AFA-based Efficient DNA Extraction and Optional Mechanical Shearing from *Mycobacteria smegmatis*

SUMMARY

We demonstrate efficient extraction and shearing of DNA from *M. smegmatis* prior to Next Generation Sequencing. Extraction of DNA from a strain of *Mycobacterium smegmatis* using the Covaris M220 Adaptive Focused Acoustics AFA instrument with a specific chemistry and set of energy conditions is shown to optimize DNA recovery.

INTRODUCTION

Covaris maintains a culture of *M. smegmatis* ATCC 19420 as a model organism. Mycobacteria are considered historically to be difficult for extraction of intracellular components. Covaris reported (ECCMID 2015) on effective peptide/protein extraction from *M. smegmatis* using AFA and Extraction Solvent (ref mix) in development of an optimized sample preparation for MALDI-TOF MS identification. We demonstrated the use of AFA and aqueous buffer conditions to process mycobacteria cultures to recover and sequence DNA.

DNA sequencing technology continues to advance into many areas of research and clinical microbiology. As the costs of sequencing are decreasing, capacity of individual laboratories to process more samples and to expand areas of research is increasing. Research projects rely on solid reproducible sequencing data. DNA Sequencing has moved from being a science to a utility. Methods of sample preparation are in need of advancements to enhance precision, reduce hands-on time and improve data quality.

The classic method of obtaining DNA from bacterial cells is the use of mechanical "bead-beater" technology. These devices may generate damaging heat and may lack precision and reproducibility. By using Adaptive Focused Acoustics (AFA) any laboratory can initiate a sequencing project more readily. In addition, the protocol can be instrument-standardized after optimization.

M. smegmatis is classified as a high GC content gram-positive bacteria with DNA content 67% GC, and 6,988,209 nucleotides. 90% of the genome (6716/6938 genes) represents coding regions that encode for 6716 proteins.

METHODS AND MATERIALS

Bacterial culture

Mycobacterium smegmatis 19420 was purchased from ATCC. The bacterium is maintained by subculture on a Middlebrook agar plates at 37° C for 48 hours. Individual colonies are selected for DNA extractions.

DNA extraction

The bacterial cell wall is disrupted physically using Covaris AFA energy under controlled temperature and power conditions. Approximately 1 mg bacterial colonies from the agar plate were transferred to 100 µl TE buffer in a microTUBE™-130 (Covaris p/n 520199) containing 25 mg glass beads. AFA was performed using a Covaris M220 Focused-ultrasonicator with Peak Incident Power (PIP) 40W, Duty Factor (DF) 50%, and varying the Cycles Per Burst (CPB) using a range 50, 200, 500 or 1000, depending on the desired DNA size. The instrument temperature maintains water temperature surrounding the microTUBE during AFA at 4° C. The AFA process duration was set to 120 seconds for each sample.

DNA clean up

The AFA-treated cell lysate was cleaned with the Qiagen QIA Quick PCR purification kit. Briefly, the lysate was mixed with 600 µl buffer PB and then cleaned according to Qiagen's protocol. The DNA was eluted in 100 ul elution buffer and quantified using a Qubit fluorometer and NanoDrop 2000.

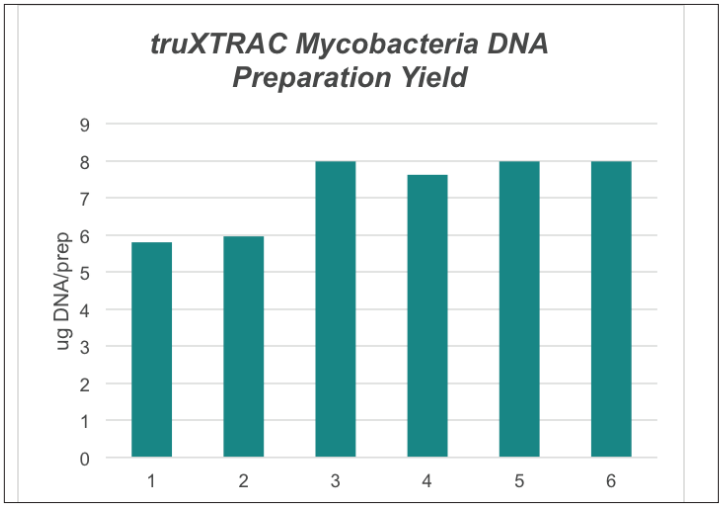
DNA sequencing

To test if the DNA extracted with AFA treatment is suitable for DNA sequencing, the DNA samples were sequenced using the Illumina MiSeq reagent V2 kit. Sequencing libraries were prepared with the Illumina TruSeq DNA PCR-Free Library Preparation Kit. Manufacturer's protocols were followed.

RESULTS AND DISCUSSION

The Covaris AFA truXTRAC™ protocol can extract sufficient DNA from *M. smegmatis* for Next Generation Sequencing. DNA was extracted from six aliquots of the same mycobacteria. The average yield was 7.22 µg/preparation (Figure 1). That amount exceeds the requirement for the Illumina NGS library preparation; 1.1 -2.2 µg is required for the Illumina TruSeq DNA PCR-Free Library Prep kit.

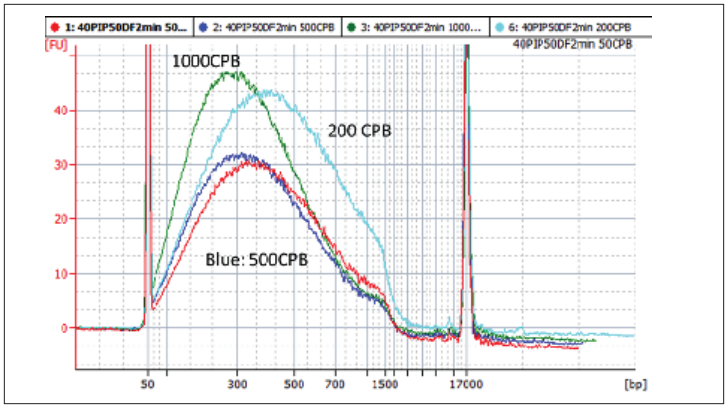
FIGURE 1. Mycobacterial DNA yield by AFA truXTRAC determined by Qubit fluorometer



DNA can be sheared to the desired size during extraction

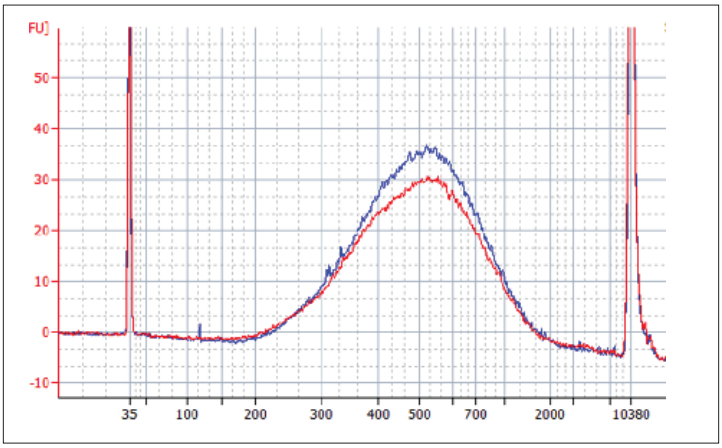
The AFA energy disrupts the cellular structure, enabling extraction, and also shears DNA. The DNA size can be controlled by varying the Cycles Per Burst (CPB). Figure 2 shows the different DNA sizes by AFA at different CPB. The yield was not affected by the different CPB values.

FIGURE 2. AFA truXTRAC DNA can be used for NGS library preparation



Two of the mycobacterial DNA samples were used for NGS library preparation with Illumina's TruSeq DNA PCR-Free Library Preparation Kit. More than 0.5 pM libraries were prepared with predicated sizes,

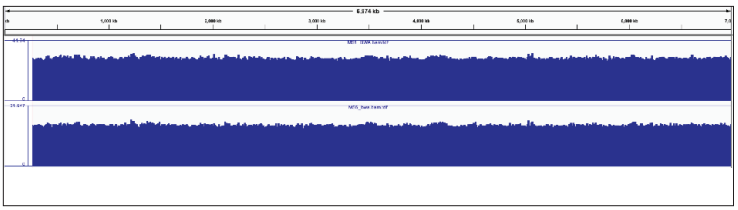
FIGURE 3. Library size. The libraries contain additional 120 bp index primers.



Sequencing by MiSeq

The two samples were applied to a MiSeq reagent V2 sequencing. Approximately 98% of the reads were aligned to the reference sequence (Figure 4)

FIGURE 4. Coverage depth map of two mycobacterial DNA samples sequenced by MiSeq.



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