

Quick Guide

DNA Shearing with LE220-plus/LE220R-plus/LE220Rsc

Introduction

This Quick Guide provides DNA Shearing protocols for the Covaris AFA-TUBE, microTUBE, and miniTUBE consumables using Covaris LE220-plus, LE220R-plus, or LE220Rsc Focused-ultrasonicator instruments.

Values are nominal values. The tolerances are as follows:

- Temperature ± 2 °C
- Sample Volume:
 - 96 AFA-TUBE™ TPX Plate: 5 to 50 μ L
 - microTUBE-15: from 15 to 20 μ L, ± 1 μ L
 - microTUBE-50: 55 μ L, ± 2.5 μ L
 - microTUBE Plate, Strip, Snap and Crimp Cap: 130 μ L, ± 5 μ L
 - microTUBE-500: 320 μ L, ± 10 μ L
 - miniTUBE: 200 μ L, ± 10 μ L

Values are nominal values. The tolerances are as follows:

- **DNA input:** microTUBE-130 and microTUBE-50 up to 5 μ g purified DNA; microTUBE-15 up to 1 μ g; microTUBE-500 minimum 320 ng and up to 5 μ g; AFA-TUBE TPX up to 100 ng/ μ L concentration
- **Buffers:** TE - 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 treatments.
- **WARNING:** DO NOT use the Covaris Adaptive Focused Acoustics® (AFA®) consumables for long term sample storage. Samples should be transferred after processing.

Instrument Setup

- Refer to the instrument manual for complete setup.
- AFA-TUBE TPX, microTUBEs, and miniTUBE consumables require specific racks.

Instrument Settings

- Recommended settings are subject to change without notice.
- Customers should perform their own QC as performances vary with analytical systems, library preparation, and sequencing methods. Typically, Covaris creates DNA shearing protocol guidelines to a target base pair of +/- 15% with a %CV < 10% as analyzed using the Agilent, HS NGS Fragment Kit (1 to 6000bp) cat# DNF-474. DNA fragment distribution will vary with analytical systems. To reach desired fragment size distribution, carry out a time course experiment based on settings provided in this document. There is size bias with different analyzers, chips, kits, buffers, and origin of DNA.
- For the current printable version of this protocol: http://www.covaris.com/wp-content/uploads/pn_010433.pdf

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AFA-TUBE TPX

Vessel	96 AFA-TUBE TPX Plate		8 AFA-TUBE TPX Strip	
Part Number (PN)	520291		520292	
Supported Sample Volume	5 to 50 μ l		5 to 50 μ l	
Rack	Rack 96 AFA-TUBE TPX plate PN 500684		Rack 8 AFA-TUBE TPX Strip PN 500685	
Plate Definition	LE220plus_500684 96 AFA-TUBE TPX Plate 1.8 offset		LE220plus-500685 8 AFA-TUBE TPX Strip 1.8 offset	
Dithering*	1 mm Y-dither at 20 mm/s		1 mm Y-dither at 20 mm/s	
Temperature ($^{\circ}$ C)	10		10	
Analytical System	Agilent, HS NGS Fragment Kit (1 to 6000bp) cat# DNF-474		Agilent, HS NGS Fragment Kit (1 to 6000bp) cat# DNF-474	
Sample Buffer	Tris-EDTA		Tris-EDTA	
Base Pair Mode (bp)	175	350	175	350
Sample Volume (μ L)	20	50	20	50
Peak Incident Power (W)	200	200	220	220
Duty Factor (%)	25	25	25	25
Cycles per Burst (cpb)	50	50	50	50
Treatment Time (sec)	200	85	200	85

* Refer to **Appendix A** for detailed instructions.


To ensure reproducible DNA shearing, centrifuge samples before and after processing. Refer to **Appendix B & C** for sample loading and centrifugation instructions for the 96 AFA-TUBE TPX Plate and 8 AFA-TUBE TPX Strips respectively.

Covaris created the protocol guidelines above for 20 μ l/175 bp and 50 μ l/350 bp.

At present, the nominal DNA shearing range in AFA-TUBE TPX is:

- 5 to 20 μ l at 175 to 550 bp
- 20 to 50 μ l at 300 to 550 bp

microTUBE-15

Vessel	8 microTUBE-15 AFA Beads Strip V2		8 microTUBE-15 AFA Beads H Slit Strip V2		
					
Part Number (PN)	520159		520241		
Sample Volume	15 µl				
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				
Dithering*	5 mm Y-dither at 20 mm/s				
Temperature (°C)	20				
Analytical System	Agilent, Bioanalyzer DNA 12000 Kit cat# 5067-1509				
Base Pair Mode (bp)	150	200	250	350	550
Peak Incident Power (W)	180	180	180	180	180
Duty Factor (%)	30	30	20	15	15
Cycles per Burst (cpb)	50	50	50	50	50
Treatment Time (sec)	250	120	105	75	40

* Refer to **Appendix A** for detailed instructions.

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

microTUBE-50 with SonoLab 8.4 or higher

	8 microTUBE-50 AFA Fiber Strip V2	8 microTUBE-50 AFA Fiber H Slit Strip V2	96 microTUBE-50 AFA Fiber Plate	96 microTUBE-50 AFA Fiber Plate Thin Foil
Vessel				
Part Number (PN)	520174	520240	520168	520232
Sample Volume	55 μ l			
Rack	Rack - XT 12 Place 8 microTUBE Strip V2 PN 500485		No Rack Needed	
Plate Definition	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
Dithering*	0.5mm X-dither & 0.5mm Y-dither at 10mm/sec			
Temperature ($^{\circ}$ C)	7			
Analytical System	Agilent, HS NGS Fragment Kit (1 to 6000bp) cat# DNF-474			
Base Pair Mode (bp)	150	350	150	350
Repeat/Iterations (#)	26	9	35	12
Repeat Process Treatment Duration (sec)	10	10	10	10
Peak Incident Power (W)	450	450	450	450
Duty Factor (%)	20	10	20	10
Cycles per Burst (cpb)	1000	1000	1000	1000
Treatment Time (sec)	260	90	350	120

* Refer to **Appendix A** for detailed instructions.


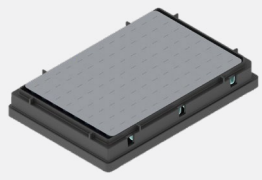
Refer to **Appendix E** for time course set-up instruction. Refer to **Appendix F** for set-up instruction of the optimized protocol.

microTUBE-50 with SonoLab 8.3 or lower

	8 microTUBE-50 AFA Fiber Strip V2	8 microTUBE-50 AFA Fiber H Slit Strip V2	96 microTUBE-50 AFA Fiber Plate	96 microTUBE-50 AFA Fiber Plate Thin Foil			
Vessel							
Part Number (PN)	520174	520240	520168	520232			
Sample Volume	55 µl						
Rack	Rack - XT 12 Place 8 microTUBE Strip V2 PN 500485		No Rack Needed				
Plate Definition	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			
Dithering*	0.5mm X-dither & 0.5mm Y-dither at 10mm/sec						
Temperature (°C)	7						
Analytical System	Agilent, Bioanalyzer DNA 12000 Kit cat# 5067-1509						
Base Pair Mode (bp)	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 (cpb)	1000	1000	1000	1000	1000	1000	1000
8-strip Treatment Time (sec)	360	160	120	79	87	74	56
Plate Treatment Time (sec)	500	200	150	100	120	90	68



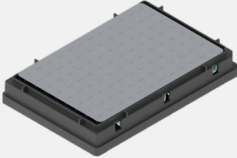
* Refer to **Appendix A** for detailed instructions.

microTUBE-130 with SonoLab 8.4 or higher


	8 microTUBE Strip V1	96 microTUBE Plate	96 microTUBE AFA Fiber Plate Thin Foil	
Vessel				
Part Number (PN)	520053	520078	520230	
Sample Volume	130 μ l			
Rack	Rack 12 Place 8 microTUBE Strip PN 500191	No Rack Needed		
Plate Definition	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	
Dithering*	No			
Temperature ($^{\circ}$ C)	7			
Analytical System	Agilent, HS NGS Fragment Kit (1 to 6000bp) cat# DNF-474			
Base Pair Mode (bp)	150	350	150	350
Repeat/Iterations (#)	26	9	41	9
Repeat Process Treatment Duration (sec)	10	10	10	10
Peak Incident Power (W)	450	450	450	450
Duty Factor (%)	30	15	25	20
Cycles per Burst (cpb)	200	200	200	200
Treatment Time (sec)	260	90	410	90

Refer to **Appendix E** for time course set-up instruction. Refer to **Appendix F** for set-up instruction of the optimized protocol.

microTUBE-130 with SonoLab 8.3 or lower

	microTUBE AFA Fiber Crimp-Cap	8 microTUBE Strip V1	96 microTUBE Plate	96 microTUBE AFA Fiber Plate Thin Foil			
Vessel							
Part Number (PN)	520052	520053	520078	520230			
Sample Volume	130 μ l						
Rack	Rack 96 Place microTUBE Crimp-Cap PN 500282	Rack 12 Place 8 microTUBE Strip PN 500191	No Rack Needed				
Plate Definition	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			
Dithering*	No						
Temperature ($^{\circ}$ C)	7						
Analytical System	Agilent, Bioanalyzer DNA 12000 Kit cat# 5067-1509						
Base Pair Mode (bp)	150	200	300	400	500	900	1500
Peak Incident Power (W)	450	450	450	450	450	450	450
Duty Factor (%)	30	30	30	15	15	5	5
Cycles per Burst (cpb)	200	200	200	200	200	200	200
Crimp Cap and 8-strip Treatment Time (sec)	420	175	60	63	46	77	17
Plate Treatment Time (sec)	490	190	80	100	75	118	20

microTUBE-500

microTUBE-500 AFA Fiber Screw-Cap	
Vessel	
Part Number (PN)	520185
Sample Volume	320 µl
Rack	Rack, 24 microTUBE-500 Screw-Cap PN 500452
Plate Definition	LE220plus_500452 Rack 24 Place microTUBE-500 Screw-Cap +6mm offset
Dithering*	No
Temperature (°C)	7
Analytical System	Agilent, Bioanalyzer High Sensitivity DNA Kit, cat# 5067-4626
Base Pair Mode (bp)	500 to 600
Peak Incident Power (W)	450
Duty Factor (%)	30
Cycles per Burst (cpb)	200
Treatment Time (sec)	65

miniTUBE

Vessel	miniTUBE		
			
Part Number (PN)	520064	520065	520066
Sample Volume	200 µl		
Rack	Rack 24 Place miniTUBE PN 500205		
Plate Definition	LE220plus_500205 24 miniTUBE +15mm offset		
Dithering*	No		
Temperature (°C)	7	20	20
miniTUBE	Clear	Blue	Red
Base Pair Mode (bp)	2000	3000	5000
Peak Incident Power (W)	50	35	100
Duty Factor (%)	20	20	20
Cycles per Burst (cpb)	1000	1000	1000
Treatment Time (sec)	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.

Additional Accessories

	Product Description	Part Number
Preparation Stations	microTUBE Prep Station Snap & Screw Cap	500330
	miniTUBE Prep Station & Stand	500207
	microTUBE-500 Screw-Cap Prep Station	500510
	8 microTUBE Strip Prep Station	500327
Centrifuge and Heat Block microTUBE 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
96 microTUBE Plate Thin Foil Seals	96 microTUBE Plate Thin Foil Seals (25); also compatible with 96 AFA-TUBE TPX Plate	520235
8 microTUBE Strip Foil Seal	(12), used to seal single microTUBE Strips	520108

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 AFA-TUBE TPX 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.

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 AFA-TUBE TPX strip (PN 520292)	0	1.0	0	20	0
96 AFA-TUBE TPX plate (PN 520291)	0	1.0	0	20	0
8 microTUBE-15 AFA Beads Strip V2 (PN 520159 and 520241)	0	5.0	0	20	0
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

Appendix B: 96 AFA-TUBE TPX Plate sample loading and centrifugation

Recommended Sequence for Use:

1. Fill the tubes:
 - Aspirate sample and dispense into the 96 AFA-TUBE TPX Plate. Dispense the samples about 2 to 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:
 - Centrifuge the plate at up to 2200rcf 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 AFA-TUBE TPX plate (PN 500684) for processing.
5. Centrifugation:
 - Centrifuge samples (up to 2200rcf for ≤ 10 seconds) before and after processing DNA using AFA-TUBE vessels. 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 AFA-TUBE TPX Plate. Do not use the plate for long term storage of the samples.

Appendix C: 8 AFA-TUBE TPX Strip sample loading and centrifugation

Recommended Sequence for Use:

1. Fill the tubes:
 - Aspirate sample and dispense into the 8 AFA-TUBE TPX Strip. Dispense the samples about 2 to 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:
 - Apply strip caps or thin foil seal to tops of tubes. If using strip caps, use the solid rack lid labeled "FOR CAP STRIP USE". If using thin foil to seal, use the open rack lid labeled "FOR FOIL SEAL USE".
3. Centrifugation:
 - Centrifuge samples (up to 2200rcf for \leq 10 seconds) before and after processing DNA using AFA-TUBE vessels. Visually inspect strip to verify that all liquid is at the bottom of each tube before proceeding to Step 4. The strip is now ready to be processed in your Covaris instrument.
4. AFA-processing:
 - The strips must be in the Rack 8 AFA-TUBE TPX Strip for LE220-plus (PN 500608) for processing.
5. Centrifugation:
 - Centrifuge samples (up to 2200rcf for \leq 10 seconds) before and after processing DNA using AFA-TUBE vessels.
6. Downstream sample handling:
 - After AFA treatment, the samples are ready for downstream processing. The strip caps or foil seal can be removed for processing in the 8 AFA-TUBE TPX Strip. Do not use the strip for long-term storage of the samples.

Appendix D: microTUBE-15 centrifugation before DNA shearing

Recommended Sequence for Use:

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 setting provided.
3. Sample Recovery:
 - Repeat the centrifuge step before recovering sample from microTUBE-15.

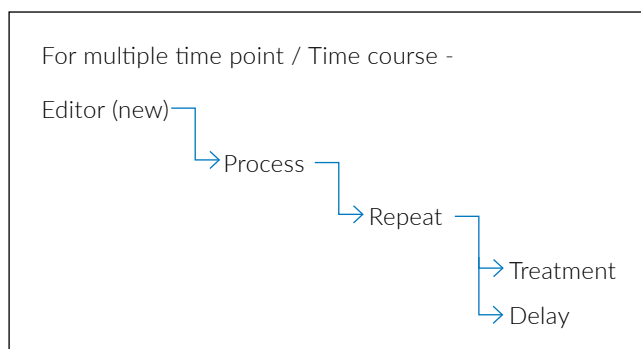
Appendix E: Set up a time course for microTUBE-50 plate vessels in SonoLab 8.4

1. Scope:

- The example described in this appendix explains how to set up a time course pulsing protocol to determine optimal shearing time with both, 96 microTUBE-50 AFA Fiber Plate (PN 520168) and 96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232). This method is compatible with SonoLab version 8.4 and above.

2. Description:

- A shearing time course is recommended to determine the optimal target of the desired DNA distribution. In general, optimizing the pulsing protocol requires to run multiple rows ($n \geq 3$) by increasing and decreasing the number of repeated treatments centered on the suggested value found in this quick guide. Each row optimization experiment with a given number of repetitions needs to have a delay at the end of the AFA treatment as summarized in the flow chart below:



The flow chart represents the button nesting order on SonoLab 8.4 that needs to be followed when creating a method.

3. Method Settings:

- In the sample example below, AFA pulsing method incorporates a time course to optimize the number of repeats for the 55 l and 350 bp protocol with time points of 100 seconds (10 repeats), 120 seconds (12 repeats), and 140 seconds (14 repeats). Each row is subjected to 10 seconds of AFA treatment followed by 20 seconds delay. The only difference in the settings for each row is the # of repeat iterations, which is the parameter that requires optimization before performing DNA shearing with real samples. Refer to the sample method screenshots below that illustrate how to set up a time course optimization experiment.

Once an optimal shearing time is determined, the actual shearing pulsing method is set up such that there is no additional delay step. Refer to **Appendix F** to set up a pulsing experiment with optimized shearing time.

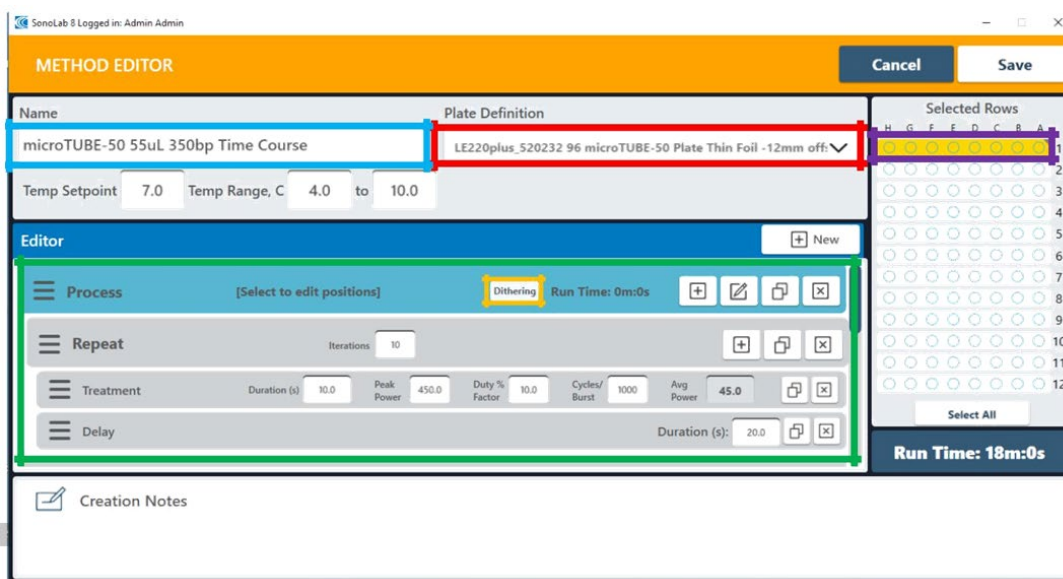
Sample volume to be processed is 55 µl in the 96 microTUBE-50 AFA Fiber Plate Thin Foil Plate: PN 520232

- Plate definition: LE220plus_520232 96 microTUBE-50 Plate Thin Foil - 12mm offset
- Temperature setpoint: 7 °C
- Peak Incident power: 450W
- Duty Factor: 10%
- Cycles per Burst: 1000
- Total AFA duration: 100 seconds, 120 seconds, 140 seconds
- Total AFA pulse duration: 10 seconds
- Delay duration: 20 seconds
- Dither parameters: +/- 0.5 mm in X and Y, Speed 10 mm/s
- # Rows/Conditions: This can be varied based on the user need. In this case, Rows 1, 2 and 3 are selected.

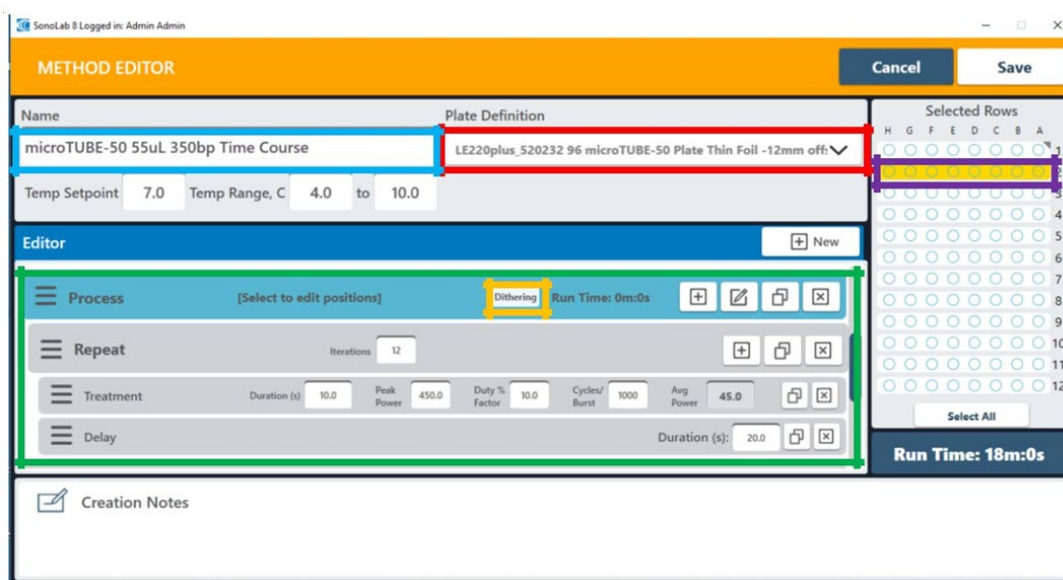
4. Details:

- Create a new method with the settings listed above. Because we are testing three time points with 1 row / iteration, to incorporate the pulsing time course we will need to have 3 repeat loops. For a 10 seconds pulse duration we need to determine the repeat iterations for given time point.

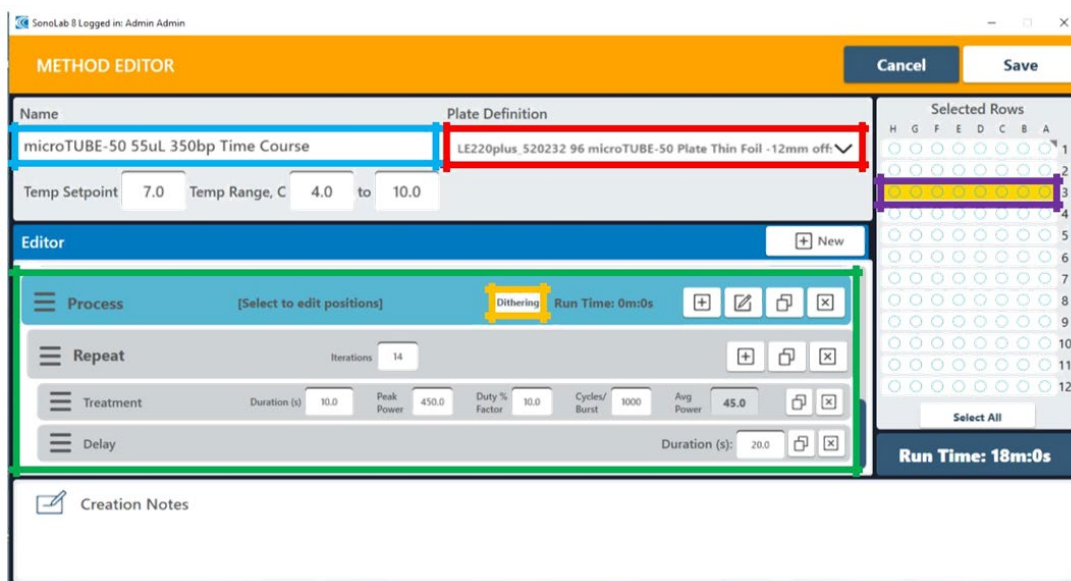
Total Duration (s)	Pulse Duration (s)	Repeat Iterations
100	10	10
120	10	12
140	10	14



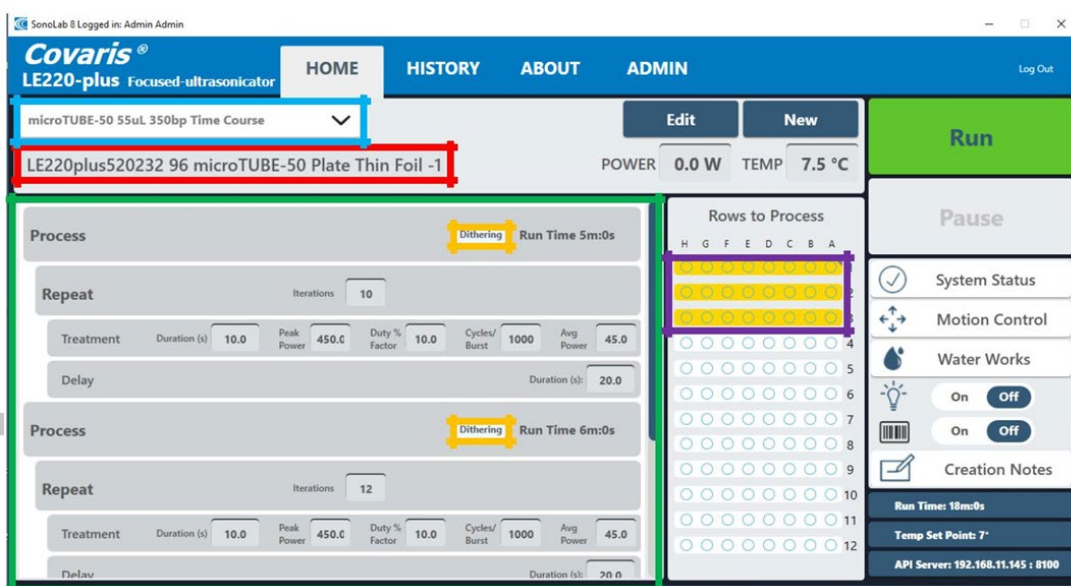
Method Editor Row 1 for Time Course method. “microTUBE-50 55µL 350bp Time Course” uses the “LE220plus_520232 96 microTUBE-50 Plate Thin Foil – 12mm offset” plate definition. The method includes repeat loops for 100 seconds, 120 seconds, and 140 seconds AFA treatment with 10 seconds pulses and 20 seconds delay for Rows 1, 2, and 3 respectively. Dithering is also included for this AFA treatment step.



Method Editor Row 2 for Time Course method. “microTUBE-50 55µL 350bp Time Course” uses the “LE220plus_520232 96 microTUBE-50 Plate Thin Foil – 12mm offset” plate definition. The method includes repeat loops for 100 seconds, 120 seconds, and 140 seconds AFA treatment with 10 seconds pulses and 20 seconds delay for Rows 1, 2, and 3 respectively. Dithering is also included for this AFA treatment step.



Method Editor Row 3 for Time Course method. “microTUBE-50 55µL 350bp Time Course” uses the “LE220plus_520232 96 microTUBE-50 Plate Thin Foil – 12mm offset” plate definition. The method includes repeat loops for 100 seconds, 120 seconds, and 140 seconds AFA treatment with 10 seconds pulses and 20 seconds delay for Rows 1, 2, and 3 respectively. Dithering is also included for this AFA treatment step.



Home Screen for Time Course method. “microTUBE-50 55µL 350bp Time Course” uses the “LE220plus_520232 96 microTUBE-50 Plate Thin Foil – 12mm offset” plate definition. The method includes repeat loops for 100 seconds, 120 seconds, and 140 seconds AFA treatment with 10 seconds pulses and 20 seconds delay for Rows 1, 2, and 3 respectively. Dithering is also included for this AFA treatment step.

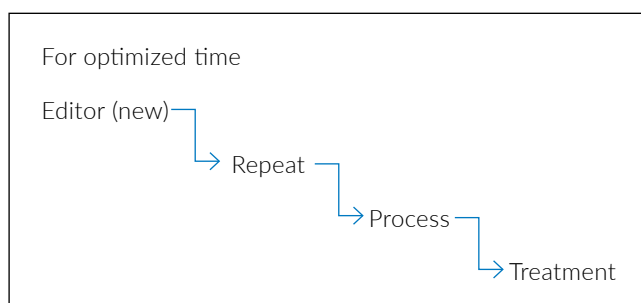
Appendix F: Set up a pulsing experiment with optimized shearing time in microTUBE-50 plate in SonoLab 8.4

1. Scope:

- The example described in this appendix explains how to set up a pulsing experiment when the optimal shearing time is known with both, 96 microTUBE-50 AFA Fiber Plate (PN 520168) and 96 microTUBE-50 AFA Fiber Plate Thin Foil (PN 520232). This method is compatible with SonoLab version 8.4 and above.

2. Description:

- To determine the optimal shearing time or if only one row needs to be processed refer to **Appendix E**. In general, the pulsing protocol with optimized time can be performed on two or more rows. Since there is only one protocol with the same number of repetitions over multiple rows, the experiment can be set up such that no additional delay is needed as shown in the flow chart below:



The flow chart represents the button nesting order on SonoLab 8.4 that needs to be followed when creating a method.

3. Method Settings:

- In this example, the AFA pulsing method incorporates an optimal shearing time of 120 seconds (12 repeats). In this instance, the 10 seconds of AFA dose is not followed by any additional delay since the treatment is cycled through the rows. Refer to the sample method screenshots below that illustrate how to set up a pulsing experiment with optimized shearing time.

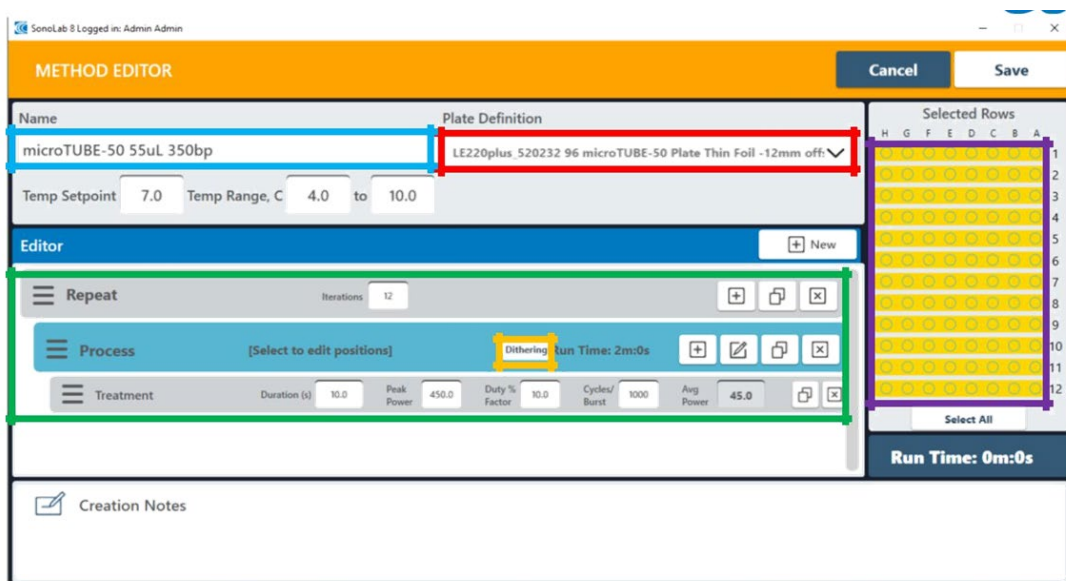
Sample volume to be processed is 55 µl in the 96 microTUBE-50 AFA Fiber Plate Thin

- Foil Plate: PN 520232
- Plate definition: LE220plus_520232 96 microTUBE-50 Plate Thin Foil - 12mm offset
- Temperature setpoint: 7 °C
- Peak Incident power: 450W
- Duty Factor: 10%
- Cycles per Burst: 1000
- Total AFA duration: 120 seconds
- Total AFA pulse duration: 10 seconds
- Delay duration: NA
- Dither parameters: +/- 0.5 mm in X and Y, Speed 10 mm/s
- # Rows with same AFA dose: $n \geq 2$

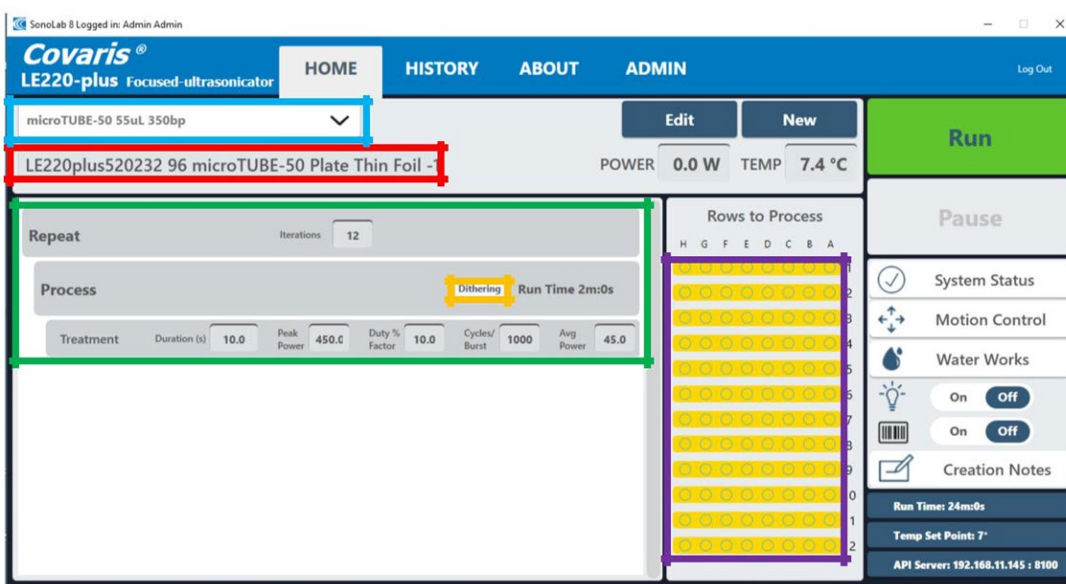
4. Details:

- Create a new method with the settings listed above. In this example we are executing one protocol, 12 repeats per rows cycled through the entire 96 microTUBE-50 AFA Fiber Plate Thin Foil. Because we are using only one optimized time point, to incorporate pulsing, we will need to have only 1 repeat loop. For a 10 seconds pulse duration we need to determine the repeat iterations for optimized time point.

Total Duration (s)	Pulse Duration (s)	Repeat Iterations
120	10	12



Method Editor for Optimized Shearing pulsing method. “microTUBE-50 55 μ L 350bp” uses the “LE220plus_520232_96 microTUBE-50 Plate Thin Foil – 12mm offset” plate definition. The method includes a single repeat loop for 120 seconds of AFA treatment with 10 seconds pulses without an added delay for a whole plate (Rows 1-12). Dithering is also included for this AFA treatment step. Note, a subset of rows can be selected for processing using this method.



Home Screen for Optimized Shearing pulsing method. “microTUBE-50 55 μ L 350bp” uses the “LE220plus_520232_96 microTUBE-50 Plate Thin Foil – 12mm offset” plate definition. The method includes a single repeat loop for 120 seconds of AFA treatment with 10 seconds pulses without an added delay for a whole plate (Rows 1-12). Dithering is also included for this AFA treatment step. Note, a subset of rows can be selected for processing using this method.

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
010433	I	3/2019	Addition microTUBE-130 plate and microTUBE-50 plate pulsing protocol
010433	J	3/2019	Addition of 8 microTUBE Strip and 8 microTUBE-50 AFA Strip V2 pulsing protocols
010433	K	4/2019	Updated name of oneTUBE to AFA-TUBE per product name change
010433	L	5/2019	Revised AFA-TUBE protocol
010433	M	7/2019	Updated volume range for AFA-TUBE
010433	N	2/2020	New Rack for AFA-TUBE with new protocol
010433	O	3/2020	Addition of 8 AFA-TUBE TPX Strip with new protocol