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Application Note

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Microbiome Sample Preparation: Efficient DNA Extraction and Optional Mechanical Shearing

BACKGROUND

Understanding microbiomes is an increasing focus of many research programs. Genome sequencing has enabled new knowledge that far exceeds capabilities of traditional microbiology culture-based techniques. Microbiome research is vital to the development of global strategies for dealing with antibiotic resistance, antibiotic development and antibiotic stewardship. In parallel with the success of fecal transplants and prescription probiotics, the demonstration of a radical new approach to infectious disease management is in motion. Empirical observations that a healthy microbiome is protective against disease can be supported by analytical data. Microbiome analysis may become mainstream for managing specific disease states, such as cure vs recurrence of *C. difficile* infections.

COVARIS AFA FOR SHEARING DNA; METAGENOMIC LIBRARY PREPARATION

Ranjan et. al., (1) described the process for mechanically shearing DNA to 300-600 bp fragments with a Covaris® S220 Focused-ultrasonicator. The shearing process was done on DNA previously extracted and frozen. To achieve high level of reproducible shearing, the Covaris S220 Focused-ultrasonicator with SonoLab software controls critical parameters such as Peak Incident Power (PIP), Duty Factor (DF), Cycles Per Burst (CPB), temperature and duration of treatment.

Each sample: 5 µg metagenomic DNA
Instrument: Covaris S220
Shearing tubes: Crimp-cap microTUBEs with AFA fiber
Sample volume: 130 µL
Operating conditions:

PIP	**140 watts**
DF	**10%**
CPB	**200**
Temperature	**7-9° C**
AFA duration	**80 seconds**

FECAL METAGENOMICS DNA ISOLATION

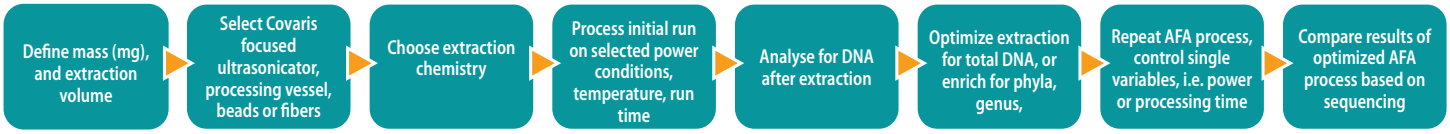
The protocol described by Ranjan et al, used Phenol/Chloroform/Isoamyl alcohol and glass beads with vortexing, followed by bead beater treatment for 5 minutes to isolate DNA from 100 mg of adult stool.

Given the range of resiliency to bead-beating and chemical lysis across the wide spectrum of species in a stool sample, a single extraction method might introduce bias by favoring a selection of organism types. Harsh chemical and mechanical lysis treatment could damage or shear DNA; while a milder extraction could result in a low yield or a biased selection of certain species. Adoption of a Covaris® Adaptive Focused Acoustics® (AFA®) extraction process could provide a carefully processed and more precise multi-stage extraction.

CONSIDERATIONS FOR USE OF AFA TO EXTRACT DNA FROM A MULTI-SPECIES SAMPLE

1. Use extraction buffers that are most compatible with your target molecule.
2. Aqueous-based buffers are preferred for AFA.
3. If detergents or surfactants are used, fill the AFA vessel to minimize foaming.
4. Consider using lower concentrations of detergents with AFA.
5. Initially define PIP and DF to visually observe effect of processing, at a set time.
6. After energy is defined, run a time course, compare results based on the needs of your experiment.
7. Consider lowering sample input to avoid over loading due to high efficiency of AFA processes.

DNA EXTRACTION FROM MIXED SPECIES USING AFA



CONCLUSION

Covaris® AFA processes are well established for DNA shearing, and can be predictably utilized for metagenomics library preparations. Depending on the sample size and source of a microbiome project, Covaris® AFA may also be used for DNA extraction of multiple species, with adjustable conditions to help better control the selection of cell types extracted.

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