Lysis and High Molecular Weight DNA Extraction from Eukaryotic and Prokaryotic Cells Powered by AFA-energetics®

Abstract

This application note describes a novel automatable approach to cell lysis and extraction of high molecular weight (HMW) DNA (>30,000 base pairs) from microbial samples, which can be readily sequenced with long-read sequencing technologies such as PacBio or Oxford Nanopore. Lysing the cells and subsequent extraction of HMW DNA from microbial samples is achieved on a Covaris LE220-plus Focused-ultrasonicator using a non-contact method mediated by Adaptive Focused Acoustics® (AFA®). Rapid lysis is achieved in less than 2 minutes for 96 individual bacteria samples and 10 to 60 minutes for 96 individual yeast samples when using a PCR-compatible 96 AFA-TUBE TPX Plate. Both electropherograms and Qubit measurements reveal that high molecular weight DNA can be efficiently extracted using AFA-mediated cell lysis.

Introduction

Efficient cell lysis is required to study many biological functions. It is an essential starting 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, soil, and even groundwater can yield valuable information:

- ***Whole Blood:*** presence of nucleic acids from fungi and bacteria quickly identifies septic patients [1]
- ***Stool:*** diversity of bacterial populations as part of the microbiome is linked to a wide range of clinical applications including infectious disease, autoimmune and autoinflammatory disorders, and responsiveness to cancer immunotherapies [2]
- ***Sputum:*** detection of nucleic acids from *M. tuberculosis* is necessary for diagnosis of tuberculosis in humans [3]
- ***Soil:*** detection of nucleic acids from bacterial spores leads to identifying harmful spores (i.e. *B. anthracis*) [4]
- ***Groundwater:*** characterization via nucleic acids of typical bacterial profiles in groundwater is important for rapid identification of contaminated water sources [5]

The significant mechanical strength of certain bacterial and yeast cell walls [6] present unique challenges to extract nucleic acids, making these organisms a great model to optimize cell lysis methodologies. Modern lysing techniques utilize mechanical agitation and/or harsh chemicals that can result in both nucleic acid degradation and poor nucleic acid recovery. Current methods, such as bead beating, have one or more of the following intrinsic limitations [7], while AFA-mediated cell lysis is able to overcome all of these limitations (***Table 1***):

Current Cell Lysis Methodologies	AFA-mediated Cell Lysis
Time-consuming, hands-on process	**Rapid, hands-free process**
Lack of thermal control – heat generated in the system denatures and damages nucleic acids and proteins	**High level of thermal control ensures biomarkers are not damaged**
Inefficient and incomplete lysis	**Efficient and tunable cell lysis**
Difficult to automate	**Simple to automate for high throughput processing**
Highly variable	**Low variability**

Table 1. Process comparison between current methodologies and AFA mediated microbial lysis.

Extrinsic limitations to the cell lysis process may include longer processing times, large volumes of reagents, and lack of automation. Additionally, chemical lysis methods using aggressive reagents can leave unwanted cell debris, and have poor compatibility with sequencing. Using a magnetic bead purification process after chemical lysis can compromise yield as some of the beads bind to debris rather than nucleic acids.

The precision and control of Covaris AFA-based extraction using the LE220-plus and LE220R-plus Focused-ultrasonicators, along with the 96 AFA-TUBE TPX Plate, enable highly reproducible and reliable lysis and nucleic acids extraction. The AFA-energetics process allows for efficient, non-contact, and temperature controlled mechanical disruption of cells, which leads to a high yield/high quality extraction while also eliminating factors that can decrease the quality of nucleic acids.

Sequencing high molecular weight DNA from bacterial genomes is preferable to lower molecular weight because higher molecular weight translates to accurate sequencing readouts and more complete coverage. PacBio RS II, using P6-C4 chemistry, is highly robust and cost-effective and should be the platform of choice for well-known, difficult-to-sequence bacterial genomes [8].

In this application note, we provide guidelines for various sample preparation protocols using the Covaris LE220-plus/LE220R-plus Focused-ultrasonicators and the 96 AFA-TUBE TPX Plate. The improved protocols enable lysis of a variety of cells, thereby increasing the extraction of HMW DNA. Compatible with PacBio SMRT or Oxford Nanopore sequencing technologies, Covaris AFA-mediated HMW DNA extraction protocols utilize high-throughput analyses. See ***Figure 1*** for a workflow example.

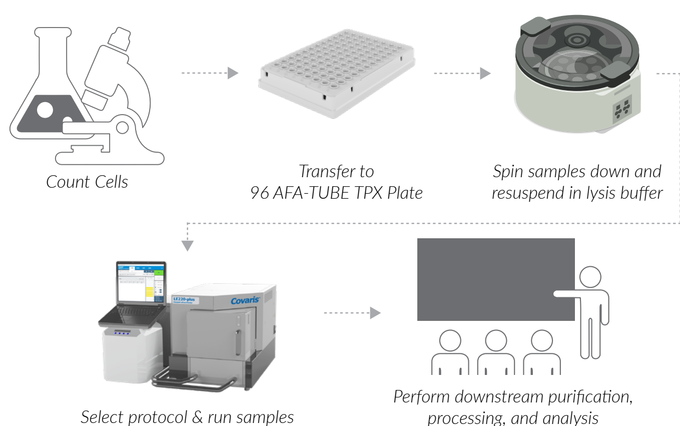

Figure 1. Simple workflow detailing sample preparation steps needed for high molecular weight DNA extraction.

Materials and Methods

Required Materials

Covaris

- LE220-plus Focused-ultrasonicator (PN 500569)
- 96 AFA-TUBE TPX Plate (PN 520272)
- Buffer BB2 contained in the truXTRAC cfDNA Kit – Magnetic Bead (PN 520221)
- Buffer WB2 contained in the truXTRAC cfDNA Kit – Magnetic Bead (PN 520221)
- 96 microTUBE Plate Thin Foil Seals (PN 520235)
- PS Rack 96 AFA-TUBE TPX Plate (PN 500622)

Other

- SpeedBeads™* magnetic carboxylate modified particles, Sigma GE45152105050250
- Covaris truPOP™ Buffer (PN 520268)

Methods

Covaris Lysis Buffer was used to resuspend yeast cells in a 96 AFA-TUBE TPX Plate. The cells were subsequentially lysed on an LE220-plus Focused-ultrasonicator according to the settings described in ***Table 2***. The DNA from lysed cells was purified with magnetic bead binding (GE SpeedBeads). The total binding volume was around 90 µl: the bind solution included lysed cells in Covaris Lysis Buffer, GE SpeedBeads, Covaris Buffer BB2, and 38% Isopropanol. The wash was performed 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. Details outlining specific times and cell numbers used is provided in ***Tables 3 & 4***. Cell numbers ranged from approximately 1 x 10^7 cells to 4 x 10^8 cells. DNA yields varied between 2.4 ng/µl to 66 ng/µl for different sample types.

Instrument	LE220-plus Focused-ultrasonicator
Vessel	96 AFA-TUBE TPX Plate
AFA Sample Volume	30 to 35 µL
Rack	PS Rack 96 AFA-TUBE TPX Plate (PN 500622)
Dithering	1.0 mm y-dither @ 20 mm/s
Temperature	12 °C
Peak Incident Power	450W
Duty Factor	50%
Cycles per Burst	200
Treatment Time	2s AFA, 2s delay repeated for 1 to 60 min per plate

Table 2. AFA Treatment Settings for Cell Lysis.

Results

Yeast Lysis

Table 3 details the cell count, yield, AFA lysis treatment time, and volumes used for each organism. The electropherograms obtained for *S. cerevisiae* and *Y. lipolytica* reveal that the size of the purified DNA is >30,000 bp, as seen in ***Figure 2 & 3***. The %CV of the yield ranges between 5 to 20% depending on the treatment time, cell count, and power level used.

Sample	Cell Number Used	Yield via AFA	Treatment Time per 96 AFA-TUBE TPX Plate	Buffer Volume
Y. lipolytica	~ 3 x 10^7 cells	~ 1.16 µg	60 minutes	30 µl
S. cerevisiae	~ 5 x 10^7 cells	~ 0.72 µg	15 minutes	30 µl

Table 3. Extraction parameters and subsequent yields from AFA-mediated cell lysis (yeast).

Sample	Cell Number Used	Yield via AFA	Treatment Time per 96 AFA-TUBE TPX Plate	Buffer Volume
E. coli (gram -)	~ 4 x 10^8 cells	~ 1.98 µg	<1 minute	30 µl
L. monocytogenes (gram +)	~ 4 x 10^8 cells	~ 1.95 µg	<1 minute	30 µl

Table 4. Extraction parameters and subsequent yields from AFA-mediated cell lysis (bacteria).

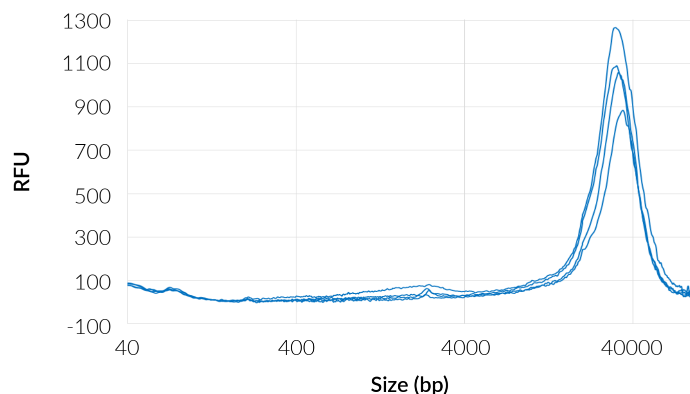

Figure 2. Superposition of electropherograms (AATI - Fragment Analyzer DNF-492-33 - SS Large Fragment) showing the fragment size distribution of DNA purified from *S. cerevisiae* lysed samples, from 40 to 90,000 bp.

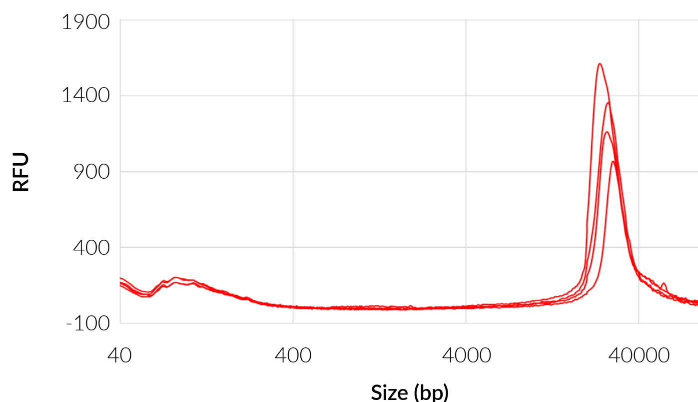

Figure 3. Superposition of electropherograms (AATI - Fragment Analyzer DNF-492-33 - SS Large Fragment) showing the fragment size distribution of DNA purified from *Y. lipolitica* lysed samples, from 40 to 90,000 bp.

Gram+ and Gram- Bacteria

Table 4 details the cell count, yields, buffer volumes, and AFA treatment time used to lyse *E. coli* and *L. monocytogenes*, respectively. The electropherograms presented in ***Figures 4 & 5*** show similar features compared to *S. cerevisiae* and *L. monocytogenes*, with the DNA size distribution centered at about 35 kb. The %CV for all samples was below 10%, indicating a high degree of repeatability.

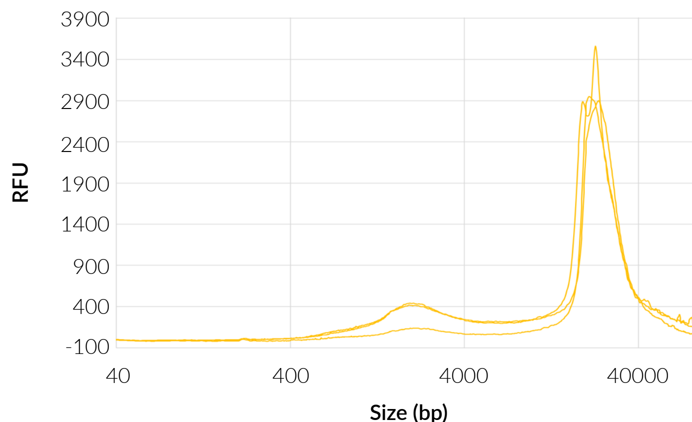

Figure 4. Superposition of electropherograms (AATI - Fragment Analyzer DNF-492-33 -SS Large Fragment) showing the fragment size distribution of DNA purified from *E. coli* lysed samples, from 40 to 90,000 bp.

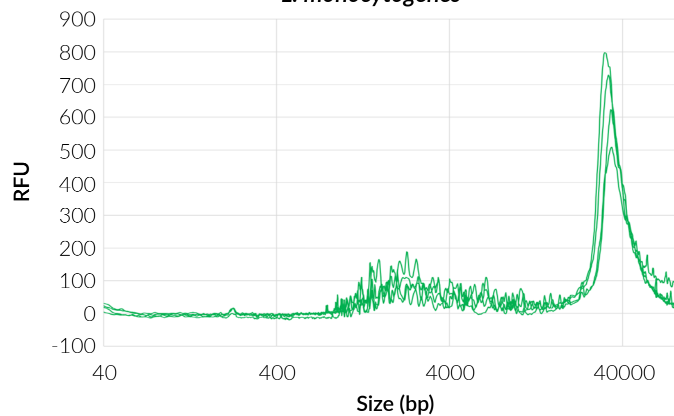

Figure 5. Superposition of electropherograms (AATI - Fragment Analyzer-DNF-492-33 - SS Large Fragment) showing the fragment size distribution of DNA purified from *L. monocytogenes* lysed samples, from 40 to 90,000 bp.

Cell Concentration and AFA Contact Time

The current methods for cell lysis and DNA extraction take from one hour to overnight to extract DNA at concentrations that are sequenceable [9]. ***Table 4*** compares specifications between Covaris lysis technology versus commercially available kits. In general, DNA yields and concentrations from competing kits are lower as compared to Covaris AFA-mediated extraction. Some kits require a higher cell count with higher volumes of elution and use more reagent volume per sample, which dramatically increases the cost of the extraction. Other methods are limited by cell count, subsequently capping the yield [10]. Even after lengthy and technically challenging protocols, there is no guarantee that the extracted DNA yield will be enough for sequencing purposes (usually 1 to 5 µg DNA requirement) or of high enough quality for a confident sequencing readout from library preparation [11].

DNA extraction using Covaris is linearly dependant, with both cell count and treatment times, exhibiting R^2 values of 0.99 and 0.98 respectively, as shown in ***Figures 6 & 7***. Yield and cell counts used are independent of the reagent volume (30 µl). The treatment times can be shorter while also providing competitive yields to other methods, by using less buffer and lysing in less time.

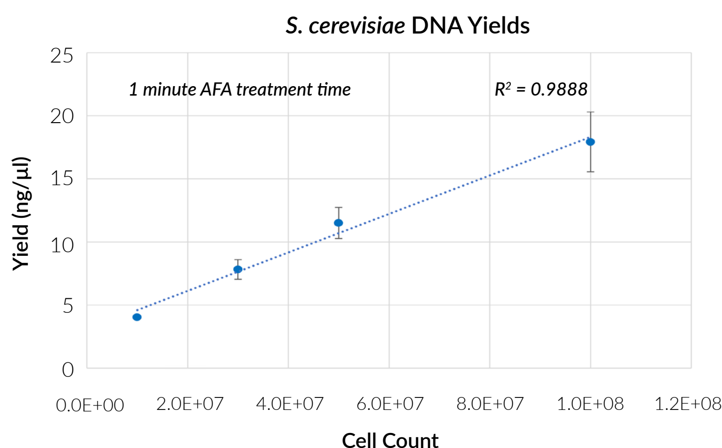

Figure 6. Plot of DNA yield as a function of cell count using 1 minute of AFA treatment. The DNA yield increases linearly as the cell counts of *S. cerevisiae* varies from 1 x 10^7 to 1 x 10^8.

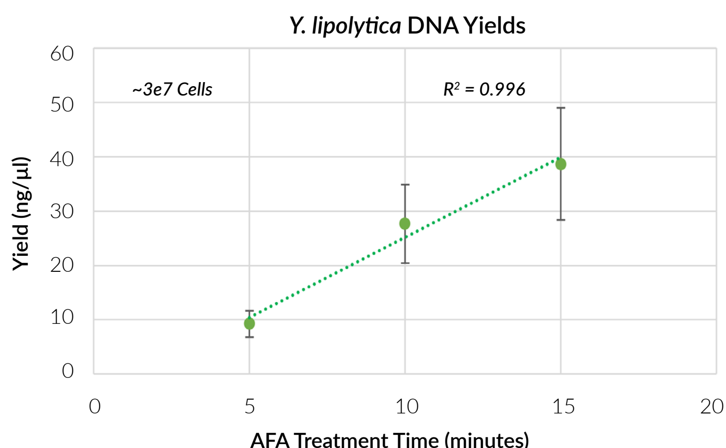

Figure 7. Plot of DNA yield as a function of AFA treatment time. The DNA yield increases linearly as AFA-mediated treatment time increases. In this experiment ~ 3 x 10^7 cells of *Y. lipolytica* were used.

Company	Kit	Prep Time	Lysis Volume	Automation Available? (Y/N)	Cell Count Used
Covaris	truPOP	Bacteria or Yeast: 10 to 30 min	30 to 40 µl	Y	Bacteria: 4.0 x 10^8 Yeast: 5.0 x 10^7
ThermoFisher [12]	Genomic DNA Extraction - PureLink™	Bacteria: 35 min Yeast: 130 min	400 µl	N	Bacteria: ≤ 2 x 10^9 Yeast: ≤ 5 x 10^7
Roche [13]	MagNA Pure 96 DNA and Viral NA Small Volume Kit	Bacteria: 60 min	300 to 450 µl	Y	Bacteria: 5 x 10^5
Promega [14]	Maxwell® RSC Cultured Cells DNA Kit	Bacteria: 45 min	400 µl	Y	Bacteria: 2 x 10^9
Qiagen [15]	QiAamp DNA Microbiome Kit	Bacteria: 120 min	410 µl	N	Bacteria: N/A

Table 5. Comparision of Covaris DNA extraction protocols as compared to other available kits. DNA yield is normalized based on recommended cell counts.

Conclusion

Historically, the extraction of high molecular weight DNA is often an unreliable, time-consuming process. We have shown reproducible and robust data for cell lysis and HMW DNA extraction from a variety of cell types using optimized protocols, as outlined in the Materials and Methods section. Cell volumes and treatment times can be adjusted to give higher DNA yields. These protocols utilize the 96 AFA-TUBE TPX Plate on the Covaris LE220-plus Focused-ultrasonicator to quickly perform cell lysis and extraction with no transfer steps until final storage of the extracted product. Protocols outlined in this technical note may be used by researchers aiming to extract high molecular weight DNA for various downstream applications.

*SpeedBeads is a trademark of Thermo Fisher Scientific or its subsidiaries.

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