AFA-energetics®-Mediated DNA Extraction from Yeast and Bacteria Using Scanning in a Labcyte 384-well Plate

Abstract

This application note describes the optimized AFA-energetics dose to lyse and extract DNA from *S. cerevisiae*, *E. coli*, & *L. monocytogenes* using a proprietary scanning method in modified Labcyte 384-well Echo-compatible plates. The maximum yield, as measured by Qubit, is obtained after 25 minutes. The electropherograms reveal that high molecular weight DNA can be extracted in a 384-well format.

Materials and Methods

Required Materials

Covaris

- LE220-plus Focused-ultrasonicator (PN 500569)
- Buffer BB2 from truXTRAC cfDNA Kit – Magnetic Bead (PN 520221)
- Buffer WB2 from truXTRAC cfDNA Kit – Magnetic Bead (PN 520221)
- 96 microTUBE Plate Thin Foil Seals (PN 520235)
- truPOP™ Cell Lysis Buffer (PN 520268)

Other

- SpeedBeads™ magnetic carboxylate modified particles Sigma (GE45152105050250)
- Labcyte 384PP 2.0 Echo Qualified Microplate (PP-0200)

Methods

Approximately 5E7 yeast cells or 4E8 bacterial cells were resuspended in truPOP Cell Lysis Buffer and lysed in the Labcyte 384-well plate on the LE220-plus Focused-ultrasonicator according to settings in ***Table 1***. Magnetic bead binding, wash, and elution was done using GE SpeedBeads. The total binding volume was around 90 µL. The bind solution included lysed cells in truPOP Cell Lysis Buffer, GE SpeedBeads, Covaris Buffer BB2, and 38% Isopropanol. The wash was done in three steps using Covaris WB2 and 80% Ethanol. Nucleic acids were eluted from the magnetic beads in 50 µL 1x Tris-EDTA Buffer and transferred to a clean tube for storage.

Instrument	LE220-plus Focused-ultrasonicator
Vessel	Labcyte 384-well Echo compatible plate
AFA Sample Volume	30 to 35 µL
Dithering	N/A
Temperature	6 °C
Peak Incident Power	450W
Duty Factor	50%
Cycles per Burst	200
Treatment Scans	90

Table 1. AFA Treatment settings.

Results and Discussion

DNA electropherograms obtained are presented in ***Figures 1 through 3***. The DNA yields obtained are presented in ***Figure 4***.

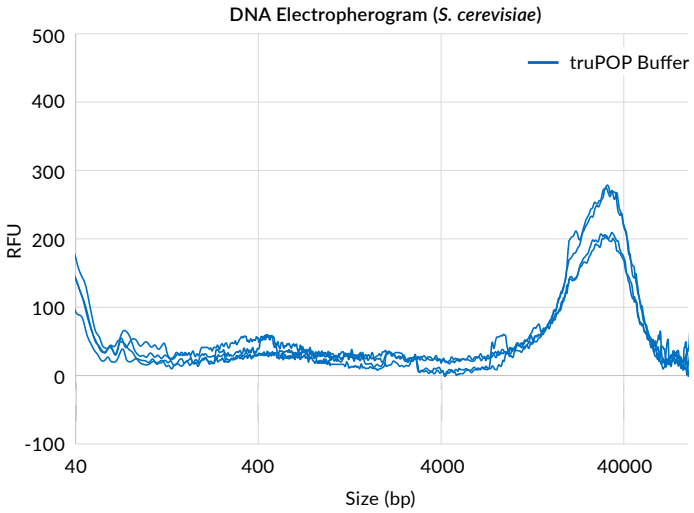

Figure 1. DNA electropherograms (Fragment Analyzer, AATI) Profile from lysed *S. cerevisiae*. Graph depicting the fragment size distribution of nucleic acid purified from lysed samples, from 40 bp to 50,000 bp, as determined by the AATI Fragment Analyzer.

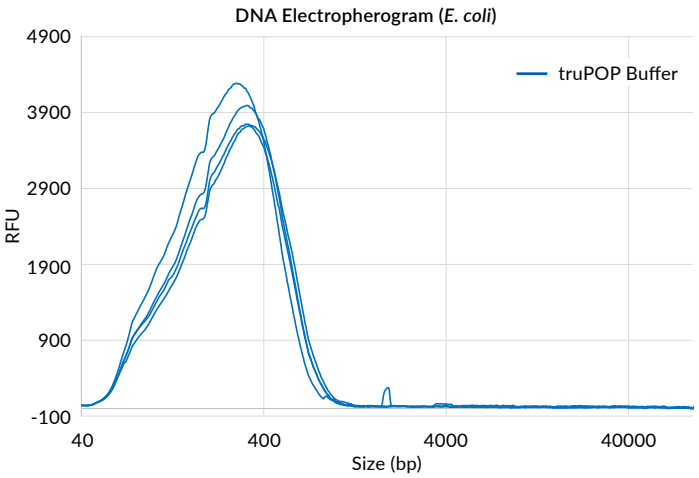

Figure 2: DNA electropherograms (Fragment Analyzer, AATI) Profile from lysed *E. coli*. Graph depicting the fragment size distribution of nucleic acid purified from lysed samples, from 40 bp to 50,000 bp, as determined by the AATI Fragment Analyzer.

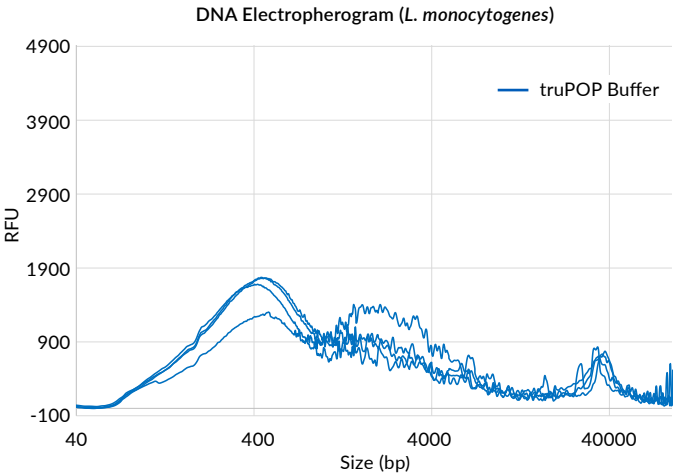

Figure 3: DNA electropherograms (Fragment Analyzer, AATI) Profile from lysed *L. monocytogenes*. Graph depicting the fragment size distribution of nucleic acid purified from lysed samples, from 40 bp to 50,000 bp, as determined by the AATI Fragment Analyzer.

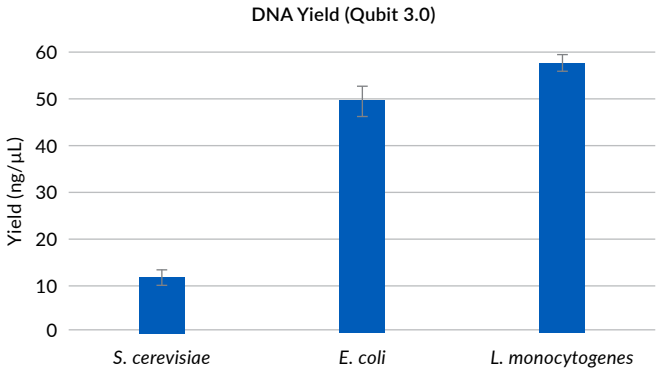

Figure 4: Yields (ng/µl) measured after AFA-mediated cell lysis & shearing. All DNA yield measurements were taken using Qubit 3.0.

Conclusion

In conclusion, we have developed a reproducible and robust protocol for lysis and nucleic acid extraction from both yeast and bacterial samples. This procedure can be used as a model system for more diverse samples from a variety of sources. By utilizing a 384-well plate format, higher throughput is achieved, on the Covaris LE220-plus Focused-ultrasonicator. Optimal AFA settings for cell lysis of yeast & bacterial samples are outlined in the Materials and Methods section. Data shows this to be an excellent application for labs aiming to develop and optimize high-throughput nucleic acid extraction from cell samples.

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