

Quick Guide:

DNA Shearing with LE220 Focused-ultrasonicator

This Quick Guide provides DNA Shearing protocols when using microTUBE, microTUBE-50, microTUBE-15, microTUBE-500, or miniTUBE and a Covaris LE220 Focused-ultrasonicator.

Revision History

Part Number	Revision	Date	Description of change
010156	N	2/16	Updated protocols for microTUBE-50
010156	O	1/17	Update template; addition of microTUBE-500 AFA Fiber Screw-Cap protocol; update additional accessories; update Appendix C
010156	P	3/17	Addition of 8 microTUBE-15 AFA Beads H Slit Strip V2 and 8 microTUBE-50 AFA Fiber H Slit Strip V2

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

- Temperature +/-2°C
- Sample volume
 - 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
- Water Level +/- 1

Sample preparation guidelines

- **DNA input:** up to 5 µg purified DNA (1 µg for the microTUBE-15; minimum 320 ng for the microTUBE-500)
- **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 microTUBE or miniTUBE for storage. Samples should be transferred after processing.**

Instrument setup




- Refer to the instrument manual for complete setup.
- microTUBE and miniTUBE have specific racks associated with them.
- LE220 protocol may require X and/or Y-dithering. Refer to Appendices A and B 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). 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.covarisinc.com/wp-content/uploads/pn_010156.pdf for updates to this document.

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) 				
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	"LE220_500282 Rack 96 Place microTUBE -4mm offset"	"LE220_500191 Rack 8 microTUBE Strip -4mm offset"	"LE220_520078 96 microTUBE Plate -4mm offset"				
	Water Level	6						
	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) 					
	Sample Volume	55 µl						
LE220	Racks	Rack – XT 12 Place 8 microTUBE Strip V2 (PN 500485)	No Rack needed					
	Plate Definitions	“LE220_500485 Rack-XT 12 Place 8 microTUBE-50 Strip V2 -12mm offset”	“LE220_520168 96 microTUBE-50 Plate -12mm offset”					
	Water Level	-2						
	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. These functions are only available on SonoLab versions 7.3 and up. 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	"LE220_500445 Rack-LV 12 Place 8 microTUBE-15 Strip V2 -4mm offset"				
	Water Level	4				
	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. This function is only available on SonoLab versions 7.3 and up. Please see Appendix B 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	"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	“LE220_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

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 techsupport@covarisinc.com

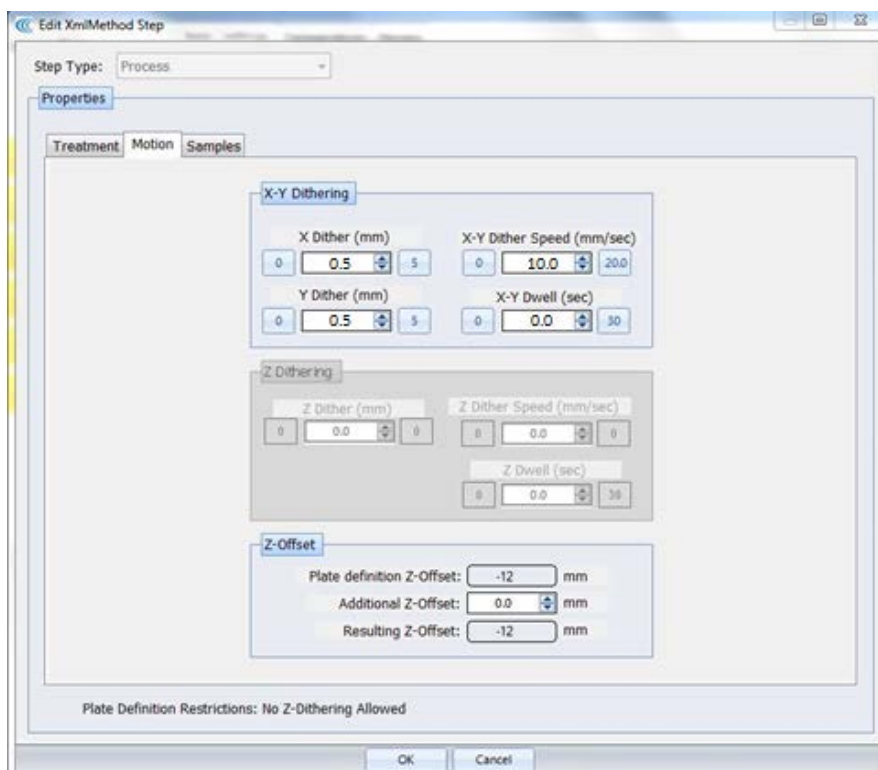
Appendix A – Using X and Y-dithering with SonoLab 7.3 and up

X and Y-dithering are required for DNA shearing with the 8 microTUBE-50 AFA Fiber Strip V2 and 96 microTUBE-50 AFA Fiber Plate

- This feature is only available on SonoLab versions 7.3 and up.
- There are dithering limitations on instruments with serial numbers below 2000.
- To obtain a copy of the SonoLab 7.3 and the Plate Definition installers, please employ the Registered Users Login on the Covaris website, www.covarisinc.com
- For any assistance in this process, please contact your local representative, or Covaris Global Technical Services at TechSupport@covarisinc.com.

Use the following steps to include X-dithering and Y-dithering in sample treatment:

1. Go into the Method Editor
2. Select 'Add Step' and enter the treatment settings for the desired fragment size
 - a. **Note:** The following steps must be done for each individual treatment
3. Select the Motion tab
4. Enter the following values into the 'X-Y Dithering' box
 - a. X Dither (mm): **0.5**
 - b. Y Dither (mm): **0.5**
 - c. X-Y Dither Speed (mm/sec): **10.0**
 - d. X-Y Dwell (sec) should be set to **0**



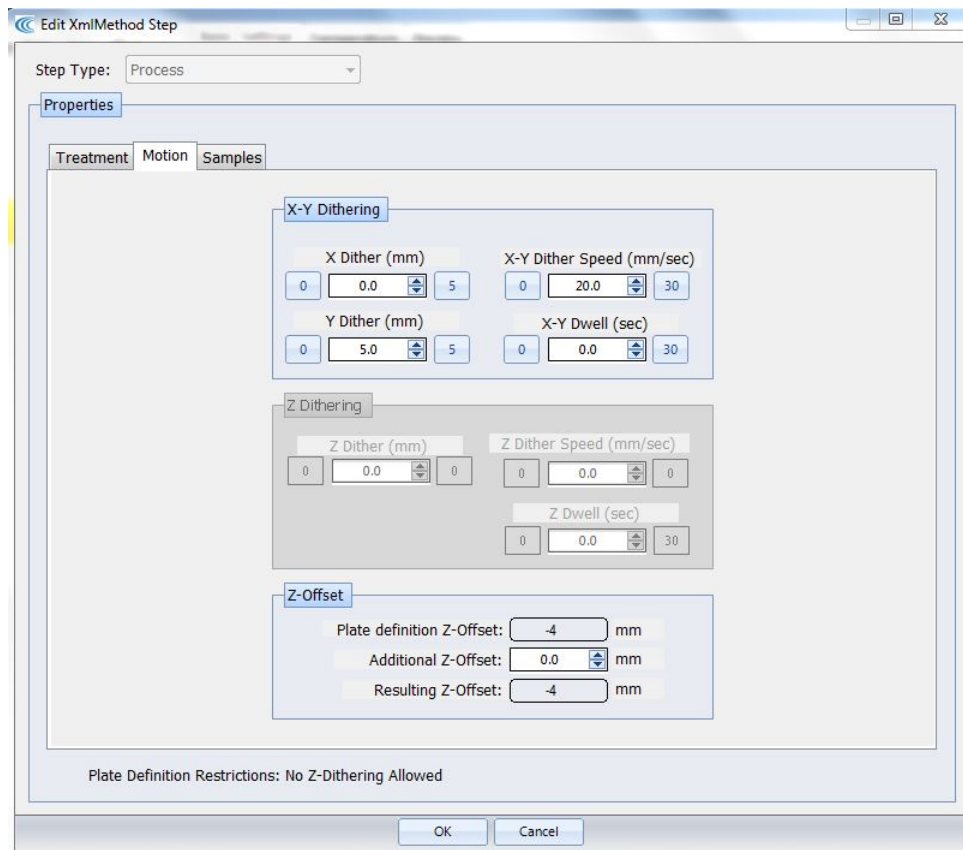
Appendix B – Using Y-dithering with SonoLab 7.3 and up

Y-dithering is required for DNA shearing with the microTUBE-15

- This feature is only available on SonoLab versions 7.3 and up.
- To obtain a copy of the SonoLab 7.3 and the Plate Definition installers, please employ the Registered Users Login on the Covaris website, www.covarisinc.com
- For any assistance in this process, please contact your local representative, or Covaris Global Technical Services at TechSupport@covarisinc.com.

Use the following steps to include Y-dithering in sample treatment:

1. Go into the Method Editor
2. Select 'Add Step' and enter the treatment settings for the desired fragment size
 - a. **Note:** The following steps must be done for each individual treatment
3. Select the Motion tab
4. Enter the following values into the 'X-Y Dithering' box
 - a. Y Dither (mm): **5.0**
 - b. X-Y Dither Speed (mm/sec): **20.0**
 - c. Both X Dither (mm) and X-Y Dwell (sec) should be set to **0**



Appendix C – microTUBE-15 centrifugation before DNA shearing

1. Sample loading and centrifugation

microTUBE-15 AFA Beads Screw-Cap

Load and centrifuge microTUBE-15 Screw-Cap as described before placing the tubes in the rack.



Carefully load sample through the septa making contact with the glass wall of the microTUBE



Load microTUBE-15 into the centrifuge using microTUBE Adapter (PN 500406)



Balance centrifuge. Spin at 3000x g (RCF) for 30 seconds

If some of the sample splashes onto the wall of the microTUBE while removing from centrifuge or placing into rack, repeat centrifuge step. All liquid should be at the bottom of the microTUBE-15 before starting the AFA treatment.

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 4.

3. Sample recovery

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



Place microTUBE-15 in Preparation Station and unscrew the cap



Retrieve the sample with a narrow bore 20 µL pipet tip. It may be necessary to push the beads aside for full recovery