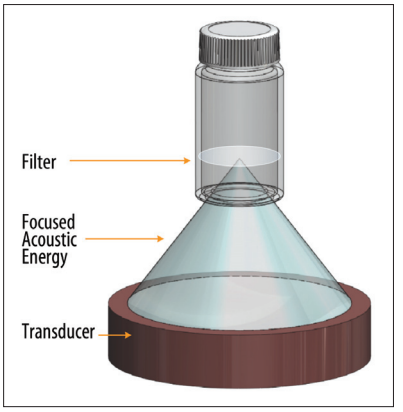
Release of Microbial Cells from a Solid Matrix, Cell Lysis, and Shearing of Nucleic Acids Performed in a One-Step Procedure

OVERVIEW

Conventional preparation of DNA from microbial cells for sequencing analysis typically requires several time-consuming procedures. For example, microbial cells in samples collected for metagenomic studies are typically released in one procedure, then lysed with another procedure. Next, DNA released from the cells is purified and sheared in yet another procedure.

This Application Note describes an Adaptive Focused Acoustics (AFA™) procedure where three of the above procedures are combined into one step (1) the microbial cells are released from a solid matrix to which they are adhered, (2) the microbial cells are lysed, and (3) the DNA released from the cells is sheared to a desired fragment size with high precision and reproducibility. Samples may be processed in batches. The procedure is compatible with downstream automated purification and sequencing of the DNA fragments. It also has potential use for sample analysis involving cells lysis of any type and detection of specific nucleic acid sequences using hybridization technology.

FIGURE 1:



Example: Microbial Cells Adhered to Filters

E. coli and *S. epidermidis* were used as models for Gram-negative and Gram-positive bacteria. A model metagenomic sample was prepared by diluting one ml aliquots (~10^9 cells) of cultured cells in PBS, then filtering through 0.45 µm pore size 25 mm diameter hydrophilic PVDF membrane filters. Filters were placed with adhered cells face down in 1.5 mL of 10 mM Tris-EDTA in 20 mL liquid scintillation vials and subjected to AFA™, as shown schematically in Figure 1. Nucleic acids were purified from post-AFA™ solutions and analyzed by gel and capillary electrophoresis. Cell release and lysis were determined by measuring protein levels in pellets and supernatants of samples that were immediately centrifuged post-AFA at 15,000 g for 10 minutes.

AFA™ Release of Microbial Cells from Filters

Table 1 shows release at different times and acoustic energies at 1,000 cycle per burst (cpb), 10% duty cycle (dc), 6-7°C. About 75% of the cells are released in one minute at 32W Peak Incident Power (PIP). Cell release increases as a function of energy level and applied time. 60W PIP for 2 min resulted in the release of at least 90% of cells, with 100% release observed on some occasions. Application of energy levels ≥ 90W PIP for 5 minutes consistently resulted in 100% release of the microbial cells from the filters.

AFA™ Lysis of Microbial Cells

Lysis of the cells at the 60W PIP level described above was typically about 15%. Application of higher energy levels with the duration held constant resulted in classic asymptotic dose response curves, as did application of a set energy level as a function of time. A summary of lysis results obtained in a series of experiments performed over the course of several months at 1,000 cpb, 10% dc, 6-7°C is shown in Table 2. Lysis of the cells at the 80% level was typically achieved in 10 min at 230 W PIP. Longer times (15-20 minutes) at the same AFA™ level resulted in lysis approaching 90%. Higher levels of lysis (≥90%) were consistently achieved in 15-20 min when 100 mg of 0.1 mm diameter glass beads were added to samples, with some samples reaching the 100% level.

TABLE 1: AFA™ Release of Microbial Cells from Filters

PIP (Watts)	Duration (minutes)	% Release (+/- 5%)
32	1	75
32	2	80
60	1	80
60	2	95
≥90	5	100

TABLE 2: AFA™ Lysis of *E. coli* or *S. epidermidis*

PIP (Watts)	Duration (minutes)	% Lysis
140	5	53
140	10	65
230	5	64
230 +Beads	5	76
230	10	80
230 + Beads	10	83
230	15	83
230 + Beads	15	90
230	20	88
230 + Beads	20	95

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Application Note

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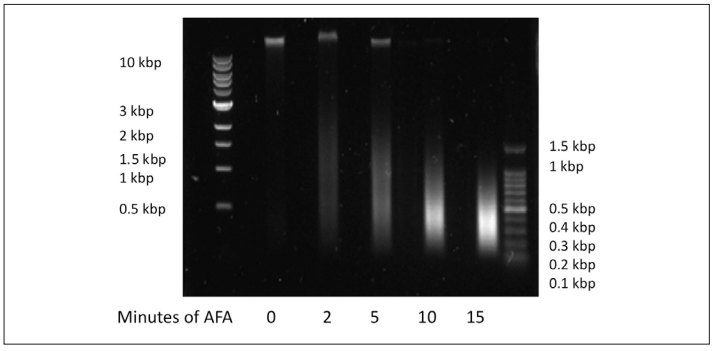
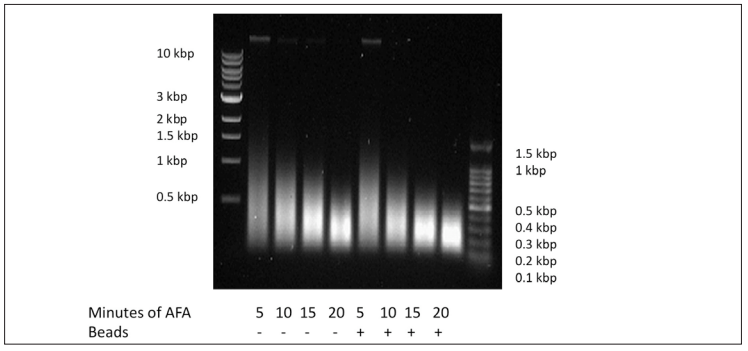
DNA fragments resulting from the use of AFA™ to simultaneously release *E. coli* cells from a membrane filter, lyse the cells, and shear the released nucleic acids.

Samples were subjected to AFA™ at 230 W PIP, 10% dc, 1,000 cpb, 6-7°C for the indicated times. The resultant DNA fragments were purified and analyzed by agarose gel and capillary electrophoresis. The larger molecular weight DNA in samples subjected to short AFA durations is from cells that were subsequently lysed in the DNA purification procedure. The "0 minutes of AFA" sample in Gel A is DNA purified from an equal volume of *E. coli* cells not subjected to AFA. 100 mg of 0.1 mm diameter glass beads were added to the four samples on the right hand side of Gel B.

GEL A



GEL B



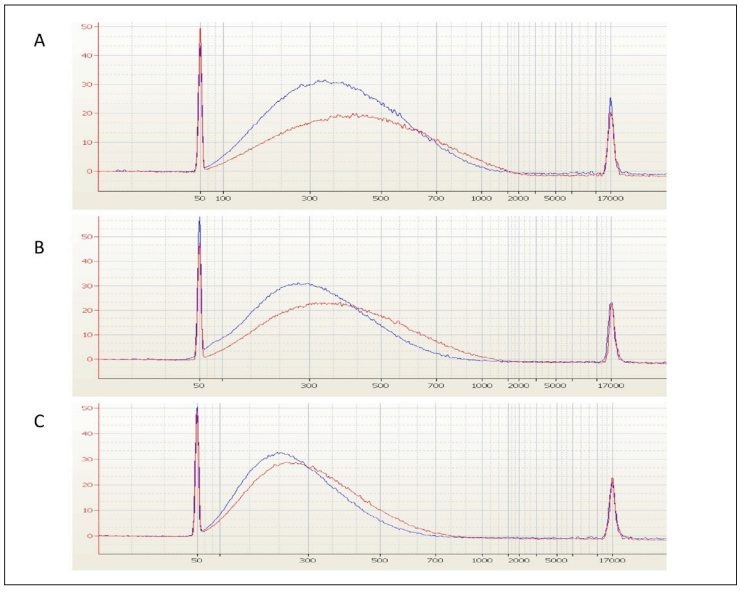
Effect of glass beads employed during AFA™ on DNA fragment size

Samples were subjected to AFA™ at 230 W PIP, 10% dc, 1,000 cpb, 6-7°C in presence (blue trace) or absence (red trace) of 100 mg of 0.1 mm diameter glass beads. The DNA fragments were purified and analyzed by capillary electrophoresis.

TABLE 3

Duration (Minutes)	Mean DNA Fragment Size Without Glass Beads (base pairs)	Mean DNA Fragment Size With Glass Beads (base pairs)
5	500	476
10	417	344
15	341	283
20	258	234

(A) 10 min AFA. (B) 15 min AFA. (C) 20 min AFA. Mean fragment sizes (+/- 10 bp) are shown in Table 3



Key Features	Benefits
• Release of microbial cells from a solid matrix, cell lysis and DNA shearing achieved in closed vessel in one step	✓ Reduction in processing times, sample loss, contamination, and operator error
• Release of *E. coli* and *S. Epidermidis* from microporous filters achieved at focused acoustic energy lower than required for DNA shearing	✓ Allows use of higher energies to release cells bound more tightly to other matrixes without excessive DNA shearing
• High precision and reproducibility of DNA shearing. • Samples may be processed in batches	✓ Post-AFA™ samples can be integrated with automated downstream purification of DNA fragments

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