AFA-based RNA Extraction from *Mycobacteria smegmatis*

SUMMARY

We demonstrate that intact RNA can be efficiently extracted from *Mycobacteria smegmatis* using the Covaris Adaptive Focused Acoustics® (AFA®) M220 or ME220 Focused-ultrasonicators. By applying precise control of AFA power conditions, RNA can be differentially extracted to favor intact subunits or shorter fragments. AFA technology can effectively replace bead-beating protocols for extraction of RNA. AFA extraction methods are more consistent, provide high quality RNA and are easy to perform.

INTRODUCTION

How proteins are expressed in microbial cells is an important area of study. Cell peptide and protein components can enable rapid identification of genus/species from culture isolates using MALDI-TOF MS. Whole Genome Sequencing provides important information about mechanisms and properties of infection and various microbiomes. Analysis of RNA enable annotation and quantification of comprehensive microbial transcripts.

METHODS AND MATERIALS

Mycobacterium smegmatis 19420 was purchased from ATCC.

Cultures were maintained on Middlebrook agar plates at 37° C for 48-72 hours.

RNA Extraction Conditions

The *M. smegmatis* cell structure was physically disrupted using Covaris AFA energy under controlled power settings and temperature. Approximately 1 mg biomass obtained from bacterial colonies grown on agar plates was transferred to 120 µl RTL buffer in a Covaris microTUBE 130 containing both 25 mg glass beads and a teflon fiber.

The AFA process was performed using a Covaris M220 Focused-ultrasonicator. Process variables controlled are Peak Incident Power (PIP), Duty Factor (DF), Cycles Per Burst (CPB), and AFA processing time. The sample temperature was held constant at 18° C. The analytical measurements indicated the relative quality of extracted RNA.

M. smegmatis RNA Extraction Results with Varying Peak Incident Power

120 µl of RTL buffer (QIAGEN RNeasy kit) was added to three Covaris microTUBEs 130 µl capacity. Each microTUBE contained both 25 mg glass beads and a teflon fiber. 1 mg of *M. smegmatis* cell biomass was added to each microTUBE using a transfer loop.

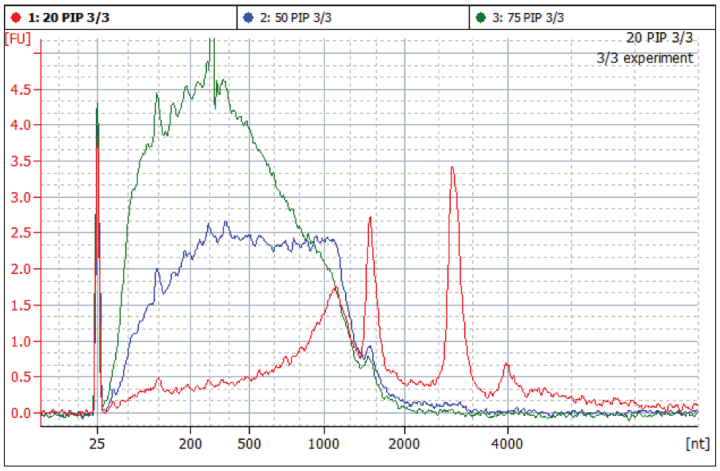
AFA was performed for 120 seconds on each microTUBE. PIP was varied, using 20, 50 and 75 watts. DF was constant at 20% and CPB was constant at 50 cycles. Sample temperature was held to 18° C.

After AFA treatment, RNA was extracted from each microTUBE according to manufacturer's instructions. Analysis was performed on an Agilent Bioanalyzer and Qubit 3.0 Fluorometer.

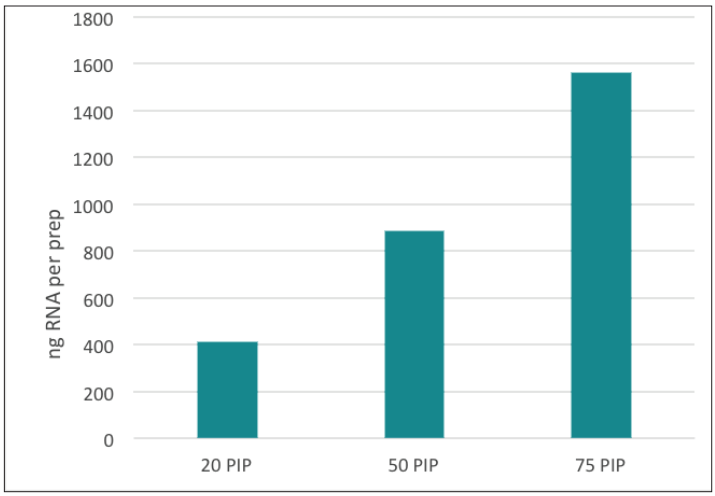
RESULTS

Higher power, at 50 and 75W PIP, provided higher yields of RNA. However, these conditions increased the amount of shearing of the RNA. Using 20W PIP, intact 16s and 23s RNA subunits are extracted, with a low level of shearing.

***M. smegmatis* RNA PIP 20-50-75**



Mycobacteria RNA yield by Qubit RNA BR



DISCUSSION & CONCLUSION

For extraction of RNA from mycobacteria, Covaris recommends starting with the following conditions:

PIP	**20W**
DF	**20%**
CPB	**50**
Temp	**18° C**
Time	**120 seconds**

Increasing duty factor, Cycles per Burst, or duration of AFA can be explored as controlled variables. Consider the importance of the quality of RNA as well as the quantity in consideration of extraction conditions.

Cell concentration and vessel volume may also affect the quantity and quality of RNA extracted.

Covaris also provides the ME220, S220, E220 and LE220 Focused-ultrasonicators. RNA Extraction conditions are predictably similar but will require verification depending on sample volume, biomass, and experimental objectives. Covaris AFA is effective for the process of RNA extraction from mycobacteria. The goals of your experiment should guide the optimization of extraction conditions using the Covaris Focused-ultrasonicator platform.

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