

Plasmid DNA from *E. coli* culture preserved in Clonestable™

Introduction

Clonestable is designed for long-term dry storage of bacterial genomic and plasmid DNA samples directly from unpurified cultures. Based on its unique stabilization properties, bacterial cells harboring selective plasmids can be applied directly into Clonestable from live overnight cultures without purification. During the drying process, the cells are inactivated and their genomic and plasmid DNA content is preserved for dry storage at ambient temperatures. Bacterial genomic and plasmid DNA is recovered by a simple one-step rehydration protocol and ready for immediate use in downstream applications without further purification. The following data demonstrates that plasmid DNA (pDNA) recovered from storage in Clonestable is fully functional in transformation assays and PCR amplification.

Materials and Methods

Sample preparation and storage:

Experiment 1: *E. coli* (DH5 α) cells containing pUC19 plasmids (2.7 kb) were grown overnight in LB containing 100 μ g/ml ampicillin. Aliquots of the culture (5 μ l) were added directly into wells of a 96-well plate containing Clonestable (Biomatrica catalog #90121-007) and allowed to dry overnight on the bench top. Identical aliquots were also added to a competitor's treated cellulose paper storage device. Both the Clonestable plate and cellulose paper storage device were then stored at room temperature for 20 months.

Experiment 2: *E. coli* (DH10 β) containing pUC19 were grown overnight in selective medium. The culture was heat treated (80°C for 3 min), and applied directly into Clonestable and allowed to dry overnight on the bench top. A 15% glycerol stock was also prepared from the culture as a control for storage at -80°C. Samples in Clonestable were stored for six months at room temperature and at 50°C to simulate accelerated aging equivalent to 3.5 years of storage at room temperature.

Plasmid recovery:

Experiment 1:

Duplicate wells containing bacterial DNA stored in Clonestable were rehydrated with 10 μ l of water. Duplicate samples stored on cellulose paper were recovered according to the manufacturer's protocol: samples were cut out of the paper, washed twice with TE⁻¹ buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) and eluted from the paper into 10 μ l of TE⁻¹ buffer. Recovered bacterial DNA was used directly in transformation assays to assess plasmid quality. Samples (10 μ l) were mixed with 100 μ l competent cells, kept on ice for 20 min, heat shocked at 42°C for 45 sec, and placed back on ice for 3 min. LB media (890 μ l) was added to the cells and incubated at 37°C for 45 min, after which 40 μ l of the transformation was plated (in triplicate) onto LB agar containing 100 μ g/ml ampicillin. The plates were grown overnight at 37°C and colonies were counted the next day (Figure 1). Transformation efficiency was determined by transforming a known amount of plasmid (1 ng) into competent cells.

Experiment 2:

Quadruplicate aliquots were brought to room temperature and rehydrated with 20 μ l of water. The frozen control glycerol stock control was thawed and the cell pellet resuspended in water, followed by heat treatment at 80°C for 3 min. Samples (10 μ l) were then used directly for transformation as described above except 20 μ l of the transformation was plated instead. Results are shown in Figure 2.

Results

After 20 months of storage at room temperature, samples stored in Clonestable resulted in more than 100-fold higher colony count than compared to samples stored on cellulose paper. This dramatic difference in recovery indicates that plasmids are stabilized and easily recovered in Clonestable compared to cellulose paper.

Stored crude *E. coli* lysates protected by Clonestable resulted in colony counts comparable to control freezer glycerol stocks. Plasmids can be recovered from Clonestable without reduced yield, even when stored at 50°C for six months, equivalent to 3.5 years at room temperature.

Clonestable is designed to protect the genomic and plasmid DNA, *not* the live *E. coli* bacteria. Extensive analysis on the viability of *E. coli* applied into Clonestable indicates cells do not survive in the dried state for more than 4 weeks; rehydrated samples inoculated into LB media did not grow (data not shown). However, rehydrated samples containing bacterial DNA mixed with transformed cells generated antibiotic resistant colonies (colonies positive for pUC19). Mini-prep analysis of pDNA recovered from Clonestable transformed back into *E. coli* resulted in restriction enzyme fragments identical to the original plasmids (data not shown).

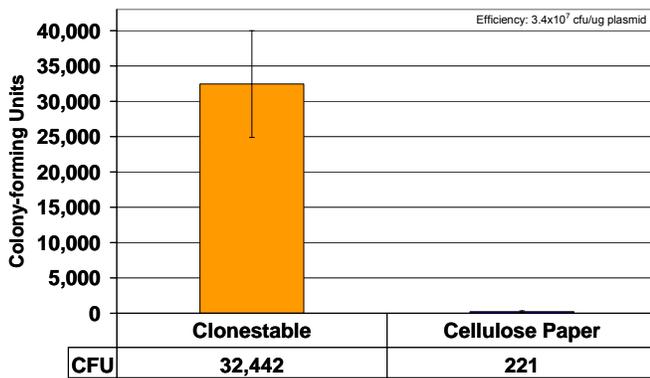


Figure 1: *E. coli* (DH5α) harboring pUC19 were stored for 20 months at room temperature in Clonestable or cellulose paper. Samples were rehydrated or used directly in transformations to recover the stored plasmids.

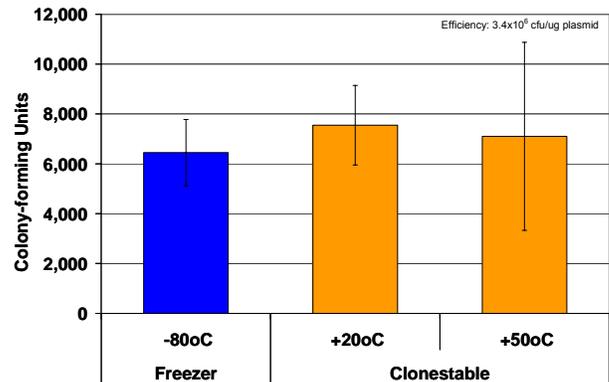


Figure 2: *E. coli* culture (DH10β containing pUC19 plasmid) was stored for 6 months at 50°C or room temperature in Clonestable or as a frozen glycerol stock at -80°C. Clonestable samples were rehydrated and used directly for transformation into competent cells. Glycerol stock samples were washed, lysed, and used for transformation.

Conclusion

The protective properties of Clonestable allow for the preservation of bacterial genomic and plasmid DNA harbored in *E. coli* cells. Overnight bacterial cultures grown in selective media can be applied directly into Clonestable without further purification and then dried for long-term storage of bacterial DNA. Recovered plasmid DNA using the one-step rehydration protocol is ready for immediate use in downstream applications including PCR amplification, transformation and large prep growth for sequencing, rolling circle amplification, and T7 transcript generation analyses. Clonestable makes bacterial DNA storage simple and easy, while protecting the sample against heat, UV, and other variable stresses encountered during storage and shipment.