Quick Guide

DNA Shearing with E220 Focused-ultrasonicator

This Quick Guide provides DNA Shearing Protocols when using microTUBE-130, microTUBE-50, microTUBE-15, microTUBE-500, or miniTUBE and a Covaris E220 Focused-ultrasonicator.

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

- **Temperature:** +/- 2 °C
- **Sample Volume:**
  - microTUBE-15: from 15 to 20 µl, +/- 1 µl
  - microTUBE-50: 55 µl, +/- 2.5 µl
  - microTUBE Plate, Strip, Snap, and Crimp Cap: 130 µl, +/- 5 µl
  - microTUBE-500: 500 µl, +/- 10 µl or 320 µl, +/- 10 µl
  - miniTUBE: 200 µl, +/- 10 µl
- **Water Level:** +/- 1

Sample Guidelines

- **DNA Input:** up to 5 µg purified DNA (1 µg for the microTUBE-15; minimum 320 ng for the microTUBE-500)
- **Buffers:** 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

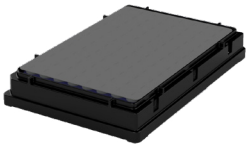
- Refer to the instrument user manual for complete setup.
- microTUBE and miniTUBE have specific holders or racks associated with them.
- E220 and E220 evolution may require the Intensifier (PN 500141). Refer to ***Appendix C*** for instructions.
- E220 and E220 evolution may require Y-dithering. Refer 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 (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.

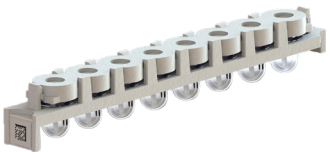
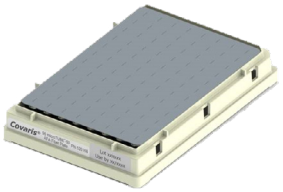
See http://www.covaris.com/wp-content/uploads/pn_010308.pdf for updates to this document.

130 µl Sample Volume: 150 to 1,500 bp

	Vessel	 microTUBE AFA Fiber Snap-Cap (PN 520045)	 microTUBE AFA Fiber Crimp-Cap (PN 520052)	 8 microTUBE Strip (PN 520053)	 96 microTUBE Plate (PN 520078) 96 microTUBE AFA Fiber Plate Thin Foil (PN 520230)
	Sample Volume	130 µl			
E220	Rack	Rack 24 Place microTUBE Snap-Cap (PN 500111)	Rack 96 Place microTUBE Crimp-Cap (PN 500282)	Rack 12 Place 8 microTUBE Strip (PN 500191)	No Rack Needed
E220	Plate Definition	"500111 24 microTUBE snap +4mm offset"	"E220_500282 Rack 96 Place microTUBE -6mm offset"	"E220_500191 8 microTUBE strip Plate -6mm offset"	"E220_520078 96 microTUBE Plate -6mm offset" "E220_520230 96 microTUBE Plate Thin Foil -6mm offset"
E220	Water Level	6			
E220	Intensifier (PN 500141)	Yes			
E220	Y-dithering	No			
E220*evolution*	Rack	Rack E220e 8 Place microTUBE Crimp and Snap Cap (PN 500433)		Rack E220e 8 microTUBE Strip (PN 500430)	Non Compatible
E220*evolution*	Plate Definition	"500433 E220e 8 microTUBE Crimp and Snap Cap -3.7mm offset"		"500430 E220e 8 microTUBE Strip -6mm offset"	N/A
E220*evolution*	Water Level	6			
E220*evolution*	Intensifier (PN 500141)	Yes			
E220*evolution*	Y-dithering	No			

All	Temperature (°C)	7							
All	Target BP (Peak)	150	200	300	400	500	800	1,000	1,500
All	Peak Incident Power (W)	175	175	140	140	105	105	105	140
All	Duty Factor (%)	10	10	10	10	5	5	5	2
All	Cycles per Burst	200	200	200	200	200	200	200	200
All	Treatment Time (s)	430	180	80	55	80	50	40	15

55 µl Sample Volume: 150 to 550 bp

	Vessel	 microTUBE-50 Screw-Cap (PN 520166)		 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						
E220	Rack	Rack 24 Place microTUBE Screw-Cap (PN 500308)		Rack 12 Place 8 microTUBE Strip (PN 500444)			No Rack needed	
E220	Plate Definition	"E220_500308 Rack 24 Place microTUBE-50 Screw-Cap +6.5mm offset"		"E220_500444 Rack 12 Place 8 microTUBE-50 Strip V2-10mm offset"			"E220_520168 96 microTUBE-50 Plate -10.5mm offset" "E220_520232 96 microTUBE-50 Plate Thin Foil -10.5mm offset"	
E220*evolution*	Rack	Rack E220e 4 Place microTUBE Screw Cap (PN 500432)		Rack E220e 8 microTUBE Strip V2 (PN 500437)			Non Compatible	
E220*evolution*	Plate Definition	"500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset"		"500437 E220e 8 microTUBE-50 Strip V2 -10mm offset"			N/A	
All	Temperature (°C)	7						
All	Water Level	6		-2			0	
All	Intensifier (PN 500141)	Yes		Yes			Yes	
All	Y-dithering	No		No			Yes (0.5mm Y-dither at 10mm/s)	
All	Target BP (Peak)	150	200	250	300	350	400	550
Screw-Cap	Peak Incident Power (W)	75	75	75	75	75	75	30
Screw-Cap	Duty Factor (%)	15	15	20	20	20	10	10
Screw-Cap	Cycles per Burst	1000	1000	1000	1000	1000	1000	1000
Screw-Cap	Treatment Time (s)	340	145	62	40	30	50	70
8-Strip	Peak Incident Power (W)	75	75	75	75	75	75	50
8-Strip	Duty Factor (%)	15	15	20	20	20	10	10
8-Strip	Cycles per Burst	500	500	1000	1000	1000	1000	1000
8-Strip	Treatment Time (s)	360	155	75	45	35	52	50
Plate	Peak Incident Power (W)	75	75	75	75	75	75	75
Plate	Duty Factor (%)	15	15	20	20	20	10	10
Plate	Cycles per Burst	1000	1000	1000	1000	1000	1000	1000
Plate	Treatment Time (s)	360	155	70	49	34	50	32

The Y-dithering function is required for shearing with 96 microTUBE-50 plate (PN 520168). This function is only available on SonoLab versions 7.3 and up. Please see ***Appendix A*** for detailed instructions.

15 µl Sample Volume: 150 to 550 bp

	Vessel	 microTUBE-15 AFA Beads Screw-Cap (PN 520145)	 8 microTUBE-15 AFA Beads Strip V2 (PN 520159) 8 microTUBE-15 AFA Beads H Slit Strip V2 (PN 520241)
	Sample Volume	15 µl	
E220	Rack	Rack 24 Place microTUBE Screw-Cap (PN 500308)	Rack 12 Place 8 microTUBE Strip V2 (PN 500444)
E220	Plate Definition	"E220_500308 Rack 24 Place microTUBE-15 Screw-Cap +15mm offset"	"E220_500444 Rack 12 Place 8 microTUBE-15 Strip V2 -1.5mm offset"
E220	Water Level	10	6
E220	Intensifier (PN 500141)	No	
E220	Y-dithering	No	
E220*evolution*	Rack	Rack E220e 4 Place microTUBE Screw Cap (PN 500432)	Rack E220e 8 microTUBE Strip V2 (PN 500437)
E220*evolution*	Plate Definition	"500432 E220e 4 microTUBE-15 Screw Cap 0.18mm offset"	"500437 E220e 8 microTUBE-15 Strip V2 -1.58mm offset"
E220*evolution*	Water Level	10	6
E220*evolution*	Intensifier (PN 500141)	No	
E220*evolution*	Y-dithering	No	

All	Temperature (°C)	20				
All	Target BP (Peak)	150	200	250	350	550
All	Peak Incident Power (W)	18	18	18	18	18
All	Duty Factor (%)	20	20	20	20	20
All	Cycles per Burst	50	50	50	50	50
All	Treatment Time (s)	300	120	80	45	22

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

Please note that microTUBE-15 requires removal of the Intensifier (PN 500141) from the E220 Focused-ultrasonicator. Please see ***Appendix C*** for instructions.

200 µl Sample Volume: 2,000, 3,000, and 5,000 bp

	Vessel miniTUBE	 Clear (PN 520064)	 Blue (PN 520065)	 Red (PN 520066)
E220	Sample Volume	200 µl		
	Rack	Rack 24 Place miniTUBE (PN 500205)		
	Plate Definition	"500205 24 miniTUBE +15mm offset"		
	Water Level	11		
	Intensifier (PN 500141)	No		
	Y-dithering	No		
E220*evolution*	Rack	Rack E220e 4 Place miniTUBE (PN 500434)		
	Plate Definition	"500434 E220e 4 miniTUBE 4.9mm offset"		
	Water Level	11		
	Intensifier (PN 500141)	No		
	Y-dithering	No		
All	Temperature (°C)	7	20	20
	Target BP (Peak)	2,000	3,000	5,000
	Peak Incident Power (W)	3	3	25
	Duty Factor (%)	20	20	20
	Cycles per Burst	1000	1000	1000
	Treatment Time (s)	900	600	600

Please note that miniTUBE requires removal of the Intensifier (PN 500141) from the E220 Focused-ultrasonicator. Please see ***Appendix C*** for instructions.

320 µl and 500 µl Sample Volumes: 150 to 600 bp

	Vessel	 microTUBE-500 AFA Fiber Screw-Cap (PN 520185)	
	Sample Volume	320 µl	500 µl
E220	Rack	Rack, 24 microTUBE-500 Screw-Cap (PN 500452)	
E220	Plate Definition	"E220_500452 Rack 24 Place microTUBE-500 Screw-Cap +6mm offset"	
E220	Water Level	6	
E220	Intensifier (PN 500141)	Yes	
E220	Y-dithering	No	

E220*evolution*	Rack	Rack E220e 4 microTUBE-500 Screw-Cap (PN 500484)
E220*evolution*	Plate Definition	"500484 E220e 4 microTUBE-500 Screw-Cap -9.9mm offset"
E220*evolution*	Water Level	6
E220*evolution*	Intensifier (PN 500141)	Yes
E220*evolution*	Y-dithering	No

All	Temperature (°C)	7				
All	Target BP (Peak)	500 to 600	150	200	350	550
All	Peak Incident Power (W)	75	175	175	175	175
All	Duty Factor (%)	25%	20%	20%	20%	5%
All	Cycles per Burst	200	200	200	200	200
All	Treatment Time (s)	75	400	180	55	110

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 to 20 kb. The only equipment needed is a compatible bench-top centrifuge.

Additional Accessories

	Product Description	Part Number
Preparation Stations	microTUBE Prep Station Snap & Screw Cap	500330
Preparation Stations	microTUBE-500 Screw-Cap Prep Station	500510
Preparation Stations	miniTUBE loading and unloading station	500207
Preparation Stations	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 ScientificTM mySPINTM 12 mini centrifuge	500541
g-TUBE	g-TUBEs (10) and prep station	520079

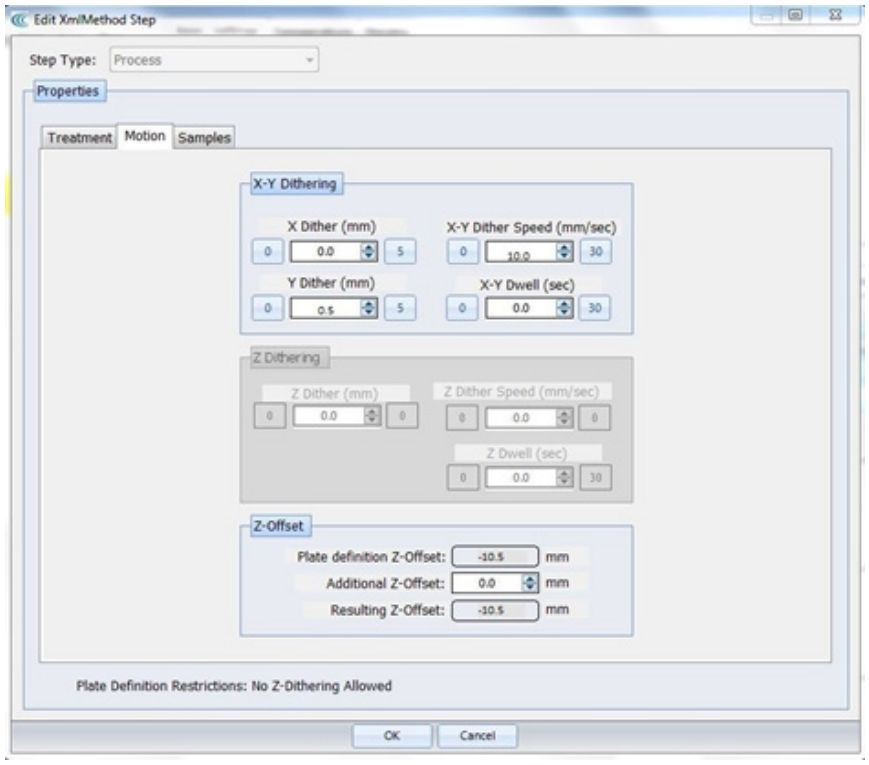
Appendix A: Using Y-dithering with SonoLab 7.3 and up

A Y-dithering step is required for DNA shearing with the 96 microTUBE-50 Plate.

- 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.covaris.com
- For any assistance in this process, please contact your local representative, or Covaris Global Technical Services at TechSupport@covaris.com.

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

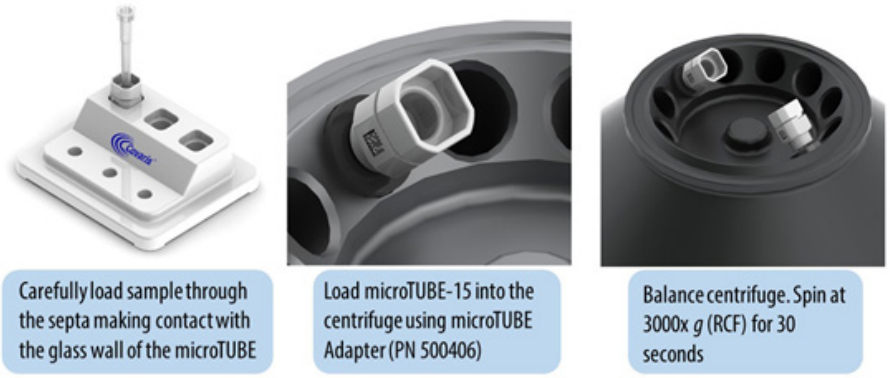
1. Go into the Method Editor
2. Select 'Add Step' and enter the treatment settings for the desired fragment size
    a. Note: The following steps must be done for each individual treatment
3. Select the Motion tab
4. Enter the following values into the 'X-Y Dithering Box'
    a. Y Dither (mm): 0.5
 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



Appendix B: microTUBE-15 Centrifugation before DNA Shearing

1. **Sample loading and centrifugation**

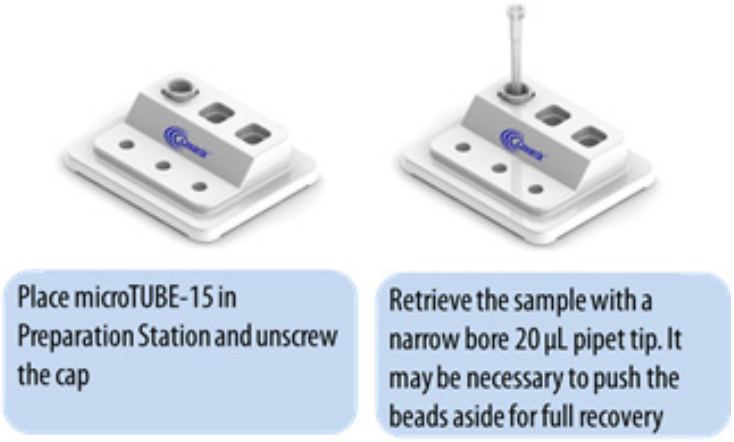
   microTUBE-15 AFA Beads Screw-Cap: Load and centrifuge microTUBE-15 Screw-Cap as described before placing the tubes in the rack.



   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™ mySPINTM 12 mini centrifuge. Place the strip in the adapter and spin for a minimum of 1 minute.

2. **Sample processing**: Use settings provided in Page 4.
3. **Sample recovery**: Repeat the centrifuge step before recovering sample from microTUBE-15.



Appendix C: Removing or Installing the Intensifier (Covaris PN 500141) from an E System

The 500141 Intensifier is a small inverted stainless steel cone centered over the E-Series transducer by four stainless wires. The wires are held by in a black plastic ring pressed into the transducer well.

If an AFA protocol requires "no intensifier", please remove the Intensifier, using the following steps:

1. Empty the water bath. Start the instrument and start the SonoLab software.
2. Wait for the homing sequence to complete (the transducer will be lowered with the rack holder at it home position, allowing easy access to the Intensifier).
3. Grasp opposite sides of plastic ring and gently pull the entire assembly out of the transducer well. Do not pull on the steel cone or the wires. The ring is a friction fit in the well – no hardware is used to hold it in place.

The 500141 Intensifier (left) shown installed in the E-Series transducer well and (right) removed. Note the "***UP***" marking at the center of the Intensifier.

If a protocol requires the Intensifier to be present, simply reverse this process:

1. Align the black plastic ring with the perimeter of the transducer well. Note that the flat side of the center cone (marked UP) should be facing up (away from the transducer).
2. Gently press each section of the ring into the well until the ring is seated uniformly in contact with the transducer, with approximately 2 mm of the ring evenly exposed above the transducer assembly. Do not press on the cone or wires. The rotation of the ring relative to the transducer assembly is not important.
3. Refill the tank. Degas and chill the water before proceeding.

Technical Assistance

- By telephone (+1 781.932.3959) during the hours of 9:00 a.m. to 5:00 p.m., 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

Revision History

Part Number	Revision	Date	Description of Change
010308	K	1/2017	Format Changes; Addition of microTUBE-500 AFA Fiber Screw-Cap protocols; update 'Additional Accessories'; update Appendix B.
010308	L	2/2017	Changes to 8 microTUBE-50 Strip V2 protocols; addition of 8 microTUBE-15 AFA Beads H Slit Strip V2 and 8 microTUBE-50 AFA Fiber H Slit Strip V2.
010308	M	5/2017	Addition of 96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232) and 130ul 96 microTUBE AFA Fiber Plate Thin Foil (PN 520230).
010308	N	7/2017	Add the names of the well plates definition for 520230 & 520232. Changed year for Rev M Date.
010308	O	12/2020	Addition of re-developed DNA shearing protocols for 150 and 200 bp in microTUBE-50 AFA-Fiber Screw-Cap (PN 520166) and the 96 microTUBE-50 AFA-Fiber Plate Thin Foil (PN 520232) for increased sample recovery.