

Quick Guide:

DNA Shearing with LE220-plus Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols when using the 96 oneTUBE-10 AFA® Plate, microTUBE, microTUBE-50, microTUBE-15, microTUBE-500, or miniTUBE with a Covaris LE220-plus Focused-ultrasonicator.

Revision History

Part Number	Revision	Date	Description of change
010433	D	3/18	Addition of glass consumables (PN 520052, PN 520053, PN 520078, PN 520230, PN 520174, PN 520240, PN 520168, PN 520232, PN 520159, PN 520241, PN 520185) and miniTUBEs (PN 520064, PN 520065, PN 520066), updated oneTUBE images

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

- Temperature +/-2°C
- Sample volume
 - o 96 oneTUBE-10 AFA Plate: 10-20 µl +/- 2 µl, 50 µl +/- 2 µl
 - o microTUBE-15: from 15 to 20 µl, +/- 1 µl
 - o microTUBE-50: 55 µl, +/- 2.5 µl
 - o microTUBE Plate, Strip, Snap and Crimp Cap: 130 µl, +/- 5 µl
 - o microTUBE-500: 320 µl, +/- 10 µl
 - o miniTUBE: 200 µl, +/- 10 µl

Sample preparation guidelines

- **DNA input:** microTUBE-130 and microTUBE-50 up to 5 µg purified DNA; microTUBE-15 up to 1 µg; microTUBE-500 minimum 320 ng and up to 5 µg; oneTUBE-10 up to 100ng/µL concentration
- **Buffer:** Tris-EDTA, pH 8.0
- **DNA quality:** Genomic DNA (> 10 kb). For lower quality DNA, Covaris recommends setting up a time dose response experiment for determining appropriate treatment times.
- **DO NOT use the oneTUBE-10, microTUBE, or miniTUBE for long term sample storage. Samples should be transferred after processing.**

Instrument setup

- Refer to the instrument manual for complete setup.
- oneTUBE-10, microTUBE, and miniTUBE have specific racks associated with them.
- LE220 protocol may require dithering. Refer to Appendix A for instructions.

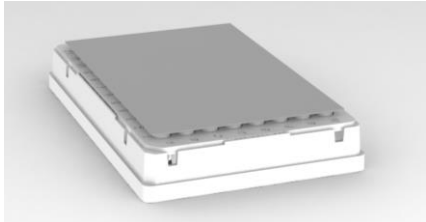
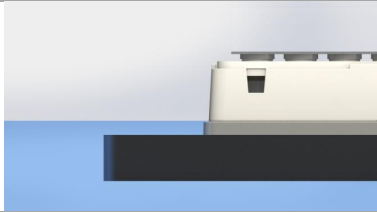
Instrument settings

- Recommended settings are subject to change without notice.
- Mean DNA fragment size distributions are based on electropherograms generated from the Agilent Bioanalyzer with the DNA 12000 Kit (cat# 5067-1509), with the exception of the 320 µl microTUBE-500 protocol (Agilent High Sensitivity DNA Kit, cat# 5067-4626) and the oneTUBE-10 (AATI Fragment Analyzer High Sensitivity NGS Fragment Kit cat# DNF-474). DNA fragment representation will vary with analytical systems.

- **Please carry out a time course experiment based on settings provided in this document to reach desired fragment size distribution.**

- See http://www.covaris.com/wp-content/uploads/pn_010433.pdf for updates to this document.

DNA Shearing with oneTUBE-10 - from 150 to 350 bp

Consumable	96 oneTUBE-10 AFA® plate PN 520249	
		
Instrument	LE220-plus	
Plate Definition	"LE220plus_520249 96 oneTUBE Plate -2.2mm offset"	
Water Level		
Rack	Rack 96 oneTUBE-10 AFA® plate PN 500588	
X and/or Y-dithering	Yes 1mm Y-dither at 20mm/s	
Temperature (°C)	20	
Sample Volume	10 µl	
Target BP (Peak)	175	350
Peak Incident Power (W)	200	200
Duty Factor	25%	25%
Cycles per Burst	50	50
Treatment Time/Duration (s)	200	40
Sample Volume	50 µl	
Target BP (Peak)	175	350
Peak Incident Power (W)	200	200
Duty Factor	25%	25%
Cycles per Burst	50	50
Treatment Time/Duration (s)	390	85



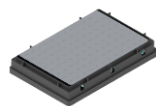


The Y-dithering function is required for shearing with the 96 oneTUBE-10 AFA® plate. Please see Appendix A for detailed instructions.


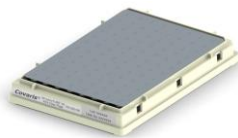


To ensure reproducible DNA shearing, it is recommended to centrifuge samples before processing DNA in a 96 oneTUBE-10 AFA® plate. Please see Appendix B for detailed sample loading and centrifugation instructions.

130 µl sample volume - from 150 to 1,500 bp

	Vessel	microTUBE AFA Fiber Crimp-Cap (PN 520052) 	8 microTUBE Strip V1 (PN 520053) 	96 microTUBE Plate (PN 520078) 96 microTUBE AFA Fiber Plate Thin Foil (PN 520230) 				
	Sample Volume	130 µl						
LE220	Racks	Rack 96 Place microTUBE Crimp-Cap (PN 500282)	Rack 12 Place 8 microTUBE Strip (PN 500191)	No Rack needed				
	Plate Definitions	"LE220plus_500282 Rack 96 Place microTUBE -4mm offset"	"LE220plus_500191 Rack 12 Place 8 microTUBE Strip - 4mm offset"	"LE220plus_520078 96 microTUBE Plate -4mm offset" "LE220plus_520230 96 microTUBE Plate Thin Foil -4mm offset"				
	X and/or Y-dithering	No						
	Temperature (°C)	7						
All	Target BP (Peak)	150	200	300	400	500	900	1,500
	Peak Incident Power (W)	450	450	450	450	450	450	450
	Duty Factor	30%	30%	30%	15%	15%	5%	5%
	Cycles per Burst	200	200	200	200	200	200	200
Crimp-Cap and 8-Strip	Treatment Time (s)	420	175	60	63	46	77	17
Plate	Treatment Time (s)	490	190	80	100	75	118	20

55 µl sample volume - from 150 to 500 bp


Vessel	8 microTUBE-50 AFA Fiber Strip V2 (PN 520174) 8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240) 	96 microTUBE-50 AFA Fiber Plate (PN 520168) 96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232) 
	Sample Volume	55 µl

LE220	Racks	Rack – XT 12 Place 8 microTUBE Strip V2 (PN 500485)	No Rack needed					
	Plate Definitions	“LE220plus_500485 Rack-XT 12 Place 8 microTUBE-50 Strip V2 - 12mm offset”	“LE220plus_520168 96 microTUBE-50 Plate -12mm offset” “LE220plus_520232 96 microTUBE-50 Plate Thin Foil -12mm offset”					
	X and/or Y-dithering	Yes 0.5mm X-dither & 0.5mm Y-dither at 10mm/sec						
	Temperature (°C)	7						
All	Target BP (Peak)	150	200	250	300	350	400	500
	Peak Incident Power (W)	450	450	450	450	450	450	450
	Duty Factor	20%	20%	15%	15%	10%	10%	10%
	Cycles per Burst	1000	1000	1000	1000	1000	1000	1000
8-Strip	Treatment Time (s)	360	160	120	79	87	74	56
Plate	Treatment Time (s)	500	200	150	100	120	90	68



The X-dithering and Y-dithering functions are both required for shearing with the 8 microTUBE-50 AFA Fiber Strip V2 and the 96 microTUBE-50 AFA Fiber Plate. Please see Appendix A for detailed instructions.

15 µl sample volume - from 150 to 550 bp

	Vessel	8 microTUBE-15 AFA Beads Strip V2 (PN 520159) 8 microTUBE-15 AFA Beads H Slit Strip V2 (PN 520241) 				
	Sample Volume	15 µl				
LE220	Rack	Rack-LV 12 Place 8 microTUBE Strip V2 (PN 500445)				
	Plate Definition	"LE220plus_500445 Rack-LV 12 Place 8 microTUBE-15 Strip V2 - 4mm offset"				
	X and/or Y-dithering	Yes 5mm Y-dither at 20mm/s				
	Temperature (°C)	20				
	Target BP (Peak)	150	200	250	350	550
	Peak Incident Power (W)	180	180	180	180	180
	Duty Factor	30%	30%	20%	15%	15%
Cycles per Burst	50	50	50	50	50	
Treatment Time (s)	250	120	105	75	40	


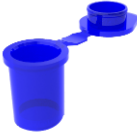



The Y-dithering function is required for shearing with 15 µl samples. Please see Appendix A for detailed instructions.




To ensure reproducible DNA shearing, it is required to centrifuge samples before processing DNA in a microTUBE-15. Please see Appendix C for instructions.

200 µl sample - 2,000; 3,000 and 5,000 bp

	Vessel	miniTUBE		
		Clear (PN 520064) 	Blue (PN 520065) 	Red (PN 520066) 
	Sample Volume	200 µl		
LE220	Rack	Rack 24 Place miniTUBE (PN 500205)		
	Plate Definition	"LE220plus_500205 24 miniTUBE +15mm offset"		
	Water Level	11		
	X and/or Y-dithering	No		
	Temperature (°C)	7	20	20
	Target BP (Peak)	2,000	3,000	5,000
	miniTUBE	Clear	Blue	Red
	Peak Incident Power (W)	50	35	100
Duty Factor	20%	20%	20%	
Cycles per Burst	1000	1000	1000	
Treatment Time (s)	900	600	600	

To fragment DNA to sizes larger than 5 kb, Covaris offers the g-TUBE: a single-use device that shears genomic DNA into selected fragments sizes ranging from 6 kb to 20 kb. The only equipment needed is a compatible bench-top centrifuge.

320 µl sample volume – average fragment size 500 to 600 bp

	Vessel	microTUBE-500 AFA Fiber Screw-Cap (PN 520185) 
	Sample Volume	320 µl
LE220	Rack	Rack, 24 microTUBE-500 Screw-Cap (PN 500452)
	Plate Definition	“LE220plus_500452 Rack 24 Place microTUBE-500 Screw-Cap +6mm offset”
	Water Level	6
	X and/or Y-dithering	No
	Temperature (°C)	7
	Target BP (Peak)	500 - 600
	Peak Incident Power (W)	450
	Duty Factor	30%
	Cycles per Burst	200
Treatment Time (s)	65	

Additional Accessories


	Product Description	Part Number
Preparation Stations	microTUBE Prep Station Snap & Screw Cap	500330
	miniTUBE loading and unloading station	500207
	microTUBE-500 Screw-Cap Prep Station	500510
	8 microTUBE Strip Prep Station	500327
Centrifuge and Heat Block microTUBE Screw-Cap Adapter	Fits microTUBE Screw-Caps into bench top microcentrifuges	500406
Centrifuge 8 microTUBE Strip V2 Adapter	Fits the 8 microTUBE Strip into a Thermo Scientific™ mySPIN™ 12 mini centrifuge	500541
g-TUBE	g-TUBEs (10) and prep station	520079
Thin foil seal	96 microTUBE Plate Thin Foil Seals (25); also compatible with 96 oneTUBE-10 AFA Plate	520235

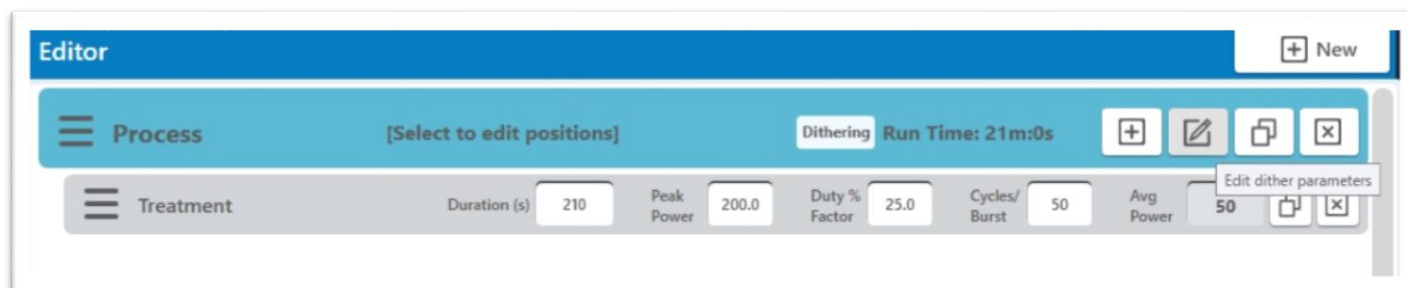
Appendix A – Using Dithering with SonoLab 8

Dithering is required for DNA shearing with the following consumables:

8 microTUBE-50 AFA Fiber Strip V2, 96 microTUBE-50 AFA Fiber Plate, 8 microTUBE-15 AFA Beads Strip V2, and 96 oneTUBE-10 AFA® plate.

Use the following steps to include dithering in sample treatment:

1. Go into the Method Editor
2. Select '[+] New' to add process and enter the treatment settings for the desired fragment size
 - a. **Note:** The following steps must be done for each individual process in a method
3. Select the Edit dither parameters icon 



4. Enter the consumable specific values (see table below) into the 'Dither Parameters' box for X Dither, Y Dither, Z Dither, Speed, and Pause Duration

Consumable	X Dither (mm)	Y Dither (mm)	Z Dither (mm)	Speed (mm/sec)	Pause Duration (s)
8 microTUBE-50 AFA Fiber Strip V2 (PN 520174 and 520240)	0.5	0.5	0	10	0
96 microTUBE-50 AFA Fiber Plate (PN 520168 and 520232)	0.5	0.5	0	10	0
8 microTUBE-15 AFA Beads Strip V2 (PN 520159 and 520241)	0	5.0	0	20	0
96 oneTUBE-10 AFA® plate (PN 520249)	0	1.0	0	20	0

Appendix B –96 oneTUBE-10 AFA® Plate Sample loading and centrifugation

Recommended Sequence for Use:

1. Fill the tubes:

Aspirate sample and dispense into the 96 oneTUBE-10 AFA® Plate. Dispense the samples about 2-3 mm above the bottom of the tube or at the bottom depending on sample volume, being careful to dispense all the sample into the bottom of the tube.

2. Seal the plate for AFA-processing:

Remove the backing from the thin foil seal and carefully align it over the plate. Using a sealing paddle or a roller (or your fingers), thoroughly press the seal on the tubes verifying that the seal is adhered to the top of each tube. The plate is now ready to be processed in your Covaris instrument.

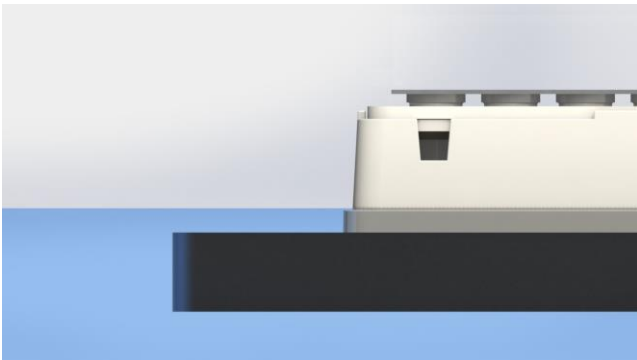
3. Centrifugation:

We recommend centrifuging the plate after sample addition to ensure the sample is at the bottom of the tube. Centrifuge the plate at up to 7,500 RPM in a benchtop centrifuge compatible with 96 well plates for ≤ 10 seconds. **DO NOT STACK PLATES IN CENTRIFUGE.** Visually inspect plate to verify that all liquid is at the bottom of each tube before proceeding to Step 4.

4. AFA-processing:

The plate must be in the Rack 96 oneTUBE-10 AFA® plate (PN 500588) for processing.

Check that the water is filled to the top of the edge of the bottom flange as depicted below:



5. Centrifugation:

It is recommended to centrifuge the plate after AFA® (up to 7,500g (RCF) for ≤ 10 seconds) before removing any sample. **DO NOT STACK PLATES IN CENTRIFUGE.**

6. Downstream sample handling:

After AFA® treatment, the samples are ready for downstream processing. The thin foil seal can be removed for processing in the 96 oneTUBE-10 AFA® Plate. Do not use the plate for long term storage of the samples.

Appendix C – microTUBE-15 centrifugation before DNA shearing

1. Sample loading and centrifugation

8 microTUBE-15 AFA Beads Strip V2

The 8 microTUBE-15 AFA Beads Strip V2 will fit into the Covaris Centrifuge 8 microTUBE Strip V2 Adapter (PN 500541) for the Thermo Scientific™ mySPIN™ 12 mini centrifuge. Place the strip in the adapter and spin for a minimum of 1 minute.

2. Sample processing

Use settings provided on page 5.

3. Sample recovery

Repeat the centrifuge step before recovering sample from microTUBE-15.

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