

## Quick Guide:

# truSHEAR Buffer DNA Shearing

This Quick Guide provides DNA Shearing protocols when using the truSHEAR Buffer and a Covaris Focused-ultrasonicator. This guide contains protocols for shearing DNA to 150 and 200bp in microTUBE-50 consumables, on the M220, ME220, S220, E220, and LE220.

### Revision History

Part Number	Revision	Date	Description of change
010401	A	5/17	Protocols for microTUBE-50, 150bp and 200bp

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

- Temperature +/-2°C
- Sample volume
  - o microTUBE-50: 55 µl, +/- 2.5 µl
- Water Level +/- 1

### Sample preparation guidelines

Consumable	Total Shearing Volume	DNA Sample Volume	truSHEAR Buffer Volume
microTUBE-50	55 µl	50 µl	5 µl

- **DNA input:** protocols are optimized for low concentrations of pure DNA up to 10ng/µl
- **DNA quality: purified genomic DNA.** For lower quality DNA, Covaris recommends setting up a time dose response experiment for determining appropriate treatment times.  
Note, truSHEAR Buffer increases yield relative to TE or low TE when shearing a purified substrate.
- **DO NOT use the microTUBE for storage. Samples should be transferred after processing.**

### Instrument setup

- Refer to the instrument manuals for complete setup.
- microTUBEs have specific racks associated with them.
- The water level is set on the RUN scale in the Start position.
- E220 and LE220 protocols 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.
- Settings developed without truSHEAR buffer maybe affected, please refer to this document for guidelines
- Mean DNA fragment size distributions are based on smear analysis (25-700bp) of fragments generated on a Fragment Analyzer (AATI) using the High Sensitivity Kit (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 hyperlink for updates to this document.

[http://covarisinc.com/wp-content/uploads/pn\\_010401.pdf](http://covarisinc.com/wp-content/uploads/pn_010401.pdf)


## M220 Focused-ultrasonicator

<b>Vessel</b>	<b>microTUBE-50 AFA Fiber Screw-Cap (PN 520166)</b> 	
<b>Holder</b>	XTU 500414	
<b>Insert</b>	PN 500488	
<b>Sample Volume</b>	55 $\mu$ l	
<b>Water Level</b>	5.5	
<b>Water Temperature</b>	20°C	
<b>Target BP (Smear)</b>	<b>150</b>	<b>200</b>
<b>Peak Incident Power (W)</b>	75	75
<b>Duty Factor</b>	10%	10%
<b>Cycles per Burst</b>	1000	1000
<b>Treatment Time (s)</b>	450	220



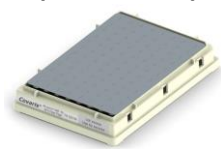
## S220 Focused-ultrasonicator

<b>Vessel</b>	<b>microTUBE-50 AFA Fiber Screw-Cap (PN 520166)</b> 	
<b>Holder</b>	500492	
<b>Sample Volume</b>	55 $\mu$ l	
<b>Water Level</b>	10	
<b>Water Temperature</b>	7°C	
<b>Target BP (Smear)</b>	<b>150</b>	<b>200</b>
<b>Peak Incident Power (W)</b>	100	75
<b>Duty Factor</b>	30%	20%
<b>Cycles per Burst</b>	1000	1000
<b>Treatment Time (s)</b>	145	105

## ME220 Focused-ultrasonicator

Vessel	<b>microTUBE-50 AFA Fiber Screw-Cap (PN 520166)</b>	<b>8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)</b>		
		<b>8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)</b>		
Rack	Rack 4-place microTUBE Screw-Cap PN 500522	Rack 8 microTUBE Strip V2 PN 500518		
Waveguide	PN 500534	PN 500526		
Sample Volume	55 $\mu$ l	55 $\mu$ l		
Water Level	5.5	5.5		
Water Temperature	9°C	9°C		
<b>Target BP (Smear)</b>	<b>150</b>	<b>200</b>	<b>150</b>	<b>200</b>
Duration (s)	170	78	280	125
Peak Power (W)	75	75	50	50
Duty Factor (%)	25%	25%	30%	30%
Cycles per Burst	1000	1000	1000	1000


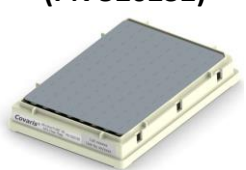
## E220 Focused-ultrasonicator

<b>Vessel</b>	microTUBE-50 Screw-Cap (PN 520166)	8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)		96 microTUBE-50 AFA Fiber Plate (PN 520168)			
		8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)		96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232)			
							
<b>Sample Volume</b>		<b>55 µl</b>					
<b>E220</b>	<b>Racks</b>	Rack 24 Place microTUBE Screw-Cap (PN 500308)	Rack 12 Place 8 microTUBE Strip (PN 500444)	No Rack needed			
	<b>Plate Definitions</b>	"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"			
<b>E220 evolution</b>	<b>Racks</b>	Rack E220e 4 Place microTUBE Screw Cap (PN 500432)	Rack E220e 8 microTUBE Strip V2 (PN 500437)	Non Compatible			
	<b>Plate Definitions</b>	"500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset"	"500437 E220e 8 microTUBE-50 Strip V2 - 10mm offset"	N/A			
<b>All</b>	<b>Temperature (°C)</b>	7					
	<b>Water Level</b>	6	-2	0			
	<b>Intensifier (PN 500141)</b>	Yes	Yes	Yes			
	<b>Y-dithering</b>	No	No	Yes 0.5mm Y-dither at 10mm/s			
	<b>Target BP (Smear)</b>	<b>150</b>	<b>200</b>	<b>150</b>	<b>200</b>	<b>150</b>	<b>200</b>
	<b>Peak Incident Power (W)</b>	100	75	75	75	75	75
	<b>Duty Factor</b>	30%	20%	15%	10%	15%	15%
	<b>Cycles per Burst</b>	1000	1000	500	500	1000	1000
	<b>Treatment Time (s)</b>	145	105	400	255	360	180



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.

## LE220 Focused-ultrasonicator

	<b>Vessel</b>	<b>8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)</b>  <b>8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)</b>  	<b>96 microTUBE-50 AFA Fiber Plate (PN 520168)</b>  <b>96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232)</b>  		
	<b>Sample Volume</b>	<b>55 µl</b>			
<b>LE220</b>	<b>Racks</b>	Rack – XT 12 Place 8 microTUBE Strip V2 (PN 500485)	No Rack needed		
	<b>Plate Definitions</b>	“LE220_500485 Rack-XT 12 Place 8 microTUBE-50 Strip V2 -12mm offset”	“LE220_520168 96 microTUBE-50 Plate -12mm offset”		
	<b>Water Level</b>	-2			
	<b>X and/or Y-dithering</b>	Yes 0.5mm X-dither & 0.5mm Y-dither at 10mm/sec			
	<b>Temperature (°C)</b>	7			
	<b>Target BP (Smear)</b>	<b>150</b>	<b>200</b>	<b>150</b>	<b>200</b>
	<b>Peak Incident Power (W)</b>	450	450	450	450
	<b>Duty Factor</b>	20%	20%	20%	20%
	<b>Cycles per Burst</b>	1000	1000	1000	1000
	<b>Treatment Time (s)</b>	360	160	540	230



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 B for detailed instructions.

## Additional Accessories

	Product Description	Part Number
<b>Preparation Stations</b>	microTUBE Prep Station Snap & Screw Cap	500330
<b>Centrifuge and Heat Block microTUBE Screw-Cap Adapter</b>	Fits microTUBE Screw-Caps into bench top microcentrifuges	500406
<b>Centrifuge 8 microTUBE Strip V2 Adapter</b>	Fits the 8 microTUBE Strip into a Thermo Scientific™ mySPIN™ 12 mini centrifuge	500541

## Technical Assistance

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- 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](mailto:techsupport@covarisinc.com)

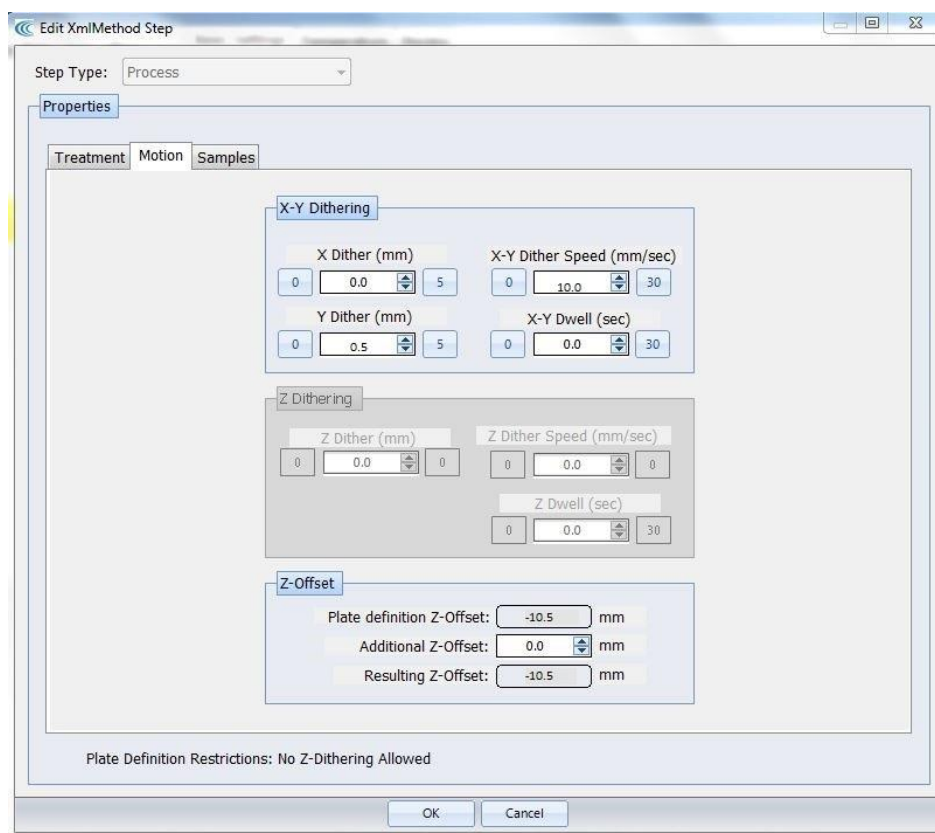
## Appendix A – Using Y-dithering with SonoLab 7.3 and up

### 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.covarisinc.com](http://www.covarisinc.com)
- For any assistance in this process, please contact your local representative, or Covaris Global Technical Services at [TechSupport@covarisinc.com](mailto: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): **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 – 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 microTUBE-50 AFA Fiber Plate

96

- 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](http://www.covarisinc.com)
- For any assistance in this process, please contact your local representative, or Covaris Global Technical Services at [TechSupport@covarisinc.com](mailto: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**

