was used for transformation of Mach1 cells. LB-ampicillin plates are pictured following an overnight incubation to visualize transformation efficiency.

Figure 3. 600ng of each sample was used for DNA sequencing with SP6 primers. No differences were detected in DNA sequences of samples stored at 76°C with or without GenTegra-DNA and frozen controls. A partial sequence comparison is pictured.

Conclusions

GenTegra-DNA tubes contain an inert chemical matrix which does not hinder downstream applications. In this study, purified plasmid DNA samples were stored in the dry state in GenTegra-DNA tubes for 18 days at room temperature, 37°C, 56°C and 76°C. Following recovery, the purity, integrity and stability of the DNA samples was assessed. Agarose gel analysis revealed no evidence of degradation at any storage temperature in samples stored with GenTegra-DNA matrix. On the other hand, samples stored
at 56°C and 76°C in the absence of GenTegra-DNA matrix showed evidence of degradation. No differences in DNA quality or integrity were observed between DNA stored frozen and DNA stored with GenTegra-DNA matrix at 76°C, an accelerated time point equivalent to 1.6 years of room temperature storage[1]. Samples in all storage conditions were successfully transformed and sequenced, with no base changes observed in samples stored in the presence of GenTegra-DNA matrix. In conclusion, GenTegra-DNA Tubes provide a valuable tool for storing plasmid DNA at room temperature and recovering DNA with high purity and integrity for immediate use in downstream applications. GenTegra-DNA tubes ensure stability and protect the DNA from degradation during long-term storage.

References

[1]Bruskov, VI. Malakhova, LV. Masalimov, ZK. Chernikov, AV. (2002) *Heat-induced formation of reactive oxygen species and 8-oxoguanine, a biomarker of damage to DNA*. Nucleic Acids Research, **6**, 1354-1363.