AFA-energetics™-Mediated RNA Extraction from Yeast

Abstract

This application note describes the optimized AFA-energetics dose to lyse and extract RNA from *S. cerevisiae*. We provide guidelines for a sample preparation protocol using the Covaris LE220-plus Focused-ultrasonicator and 96 oneTUBE-10 AFA Plate that can be used by investigators to lyse *S. cerevisiae* and extract RNA, along with supporting data.

Introduction

Cell lysis is key to studying many biological functions and is a vital step for molecular diagnostics, immunoassays, protein purification, cancer diagnostics, drug screening, and mRNA transcriptome determination. More specifically, DNA and RNA extracted from microbes collected from sources such as whole blood, stool, sputum, and even groundwater can each yield valuable information:

- ***Whole Blood***: presence of nucleic acids of fungi and bacteria quickly identifies septic patients [1]
- ***Stool***: diversity of bacterial populations indicates responsiveness to cancer immunotherapies [2]
- ***Sputum***: detection of nucleic acids from *M. tuberculosis* is necessary for diagnosis of tuberculosis in humans [3]
- ***Groundwater***: characterization via nucleic acids of typical bacterial profiles in groundwater is important for rapid identification of contaminated water sources [4]

The significant mechanical strength of the yeast cell wall [5] causes a special challenge in the recovery of its nucleic acids, making it a great model system to optimize cell lysis methodologies.

Modern lysing techniques utilize mechanical agitation and/or harsh chemicals that can result in nucleic acid degradation and poor nucleic acid recovery. Rigorous mechanical methods such as bead beating have intrinsic limitations:

1. Poor repeatability for advanced bioanalysis
2. Lack of precise thermal control, leading to an aggregation of heat in the system and denaturation of nucleic acids and proteins [6]

Chemical methods using detergents can leave cell debris, compromising yield from magnetic bead purification, as some of the beads bind to debris preferentially over nucleic acids.

In contrast to the disadvantages of other popular lysis methods, the precise control of a Covaris AFA-based (Adaptive Focused Acoustics®) extraction protocol using the oneTUBE-10™ system enables highly reproducible lysis and extraction of nucleic acids. The efficient non-contact isothermal mechanical disruption of cells by AFA leads to a high degree of extraction reproducibility while eliminating factors that can decrease quality of the nucleic acid product. *S. cerevisiae* was chosen because it is one of the major model systems in genetic engineering (i.e. expression vectors, protein engineering etc.) and biopathway engineering.

Materials and Methods

Required Materials

Covaris

- LE220-plus Focused-ultrasonicator (PN 500569)
- 96 oneTUBE-10 AFA Plate (PN 520249)
- Buffer BB2 from truXTRAC® cfDNA Kit – Magnetic Bead (PN 520221)
- Buffer WB2 from truXTRAC cfDNA Kit – Magnetic Bead (PN 520221)

Other

- SpeedBeads™ magnetic carboxylate modified particles Sigma GE45152105050250
- Buffer RLT Qiagen ID 79216

Methods

Approximately 1E7 *S. cerevisiae* cells were resuspended in RLT buffer and lysed using the 96 oneTUBE-10 AFA plate on the LE220-plus Focused-ultrasonicator according to the settings in ***Table 1***. Magnetic bead binding, wash, and elution were performed using GE SpeedBeads. The total binding volume was around 90 µL. The bind solution included lysed cells in Buffer RLT, GE SpeedBeads, Covaris Buffer BB2, and 38% Isopropanol. Wash steps were done 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	96 oneTUBE-10 AFA Plate
AFA Sample Volume	30 µL
Rack	Rack 96 oneTUBE-10 AFA plate (PN 500588)
Dithering	1.0 mm y-dither @ 20 mm/s
Temperature	12C
Peak Incident Power	450 W
Duty Factor	50%
Cycles per Burst	200
Treatment Time	2s + AFA, 1s delay repeated for up to 25 min

Table 1. AFA treatment settings for *S. cerevisiae* lysis.

Results and Discussion

The superposition of RNA electropherograms obtained by applying different AFA times is presented in ***Figure 1***. The diversity of RNA fragments can be rationalized according to:

1. 125 to 170 nt: tRNA, 5S rRNA and 5.8S rRNA
2. >200 nt: mRNA
3. 1800 nt: rRNA 18S
4. 3300 nt: rRNA 28S
5. Around 11000 nt: double stranded viral RNA P1 [7-9]

As the AFA-mediated lysis contact increases, shearing of longer RNA fragment occurs, making it possible for longer fragments to be sequenced using the Illumina technology. In general, the DV200 decreases as a function of AFA exposure time (***Figure 2***), thus allowing the user to fine-tune optimal conditions for downstream applications.

Approximately 1x10E7 cells were used per assay. Considering that *S. cerevisiae* has 7.1x10E-7 µg of RNA per cell [10], the maximum calculated extraction yield is around 7.5 µg per assay. ***Figure 2*** shows the RNA yield as measured by Qubit (green dots) and DV200 (red dot) as a function of AFA contact time. Full lysis is achieved after 15 to 20 minutes of continuous treatment, producing around 8 µg of total RNA.

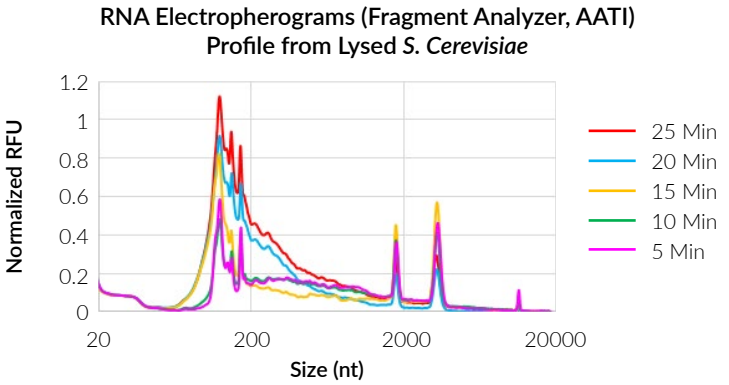

Figure 1. Graph depicting the fragment size distribution of nucleic acid purified from lysed samples, from 20 nt to 20,000 nt, as determined by the AATI Fragment Analyzer. AFA treatment time is indicated by the color of the curve according to the legend, and magnitude of RNA at each size is shown with greater magnitude corresponding to greater 'Normalized RFU'.

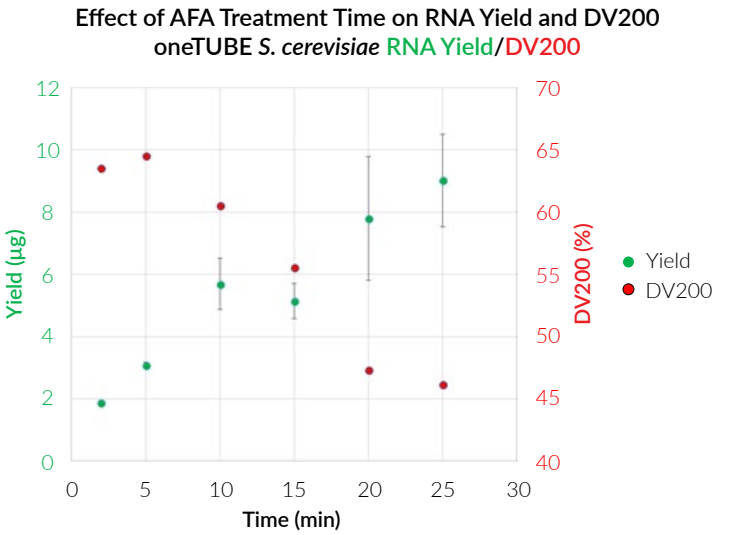

Figure 2. Graph superimposing RNA yield in µg on DV200 in % as AFA treatment time is varied. DV200 measures the percentage of the RNA sample which has a length of >200 nt.

Conclusion

In conclusion, we have developed a reproducible and robust protocol for cell lysis and nucleic acid extraction from *S. cerevisiae* samples as a model system for more diverse samples from a variety of sources. This protocol utilizes the 96 oneTUBE-10 AFA Plate, on the Covaris LE220-plus Focused-ultrasonicator, to quickly perform this protocol with no transfer steps until final storage of the extracted product. Optimal AFA settings for lysis of *S. cerevisiae* samples were determined, as outlined in the Materials and Methods section. This approach to lysing *S. cerevisiae* is easily adaptable to other yeast species, such as *Candida*, by doing an energy and/or time course study to obtain the appropriate settings for the specific yeast. After lysis, this protocol produces up to 100% extraction of RNA. The protocol outlined in this application note is an excellent application for other labs aiming to develop and optimize extraction of nucleic acid from cell samples.

References

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USA: Covaris, Inc. | Tel: +1 781.932.3959 | Fax: +1 781.932.8705 | Email: customerservice@covaris.com
Europe: Covaris Ltd. | Tel: +44 (0)845 872 0100 | Fax: +44 (0)845 384 9160 | Email: emeacustomerservice@covaris.com
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