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.

Revision History

Part Number	Revision	Date	Description of change
010308	K	1/17	Format Changes; Addition of microTUBE-500 AFA Fiber Screw-Cap protocols; update 'Additional Accessories'; update Appendix B
010308	L	2/17	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	5/17	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	7/17	Add the names of the well plates definition for 520230 & 520232. Changed year for Rev M Date.

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)
- **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 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.

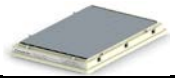
See http://www.covaris.com/wp-content/uploads/pn_010308.pdf for updates to this document.

130 µl sample volume - from 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	**Racks**	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
	Plate Definitions	"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"
	Water Level	6			
	Intensifier (PN 500141)	Yes			
	Y-dithering	No			
E220 evolution	**Racks**	Rack E220e 8 Place microTUBE Crimp and Snap Cap (PN 500433)		Rack E220e 8 microTUBE Strip (PN 500430)	Non Compatible
	Plate Definitions	"500433 E220e 8 microTUBE Crimp and Snap Cap -3.7mm offset"		"500430 E220e 8 microTUBE Strip -6mm offset"	N/A
	Water Level	6			
	Intensifier (PN 500141)	Yes			
	Y-dithering	No			
All	**Temperature (°C)**	7			

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

55 µl sample volume - from 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	**Racks**	Rack 24 Place microTUBE Screw-Cap (PN 500308)	Rack 12 Place 8 microTUBE Strip (PN 500444)	No Rack needed
	Plate Definitions	"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	**Racks**	Rack E220e 4 Place microTUBE Screw Cap (PN 500432)	Rack E220e 8 microTUBE Strip V2 (PN 500437)	Non Compatible
	Plate Definitions	"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		
	Water Level	6	-2	0
	Intensifier (PN 500141)	Yes	Yes	Yes
	Y-dithering	No	No	Yes (0.5mm Y-dither at 10mm/s)

	Target BP (Peak)	**150**	**200**	**250**	**300**	**350**	**400**	**550**
Screw-Cap	**Peak Incident Power(W)**	100	75	75	75	75	75	30
	Duty Factor	30%	20%	20%	20%	20%	10%	10%
	Cycles per Burst	1000	1000	1000	1000	1000	1000	1000
	Treatment Time (s)	130	95	62	40	30	50	70
8-Strip	**Peak Incident Power (W)**	75	75	75	75	75	75	50
	Duty Factor	15%	15%	20%	20%	20%	10%	10%
	Cycles per Burst	500	500	1000	1000	1000	1000	1000
	Treatment Time (s)	360	155	75	45	35	52	50
Plate	**Peak Incident Power (W)**	100	100	75	75	75	75	75
	Duty Factor	30%	30%	20%	20%	20%	10%	10%
	Cycles per Burst	1000	1000	1000	1000	1000	1000	1000
	Treatment Time (s)	145	90	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 - from 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	**Racks**	Rack 24 Place microTUBE Screw-Cap (PN 500308)			Rack 12 Place 8 microTUBE Strip V2 (PN 500444)	
	Plate Definitions	"E220_500308 Rack 24 Place microTUBE-15 Screw-Cap +15mm offset"			"E220_500444 Rack 12 Place 8 microTUBE-15 Strip V2 -1.5mm offset"	
	Water Level	10			6	
	Intensifier (PN 500141)	No				
	Y-dithering	No				
E220 evolution	**Racks**	Rack E220e 4 Place microTUBE Screw Cap (PN 500432)			Rack E220e 8 microTUBE Strip V2 (PN 500437)	
	Plate Definitions	"500432 E220e 4 microTUBE-15 Screw Cap 0.18mm offset"			"500437 E220e 8 microTUBE-15 Strip V2 -1.58mm offset"	
	Water Level	10			6	
	Intensifier (PN 500141)	No				
	Y-dithering	No				
All	**Temperature (°C)**	20				
	Target BP (Peak)	**150**	**200**	**250**	**350**	**550**
	Peak Incident Power (W)	18	18	18	18	18
	Duty Factor	20%	20%	20%	20%	20%
	Cycles per Burst	50	50	50	50	50
	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 - 2,000; 3,000 and 5,000 bp

	Vessel	miniTUBE		
		Clear (PN 520064) 	Blue (PN 520065) 	Red (PN 520066) 
	Sample Volume	**200 µl**		
E220	**Racks**	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	**Racks**	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**
	miniTUBE	**Clear**	**Blue**	**Red**
	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 volume – from 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)				
	Plate Definition	"E220_500452 Rack 24 Place microTUBE-500 Screw-Cap +6mm offset"				
	Water Level	6				
	Intensifier (PN 500141)	Yes				
	Y-dithering	No				
E220 evolution	**Rack**	Rack E220e 4 microTUBE-500 Screw-Cap (PN 500484)				
	Plate Definition	"500484 E220e 4 microTUBE-500 Screw-Cap -9.9mm offset"				
	Water Level	6				
	Intensifier (PN 500141)	Yes				
	Y-dithering	No				
All	**Temperature (°C)**	7				
	Target BP (Peak)	**500 - 600**	**150**	**200**	**350**	**550**
	Peak Incident Power (W)	75	175	175	175	175
	Duty Factor	25%	20%	20%	20%	5%
	Cycles per Burst	200	200	200	200	200
	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 kb 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
	microTUBE-500 Screw-Cap Prep Station	500510
	miniTUBE loading and unloading station	500207
	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

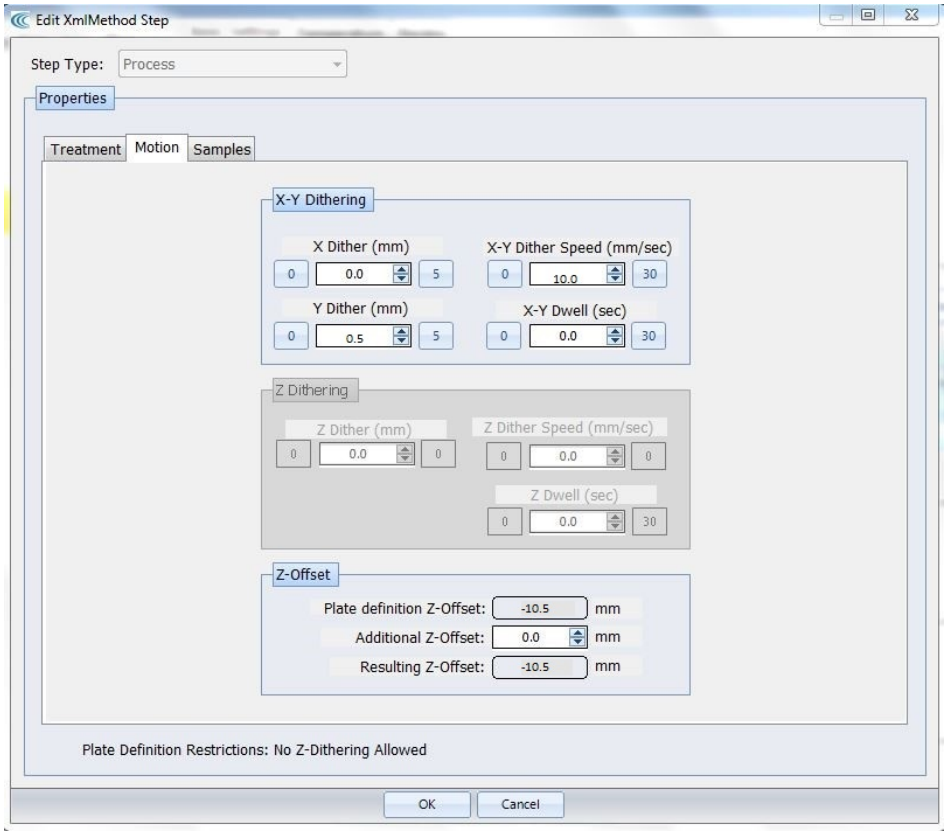
Appendix A – Using Y-dithering with SonoLab 7.3 and up

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.

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 in 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

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 E System 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 System 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 9:00am 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@covaris.com