Quick Guide:

RNA Shearing with S, E, and LE-Series Focused-ultrasonicators

This Quick Guide provides recommendations for shearing single stranded Nucleic Acids (RNA, mRNA or ssDNA) with a Covaris S-, E-, or LE-Series Focused-ultrasonicator.

Revision History

Part Number	Revision	Date	Description of change
010304	B	03/18	Format changes

Values mentioned in this Quick Guide are nominal values. The tolerances are as follow:

- Temperature +/-2°C
- Sample volume
  - microTUBE-15: from 15 to 20 µl, +/- 1 µl
  - microTUBE-50: 55 µl, +/- 2.5 µl
  - microTUBE Plate, Strip, Crimp-Cap, and Snap-Cap: 130 µl, +/- 5 µl
  - microTUBE-500: 500 µl, +/- 10 µl or 320 µl, +/- 10 µl
- Water Level +/- 1

Sample guidelines

- **RNA input:** Up to 5 µg of total RNA or ssDNA. (1 µg for the microTUBE-15).
- **Buffer:** Tris-EDTA, pH 8.0.
- **RNA quality:** High quality total RNA, mRNA, or ssDNA.

  DO NOT use the microTUBE for storage. Samples should be transferred after processing.

Instrument setup

- Refer to the instrument manual for complete setup.
- microTUBEs have specific holders or racks associated with them.
- E220 and E220evolution may require the Intensifier (PN 500141).
- E220, E220evolution, and LE220 may require X and/or Y-dithering.

Instrument settings

- Recommended settings are subject to change without notice.
- Mean RNA fragment size distributions are based on electropherograms generated from the Agilent Bioanalyzer with RNA 6000 Nano Kit (cat# 5067-1511). RNA fragment representation will vary with analytical systems, please carry out a time course based on settings provided in this document to reach desired fragment size distribution[(1)].

See http://www.covaris.com/wp-content/uploads/pn_010304.pdf for updates to this document.

RNA Shearing

Covaris recommends using settings developed for DNA Shearing as a starting point to develop a robust RNA Shearing protocol. Please refer to the instrument specific Quick Guide, and choose the appropriate consumable based on your sample volume and desired throughput.

Specifically, use the settings provided for 200 bp DNA Shearing (or alternatively 250 bp if 200 bp is not available), and perform a time course experiment to determine appropriate treatment times for your RNA sample.

Due to the variability in sample input, Covaris recommends setting up a time course experiment for determining appropriate treatment times (15% increments of time, up to +/- 60% of suggested treatment time). For total RNA, treatment time should be increased, whilst for mRNA, treatment time should be reduced. Please see Appendix A and B for example traces.

DNA Shearing with the S220 Focused-ultrasonicator - http://covaris.com/wp-content/uploads/pn_010368.pdf

DNA Shearing with the E220 Focused-ultrasonicator - http://covaris.com/wp-content/uploads/pn_010308.pdf

DNA Shearing with the LE220 Focused-ultrasonicator - http://covaris.com/wp-content/uploads/pn_010156.pdf

Example S220 settings - 130 µl sample volume

	Vessel	microTUBE AFA Fiber Snap-Cap (PN 520045) 	microTUBE AFA Fiber Crimp-Cap (PN 520052) 
	Sample Volume	**130 µl**	
S220	**Holder**	S-Series Holder microTUBE (PN 500114)	
	Water Level	12	
	Temperature (°C)	7	
	Target BP (Peak)	**200**	
	Peak Incident Power (W)	175	
	Duty Factor	10%	
	Cycles per Burst	200	
	Treatment Time (s)	~180(1)	

Technical Assistance

- By telephone (+1 781 932 3959) during the hours of 9:00am to 5:00pm, Monday through Friday, United States Eastern Standard Time (EST) or Greenwich Mean Time (GMT) minus 05:00 hours
- By e-mail at applicationsupport@covaris.com

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Appendix A - mRNA Fragmentation

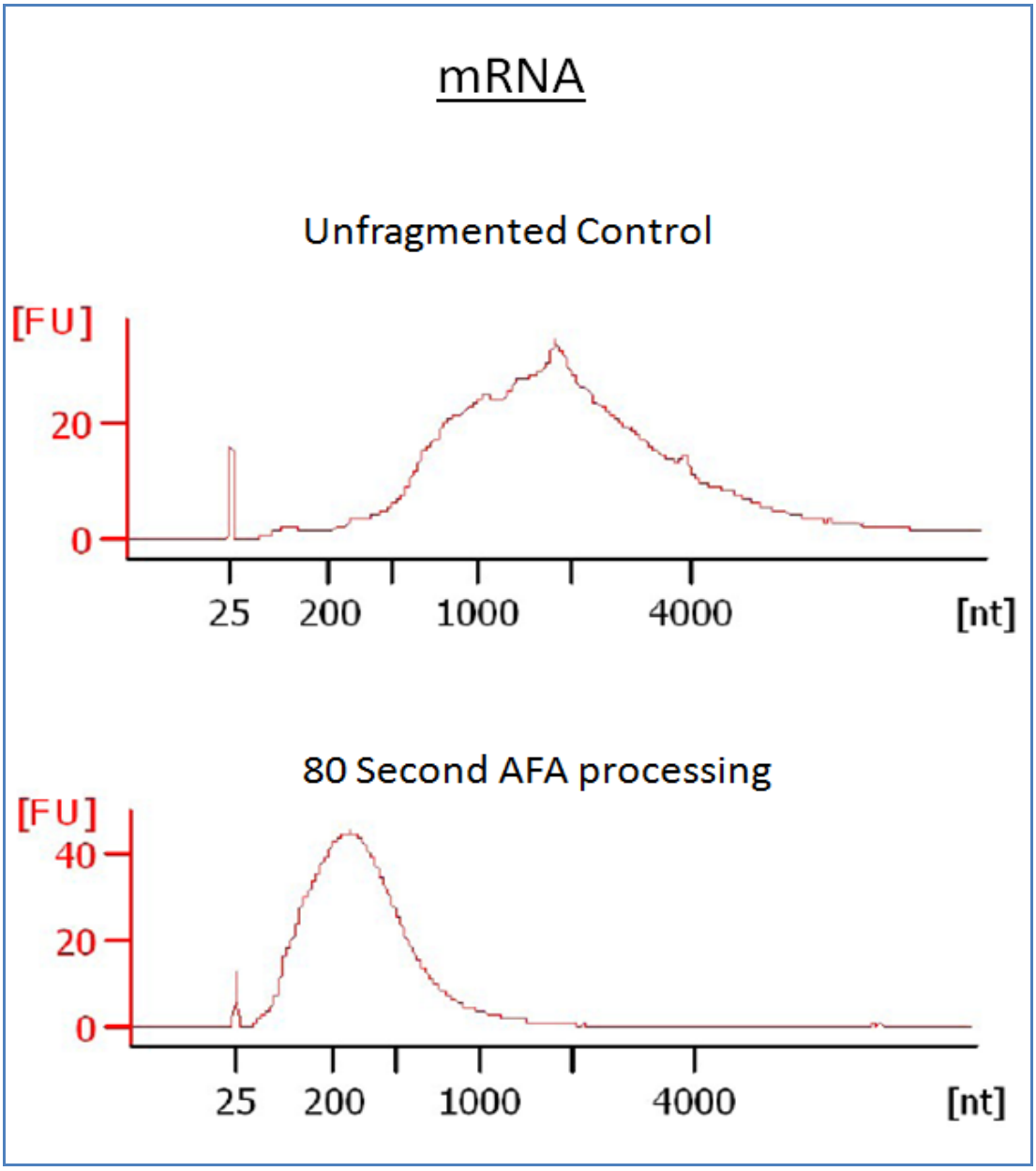

Figure 1 - Agilent BioAnalyzer electropherogram of 100ng of mRNA processed in a volume of 130ul of TE buffer in a microTUBE-130, showing the mean fragment size at 200 bases.

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Appendix B - Total RNA fragmentation

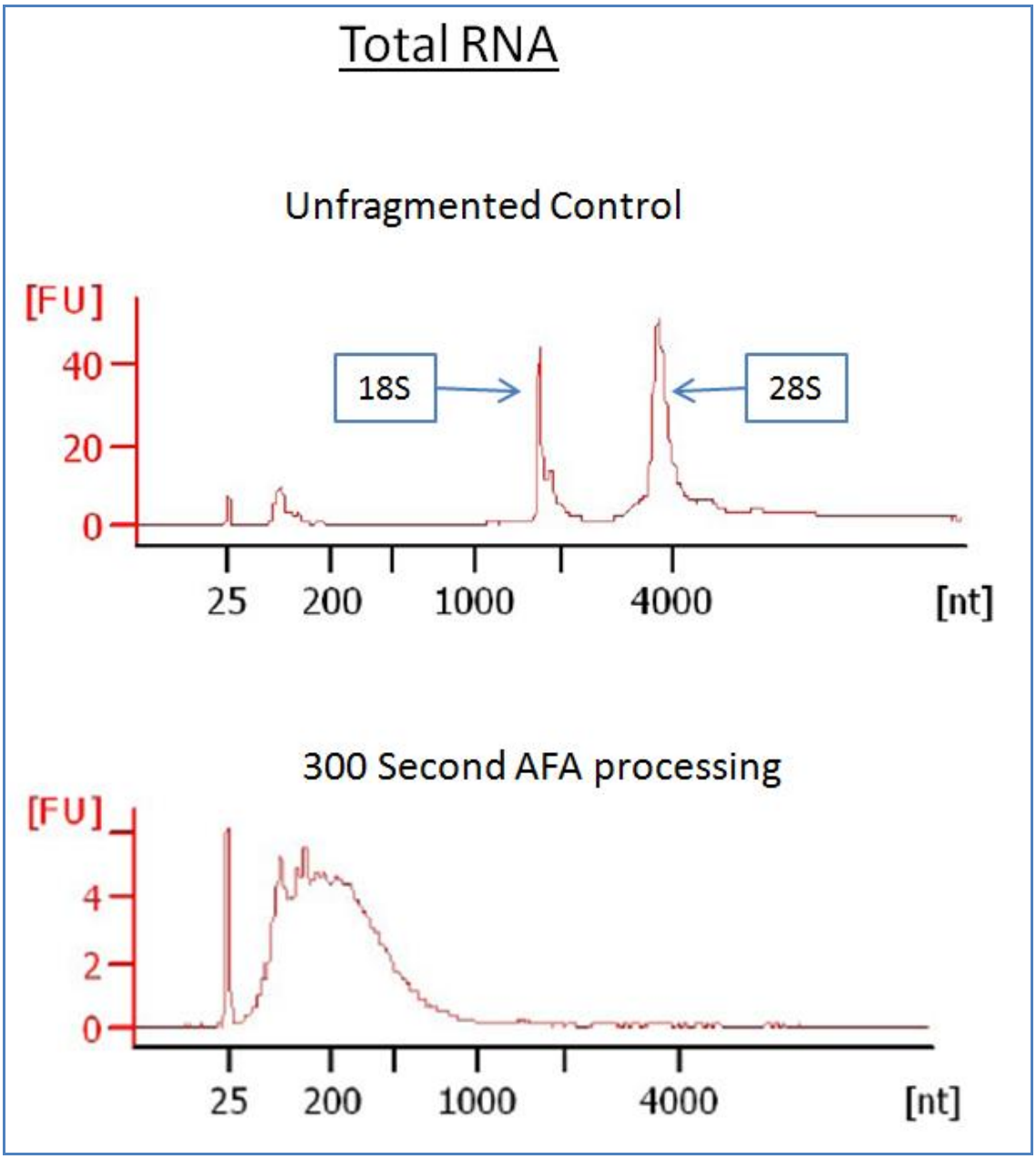

Figure 2 - Agilent BioAnalyzer electropherogram of 5µg of mRNA processed in a volume of 130ul of TE buffer in a microTUBE-130, showing the mean fragment size at 200 bases.

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