PROTOCOL

Covaris

Fragmentation of Total RNA with M220

INTRODUCTION

Fragmentation of Total RNA with the Covaris M220 Focused-ultrasonicator is a highly efficient and reproducible process. RNA strands are physically sheared by the acoustic microstreaming generated by the ultrasonic energy. Since this process is rate-limited; the size of the RNA fragments generated is affected by the treatment duration (see Figure 1). Longer treatment time will produce smaller RNA fragments.

Mean Fragment Size	**200 nt**	**200 nt**
microTUBE AFA Fiber	Screw-Cap	Snap-Cap
Sample volume	50 µl	130 µl
Peak Incident Power	50 W	50 W
Duty Factor	20 %	20 %
Cycles per Burst	200	200
Treatment Time	420 s	240 s
Temperature	7°C	7°C

Table 1 – M220 Instrument settings for RNA fragmentation

Note:

The value for treatment time listed in Table 1 is a recommended guideline. Actual results may vary depending on the concentration, viscosity, and the quality of the starting material. Depending of your application, Covaris recommends performing a time course experiment to determine the appropriate treatment time settings (for example 180, 240, 300, 360 seconds for 130 µl samples in microTUBE Snap-Cap).

The above conditions were optimized using total RNA; with mRNA, the treatment time is reduced. For example, the mRNA sample time course optimization should be centered at 120 seconds for 50 µl samples and 70 seconds for 130 µl samples.

MATERIALS

Covaris M220 Focused-ultrasonicator and Covaris M-Series microTUBE holder insert
Sample Vessel: Covaris microTUBE Snap-Cap for 130 µl samples or microTUBE Screw-Cap for 50 µl samples.
Buffer: Water, Tris EDTA, pH8, or 10mM Tris-HCl, pH 8.5
Sample Concentration: < 300 pg / µl

SUPPLIES

Covaris microTUBE	microTUBE AFA Fiber Snap-Cap (25)	520045
	microTUBE AFA Fiber Screw-Cap (25)	520096
Tube Holder	M-Series microTUBE Holder Insert	500301
Kit	M220 Accessory Kit for microTUBE	500296
	Includes: microTUBE holder insert microTUBE Snap-Cap (25) microTUBE Screw-Cap (25) microTUBE prep station with tool	
Instrument cleaning	M-Series Fill & Drain Accessory Kit	500299
	M-Series Swab Cleaning Kit	500298
AFA-grade Water		520101
Preparation station	microTUBE prep station with tool	500330

PROCEDURE

1. Turn the power switch at the back of M220 instrument to ON position, power on the computer, and start the SonoLab software.
2. Open the safety cover and place the M-Series microTUBE Holder insert into the water bath housing.
3. Fill the provided wash bottle with the AFA-grade water (use either Covaris AFA-grade water PN 520101 or highly purified water).
4. Using the wash bottle, add water in the water bath housing. The water level should reach the top surface of the Tube Holder and water level indicator in SonoLab should turn to green.
5. Select an existing method in SonoLab or create your own following settings in Table 1.
6. Wait until the water temperature in the water bath reaches 7 °C and the water temperature indicator in SonoLab turns green.
7. Load the sample into microTUBE by pushing the pipette tip through the slit in the cap (Snap-Cap microTUBE) or directly into Screw-Cap microTUBE and carefully dispense the fluid. Make sure that no air bubbles are in the fluid sample as they will interfere with the acoustic energy.
8. Open the safety cover, lift the Sliding Weight and rotate it into the loading position.
9. Place the microTUBE in the central opening of the microTUBE Holder insert and place the Sliding Weight on the top of microTUBE.
10. Close the Safety Cover and click “Run” button in the SonoLab software to start the process.
11. After the treatment is completed, lift the Sliding Weight and remove the microTUBE from the Tube Holder.

NOTE: With 50 µl samples the tube should be centrifuged gently to concentrate the sample into the bottom of the microTUBE before sample is removed.

QUALITY CONTROL NOTE

In addition to the physical process of RNA fragmentation, the analytical process used to determine fragment size is a potential source of variation in the peak mean value and fragment distribution. Agilent Bioanalyzer electropherogram results can differ from the results obtained using the gel electrophoresis. Therefore, fragment distributions and peak values may differ depending on the analytical method employed. Furthermore, column purification and concentration of the sheared RNA can generate biased fragment distribution profiles due to the inherent loss of smaller RNA fragments during the process. Fragment analysis should be conducted prior to column purification or concentration of the sheared RNA samples.

SUPPLEMENTARY DATA

Figure 1 - Time course of total RNA fragmentation using Covaris M220 Focused-ultrasonicator

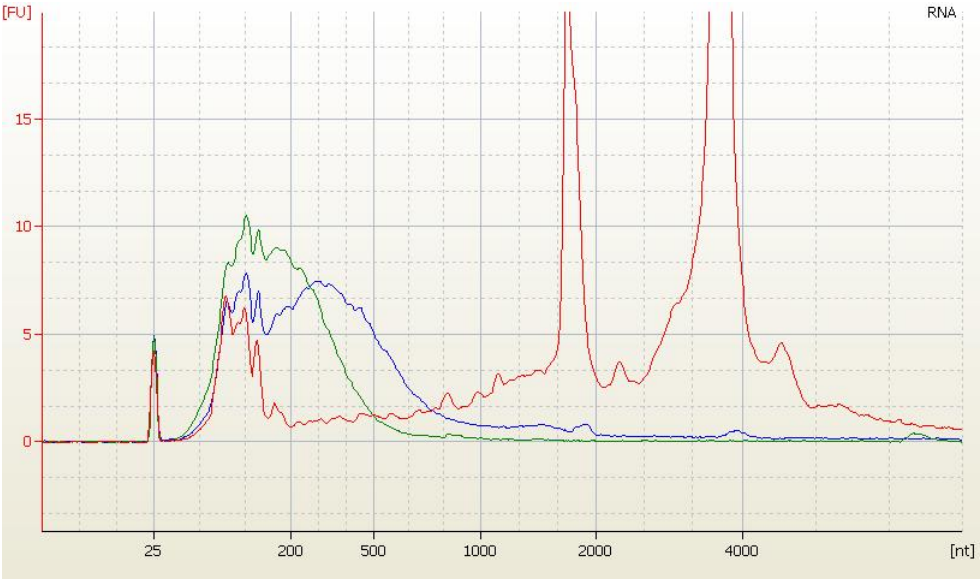

Figure 1A: 130 µl samples of total RNA were treated in the Snap-Cap microTUBE using the following conditions: DF 20%, PIP 50, cpb 200 for, 0 sec (Red trace), 60 sec (Blue trace) and 240 sec (Green trace). RNA was analyzed using Agilent Eukaryotic Total RNA Nano kit.

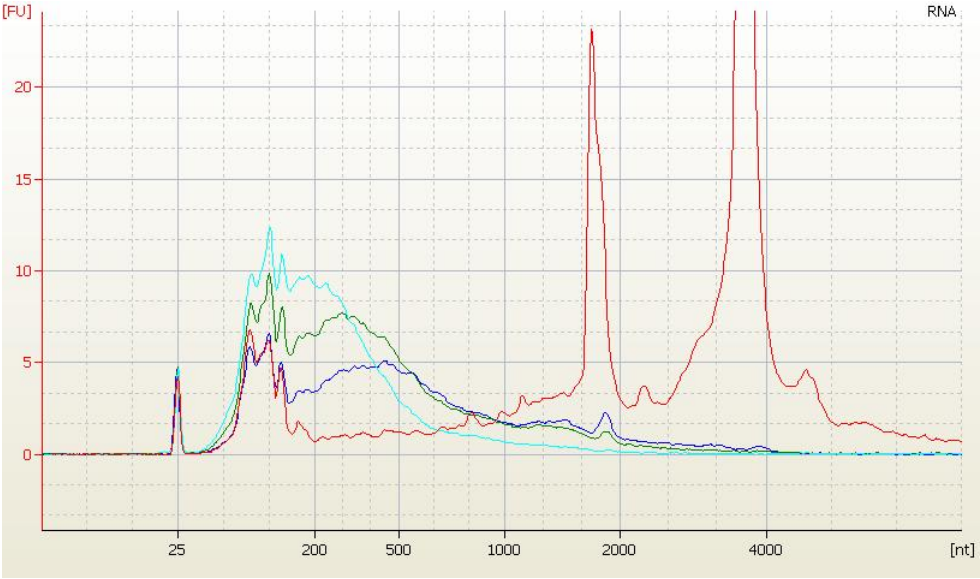

Figure 1B: 50 µl samples of total RNA were treated in the Screw-Cap microTUBE using the following conditions: DF 20%, PIP 50, cpb 200 for, 0 sec (Red trace), 60 sec (Blue trace), 180 sec (Green trace) and 420 sec (Light blue trace). RNA was analyzed using Agilent Eukaryotic Total RNA Nano kit

Figure 2 - Reproducibility of RNA fragmentation using Covaris M220 Focused-ultrasonicator

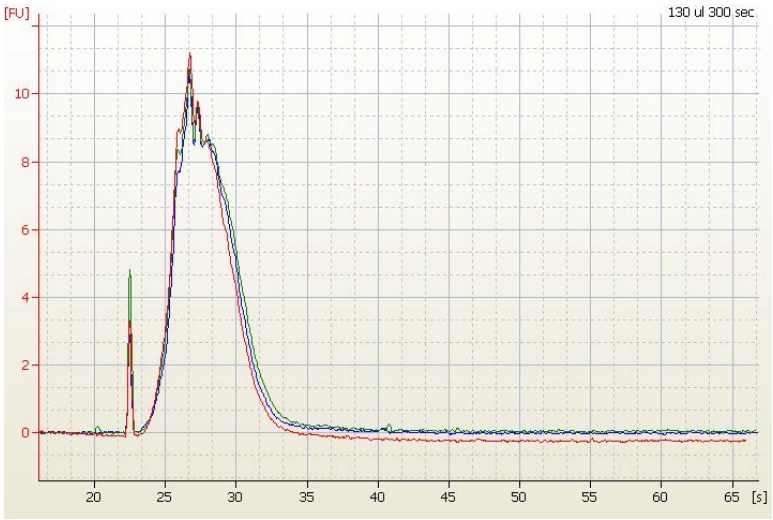

Figure 2A: In three separate experiments 130 µl RNA samples were sheared microTUBE AFA-Fiber Snap-Cap using the following AFA conditions: DF 20%, PIP 50, cpb 200 for 240 sec. Fragmented RNA was analyzed using Agilent Eukaryotic Total RNA Nano kit.

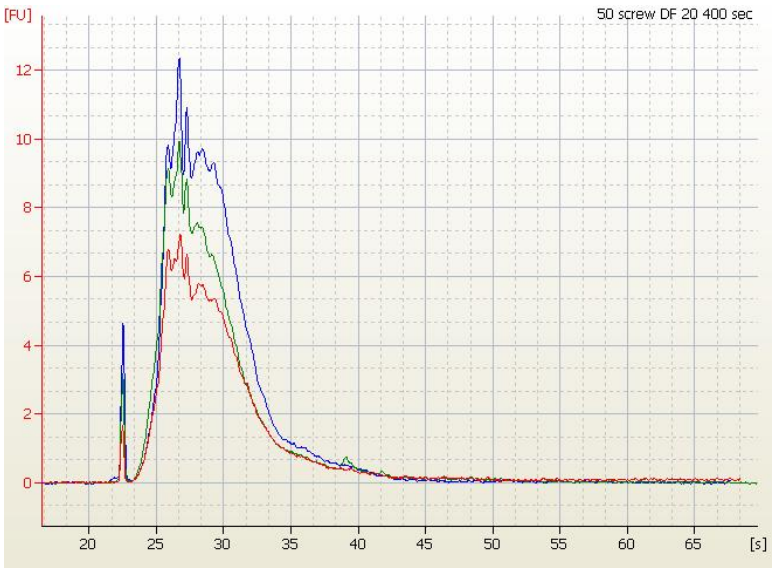

Figure 2B: In three separate experiments 50 µl RNA samples were sheared in microTUBE AFA-Fiber Screw-Cap using the following AFA conditions: DF 20%, PIP 50, cpb 200 for 420 sec. Fragmented RNA was analyzed using Agilent Eukaryotic Total RNA Nano kit.