

Quick Guide

DNA Shearing with R230 Focused-ultrasonicator

For Research Only

Software: SonoLab 10

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This Quick Guide provides DNA Shearing protocols for the R230 Focused-ultrasonicator Instrument and associated consumables.

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

- **Temperature:** +/- 3 °C
- **Sample Volume:**
 - 96 AFA-TUBE TPX Plate and 8 AFA-TUBE TPX Strip for ≥ 300 bp: 50 μ L, ± 2.5 μ L
 - 96 AFA-TUBE TPX Plate and 8 AFA-TUBE TPX Strip for ≤ 300 bp: 20 μ L, ± 2.5 μ L
 - 384 AFA-TUBE 5 PP Plate: 3.5 μ L, ± 1.5 μ L
 - 96 microTUBE Plate: 50 μ L ± 2.5 μ L or 130 μ L ± 2.5 μ L
 - 96 AFA-TUBE PS Plate: 50 μ L, ± 2.5 μ L
 - 8 microTUBE Strip: 50 μ L ± 2.5 μ L, 130 μ L ± 5 μ L
- **Water Level:** +/- 0.5

Sample Guidelines

- **DNA Input:**
 - 96 AFA-TUBE TPX Plate, 8 AFA-TUBE TPX Strip, 96 microTUBE Plate, 96 AFA-TUBE PS Plate, and 8 microTUBE Strip up to 5 μ g purified DNA
 - 384 AFA-TUBE 5 PP Plate up to 350 ng purified DNA
 - 96 AFA-TUBE PS Plate up to 1 μ g of purified DNA
- **Buffers:** TE - Tris-EDTA, pH 8.0
 - Water is not a suitable diluent for DNA shearing in AFA-TUBE Consumables.
- **DNA Quality:** Genomic DNA (> 10 kb). For lower quality DNA, Covaris recommends setting up a time dose response experiment for determining appropriate treatment times.
- **WARNING:** DO NOT use the 96 AFA-TUBE TPX Plates, 8 AFA-TUBE TPX Strip, 96 microTUBE Plates, 96 AFA-TUBE PS Plate, 8 microTUBE Strip, or 384 AFA-TUBE 5 PP Plates for long-term sample storage. Samples should be transferred after processing to an appropriate storage tube or plate with low binding properties.

Instrument Setup

- Refer to the instrument manual for complete setup.
- Check for SonoLab updates and use the latest available version.
 - Plates may require a SonoLab update to run protocols and current versions are found at www.covaris.com.
- Consumables have specific accessories (racks and/or plate weights) associated with them.
- Covaris-certified Consumables contain an RFID tag for use on the R230 Focused-ultrasonicator.


Instrument Settings

- Recommended settings are subject to change without notice.
- Covaris recommends a time course experiment to reach the desired fragment size distribution. Please see **Appendices A and B** for assistance in programming the R230 for a time course experiment.

NOTE: DNA fragment representation will vary with analytical systems. Time course experiments can be performed by adjusting the number of iterations. Please contact ApplicationSupport@covaris.com regarding details on how to set up a method for time course experiment.

Follow [this link](#) for updates to this document.

96 AFA-TUBE TPX Plate with SonoLab 10.0.0 or Higher

	96 AFA-TUBE TPX Plate (PN 520291)	
Vessel		
Suggested Sample Volume	10 to 50 μ L (see sample volume guidelines below)	
Rack	R230 Rack AFA-TUBE TPX Plate (PN 500668)	
Plate Definition	R230_520291 96 AFA-TUBE TPX Plate +0.5 offset	
Dithering	3 mm Y at 20 mm/s	
Temperature ($^{\circ}$ C)	10	
Analytical System	Agilent High Sensitivity NGS Fragment Analyzer Kit DNF-474	
Base Pair Mode (bp)	175	350
Repeat/Iterations (#)	13	5
Repeat Process Treatment Duration (sec)	10	10
Peak Incident Power (W)	280	280
Duty Factor (%)	25	25
Cycles per Burst (#)	50	50
Delay* Duration (sec)	10	10
Total Treatment Duration (sec)	130	50
Sample Volume (μ L)	20	50

***NOTE:** The delay step is required only for time course experiments and in run scenarios when only 1 column is being tested. For shearing in more than one column with optimized final setting, delay step is not required. 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 or 20 to 50 μ L at 300 to 550 bp.

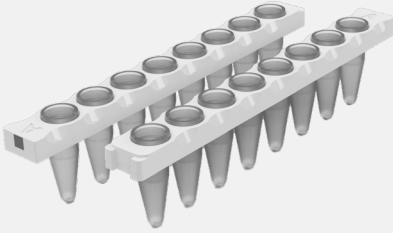
An adhesive foil seal is recommended for sealing the plate and one is provided with the plate. Heat sealing is not compatible with the 96 AFA-TUBE TPX Plate. Covaris recommends a time course experiment to reach the desired fragment size distribution. Time course experiments are typically performed by changing the number of iterations. See **Appendices A and B** for how to program 1 column at a time on the R230.

Please contact ApplicationSupport@covaris.com regarding details on how to set up a method for time course experiment.

Additional Accessories

Product	Part Number	Product Description
R230 Rack AFA-TUBE TPX Plate	500668	This rack is compatible for use with the 96 AFA-TUBE TPX Plate on the R230 Focused-ultrasonicator
8 AFA-TUBE TPX Strip Caps	500639	Use with 8 AFA-TUBE TPX Strip, PN 520292 and 96 AFA-TUBE TPX Plate, PN 520291. Pack of 12

8 AFA-TUBE TPX Strip with SonoLab 10.0.1 or Higher

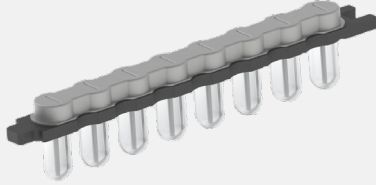
Vessel	8 AFA-TUBE TPX Strip (PN 520292)	
		
Suggested Sample Volume	10 to 50 µL (see sample volume guidelines below)	
Rack	Rack R230 AFA-TUBE TPX Plate & Strip (PN 500668)	
Tube Holder	PSU Rack R230 8 AFA-TUBE TPX Strip (PN 500723)	
Plate Definition	R230_500723 PSU Holder 8 AFA-TUBE TPX Strip +0.5 offset	
Dithering	3 mm Y at 20 mm/s	
Temperature (°C)	10	
Analytical System	Agilent High Sensitivity Fragment Analyzer Kit DNF-474	
Base Pair Mode (bp)	175	350
Repeat/Iterations (#)	13	5
Repeat Process Treatment Duration (sec)	10	10
Peak Incident Power (W)	280	280
Duty Factor (%)	25	25
Cycles per Burst (#)	50	50
Delay* Duration (sec)	10	10
Total Treatment Duration (sec)	130	50
Sample Volume (µL)	20	50

***NOTE:** The Delay step is required only for time course experiments and in run scenarios when only 1 column is being tested. For shearing in more than one column with optimized final setting, delay step is not required. A handheld RFID Reader is required when running the 8 AFA-TUBE TPX Strip. Please see R230 Setup & Instruction Manual (PN 010480) Revision E or higher for instructions on how to install and use the handheld RFID Reader. Covaris created the protocol guidelines above for 20 µL/175 bp and 50 µL/350 bp. At present, the nominal DNA shearing range in AFA-TUBE TPX is 5 to 20 µL at 175 to 550 bp or 20 to 50 µL at 300 to 550 bp. Covaris recommends a time course experiment to reach the desired fragment size distribution. Time course experiments are typically performed by changing the number of iterations. See **Appendices A and B** for how to program 1 column at a time on the R230. Please contact ApplicationSupport@covaris.com regarding details on how to set up a method for time course experiment.

Additional Accessories

Product	Part #	Product Description
R230 Rack AFA-TUBE TPX Plate & Strip	500668	This rack is compatible for use with the 96 AFA-TUBE TPX Plate and 8 AFA-TUBE TPX Strip on R230.
PSU Rack R230 AFA-TUBE TPX Strip	500723	This rack is required in addition to the 500668 rack for use with the 8 AFA-TUBE TPX Strip on R230.
GAO ProMag 13.56 MHz Smart Label Handheld RFID Reader	SKU 223019	This device is required for use with the 8 AFA-TUBE TPX Strip on R230 Focused-ultrasonicator.
8 AFA-TUBE TPX Strip Caps	500639	Package of 12 Strip Caps. For use with 520291 and 520292.

8 microTUBE-130 Strip with SonoLab 10.1.0 or Higher

Vessel	8 microTUBE Strip (PN 520053)			
				
Rack	PSU Rack R230 8 microTUBE Strip (PN 500803) and PSU Rack R230 TPX Plate & 130 Plate (PN 500750)			
Plate Definition	R230_500803 PSU Rack 8 microTUBE Strip +0.5 offset.plt			
Dithering	1.5 mm Y @ 10 mm/s			
Temperature (°C)	10			
Analytical System	Agilent High Sensitivity NGS Fragment Analyzer Kit DNF-474			
Base Pair Mode (bp)	150		350	
Sample Volume (µL)	50	130	50	130
Repeat/Iterations (#)	23	38	5	7
Repeat Process Treatment Duration (sec)	10	10	10	10
Peak Incident Power (W)	450	450	450	450
Duty Factor (%)	25	25	20	24
Cycles per Burst (#)	600	600	600	600
Delay Duration* (sec)	10	10	10	10
Total Treatment Time per Sample(s)	230	380	50	70


***NOTE:** The Delay step is required only for time course experiments and in run scenarios when only 1 column is being tested. For shearing in more than one column with optimized final setting, delay step is not required. Covaris created the protocol guidelines above for 50 µL and 130 µL. An adhesive foil seal is in place to keep the AFA Fibers in place. This product contains the required foil seals to reseal the 8 microTUBE Strip after sample addition. To order additional foil seals, please see the additional accessories below.

Covaris recommends a time course experiment to reach the desired fragment size distribution. Time course experiments are typically performed by changing the number of iterations only. See **Appendices A and B** for how to program 1 column at a time on the R230. Please contact ApplicationSupport@covaris.com regarding details on how to set up a method for a time course experiment.

Additional Accessories

Product	Part Number	Product Description
PSU Rack R230 8 microTUBE Strip	500803	This rack is compatible for use with the 8 microTUBE-130 V1 Strip on R230.
PSU Rack R230 TPX Plate & 130 Plate	500750	This rack is required in addition to the 500803 rack for use with the 8 microTUBE-130 V1 Strip on R230.
8 microTUBE Strip Foil Seal (12)	520108	Package of 12 Foil Seals. For use with 520053.

384 AFA-TUBE 5 PP Plate with SonoLab 10.0.1 or Higher

	384 AFA-TUBE 5 PP Plate (PN 520302)
Vessel	
Suggested Sample Volume	2 to 3.5 μ L
Rack	PSU Rack R230 384 AFA-TUBE PP Plate (PN 500708)
Plate Weight	Weight AFA-TUBE PP Plate (PN 500710)
Plate Definition	R230_500708 PSU Rack 384 AFA-TUBE 5 PP Plate +0.5 offset
Dithering	1 mm Y at 10 mm/s
Temperature ($^{\circ}$ C)	10
Analytical System	Agilent High Sensitivity Fragment Analyzer Kit DNF-474
Base Pair Mode (bp)	200
Repeat/Iterations (#)	100
Peak Incident Power (W)	280
Duty Factor (%)	25
Cycles per Burst (#)	50
Scanning Speed (mm/s)	5
Total Treatment Duration (min)	40
Sample Volume (μ L)	3.5

NOTE: Scanning will treat all samples of the plate in parallel. Please see **Appendix E** for examples of a Scanning Method in SonoLab 10.0.1 or higher. An adhesive foil seal or pierceable foil heat seal is recommended for sealing the plate. Samples should be transferred after processing to an appropriate storage tube or plate with low binding properties.

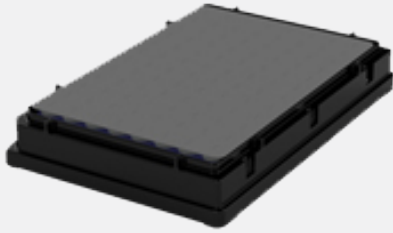
Covaris recommends a time course experiment to reach the desired fragment size distribution.

Please contact ApplicationSupport@covaris.com for details on how to set up a method for time course experiment in scanning mode.

Additional Accessories

Product	Part Number	Product Description
PSU Rack R230 384 AFA-TUBE 5 PP Plate	500708	This rack is compatible for use with the 384 AFA-TUBE 5 PP Plate on R230 Focused-ultrasonicator
Weight AFA-TUBE PP Plate	500710	This weight is required for use with the 384 AFA-TUBE 5 PP Plate on R230 Focused-ultrasonicator
96 microTUBE Plate Thin Foil Seals (25)	520235	This is a compatible seal for use with the 384 AFA-TUBE 5 PP Plate on R230 Focused-ultrasonicator

96 microTUBE Plate with SonoLab 10.0.1 or Higher

Vessel	96 microTUBE Plate (PN 520078)		
			
Suggested Sample Volume	50 µL and 130 µL		
Rack	PSU Rack R230 TPX Plate & 130 Plate (PN 500750)		
Plate Definition	R230_500750 PSU Rack 96 microTUBE Plate +0.5 offset		
Dithering	1.5 mm Y at 10 mm/s		
Temperature (°C)	10		
Analytical System	Agilent High Sensitivity Fragment Analyzer Kit DNF-474		
Base Pair Mode (bp)	150		350
Repeat/Iterations (#)	20	38	7
Repeat Process Treatment Duration (sec)	10	10	10
Peak Incident Power (W)	450	450	450
Duty Factor (%)	25	25	25
Cycles per Burst (#)	600	600	600
Delay* Duration (sec)	10	10	10
Total Treatment Duration (sec)	200	380	70
Sample Volume (µL)	50	130	130


NOTE: *The Delay step is required only for time course experiments and in run scenarios when only 1 column is being tested. For shearing in more than one column with optimized final setting, delay step is not required. An adhesive foil seal is recommended for sealing the plate and one is provided with the plate.

Please contact ApplicationSupport@covaris.com regarding details on how to set up a method for time course experiment. Time course experiments are typically performed by changing the number of iterations. See **Appendices A and B** for how to program 1 column at a time on the R230.

Additional Accessories

Product	Part Number	Product Description
PSU Rack R230 TPX Plate & 130 Plate	500750	This rack is compatible for use with the 96 microTUBE Plate on the R230 Focused-ultrasonicator

96 AFA-TUBE PS Plate with SonoLab 10.0.0 or Higher

	96 AFA-TUBE PS Plate (PN 520311)
Vessel	
Suggested Sample Volume	50 μ L
Plate Weight	Weight AFA-TUBE PP Plate (PN 500710)
Plate Definition	R230_520311 96 AFA-TUBE PS Plate +0.5 offset
Dithering	N/A
Temperature ($^{\circ}$ C)	30
Analytical System	Agilent High Sensitivity Fragment Analyzer Kit DNF-464
Base Pair Mode (bp)	3 kb (3000 bp)
Repeat/Iterations (#)	42
Peak Incident Power (W)	8
Duty Factor (%)	40
Cycles per Burst (#)	50
Scanning Speed (mm/s)	1
Total Treatment Duration (sec)	80
Sample Volume (μ L)	50

NOTE: Scanning will treat all samples of the plate in parallel. Please see **Appendix E** for examples of a Scanning Method in SonoLab 10.0.1 or higher. The plate is pre-sealed with a pierceable heat seal. Once pierced an adhesive foil seal or additional foil heat seal is recommended for re-sealing the plate and an adhesive seal is provided with the plate.

Covaris recommends a time course experiment to reach the desired fragment size distribution. This consumable requires optimization with support from Applications at Covaris.

Please contact ApplicationSupport@covaris.com for details on how to set up a method for time course experiment in scanning mode.

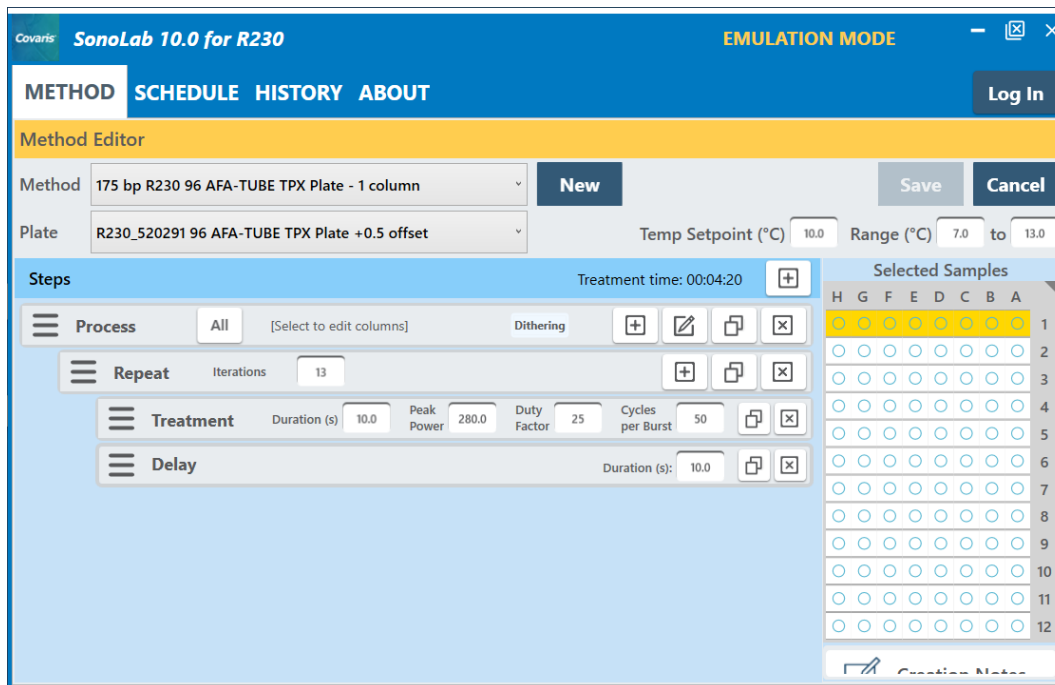
Additional Accessories

Product	Part Number	Product Description
Weight AFA-TUBE PP Plate	500710	Weight is required for use with the 96 AFA-TUBE PS Plate on R230 Focused-ultrasonicator

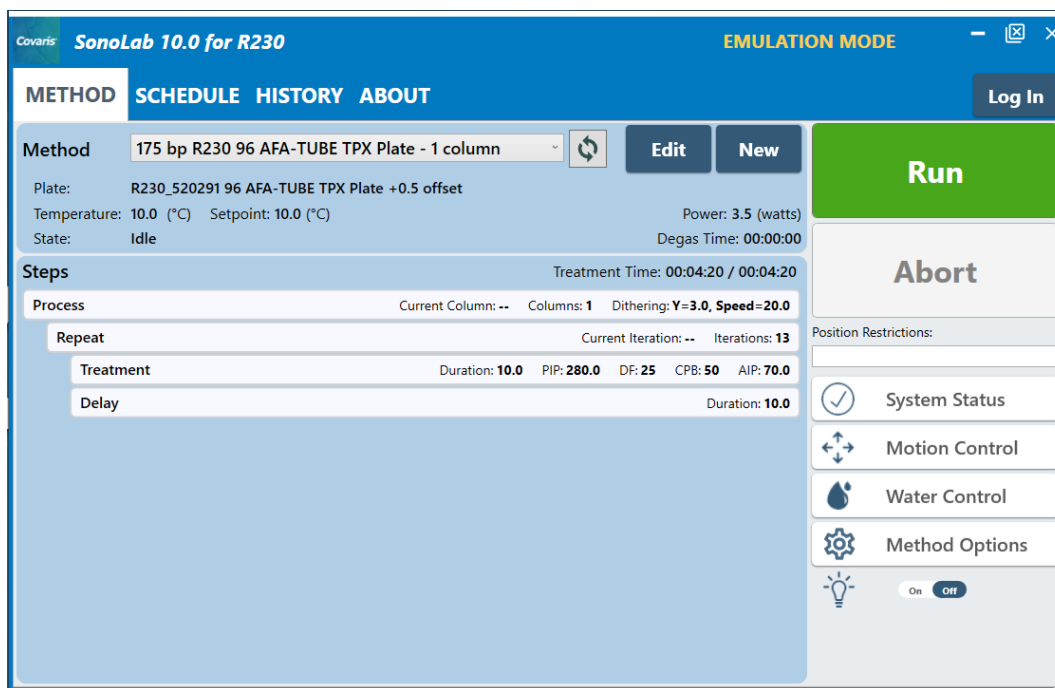
Appendix A: 175 bp Protocol on SonoLab 10.0.0 or Higher for R230 – 1 column

Below are some example screenshots for processing of 175 bp protocol with 1 column of the AFA-TUBE TPX Plate in the Method Editor and Method Screen. Please note that a Delay step is added when processing only 1 column. For a time course experiment, copy these 4 steps for each individual column. Typically only the number of iterations will change from the initial protocol.

Shearing Protocol in Method Editor



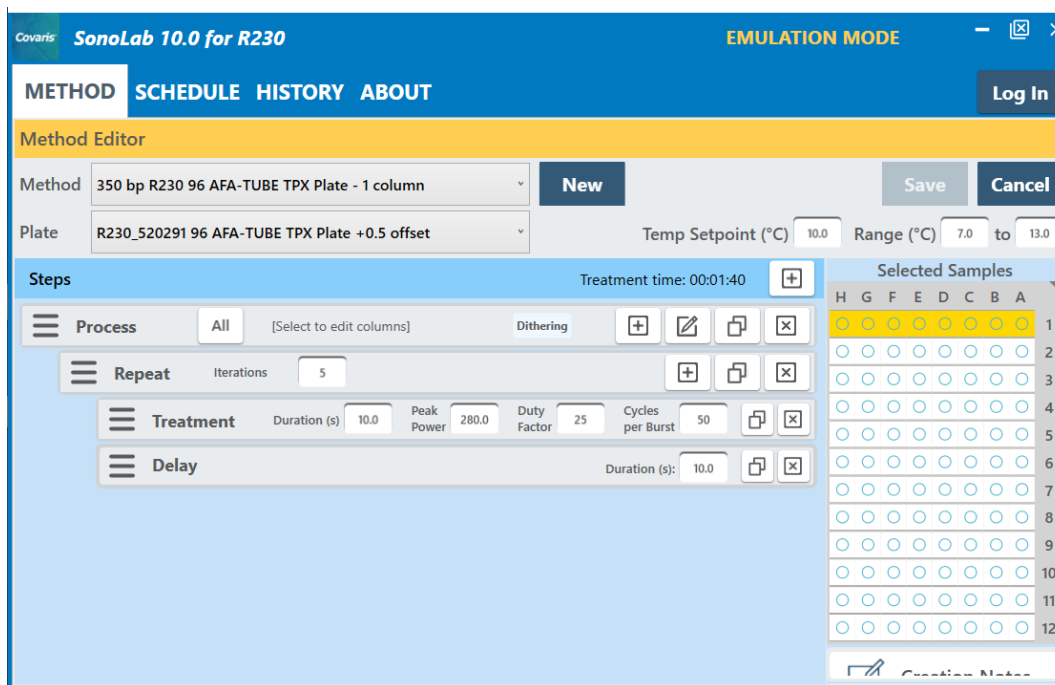
Shearing Protocol in Method Screen



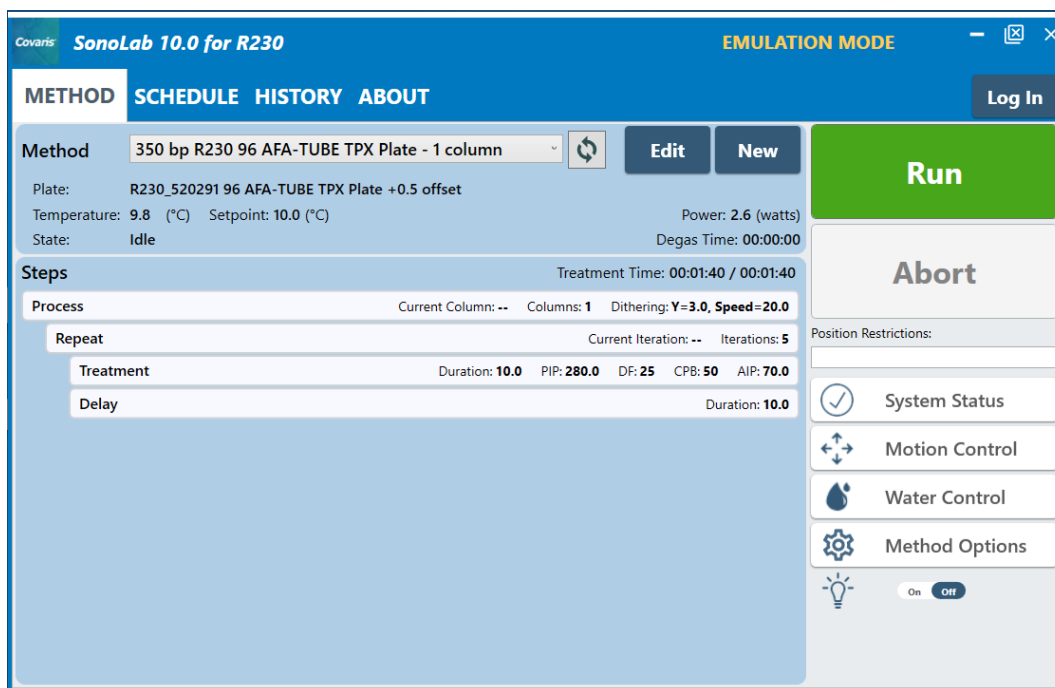
Appendix B: 350 bp Protocol on SonoLab 10.0.0 or Higher for R230 – 1 column

Below are some example screenshots for processing of 350 bp protocol with 1 column of the AFA-TUBE TPX Plate in the Method Editor and Method Screen. Please note that a Delay step is added when processing only 1 column. For a time course experiment, copy these 4 steps for each individual column. Typically only the number of iterations will change from the initial protocol.

Shearing Protocol in Method Editor



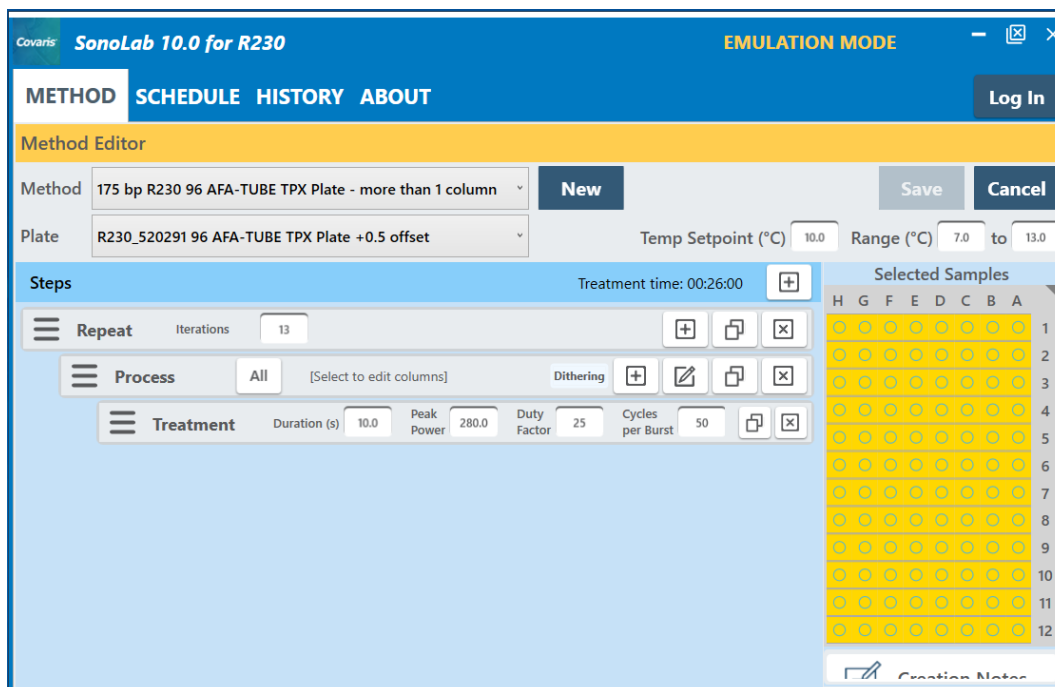
Shearing Protocol in Method Screen



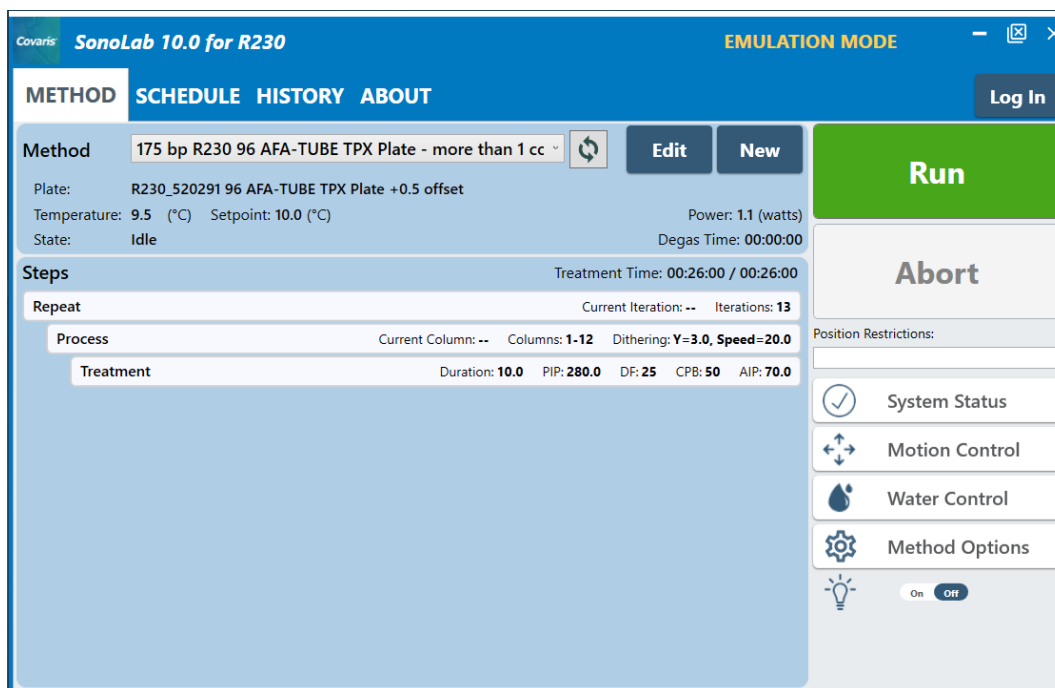
Appendix C: 175 bp Protocol on SonoLab 10.0.0 or Higher for R230 – more than 1 column

Below are some example screenshots for processing of 175 bp protocol with more than 1 column of the AFA-TUBE TPX Plate in the Method Editor and Method Screen. Please note that a Delay step is not required when processing more than 1 column.

Shearing Protocol in Method Editor



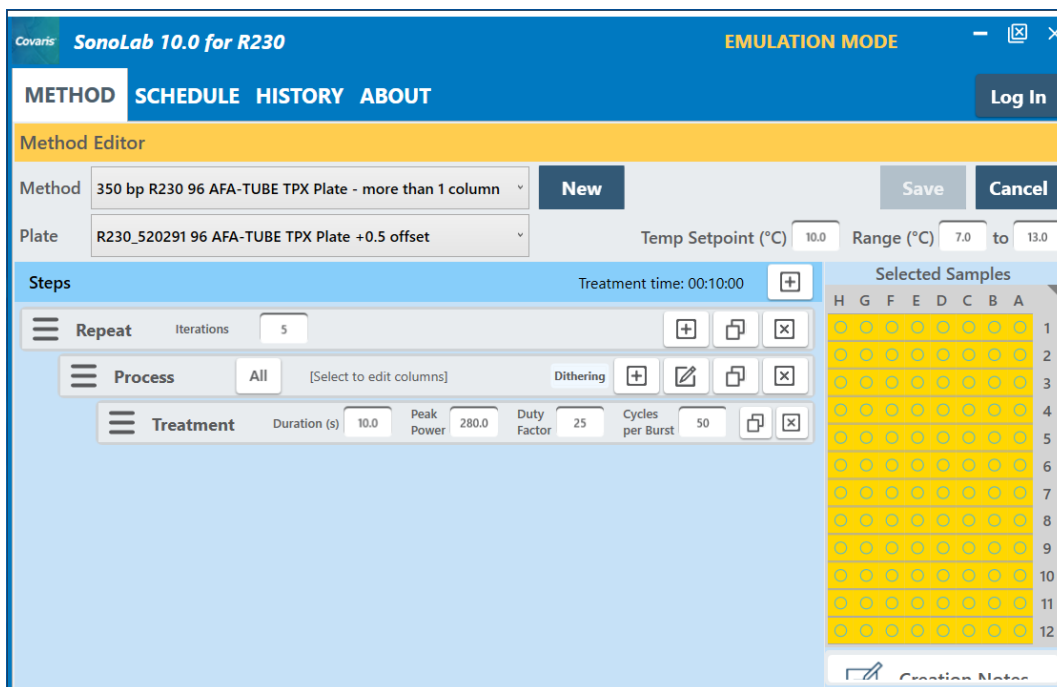
Shearing Protocol in Method Screen



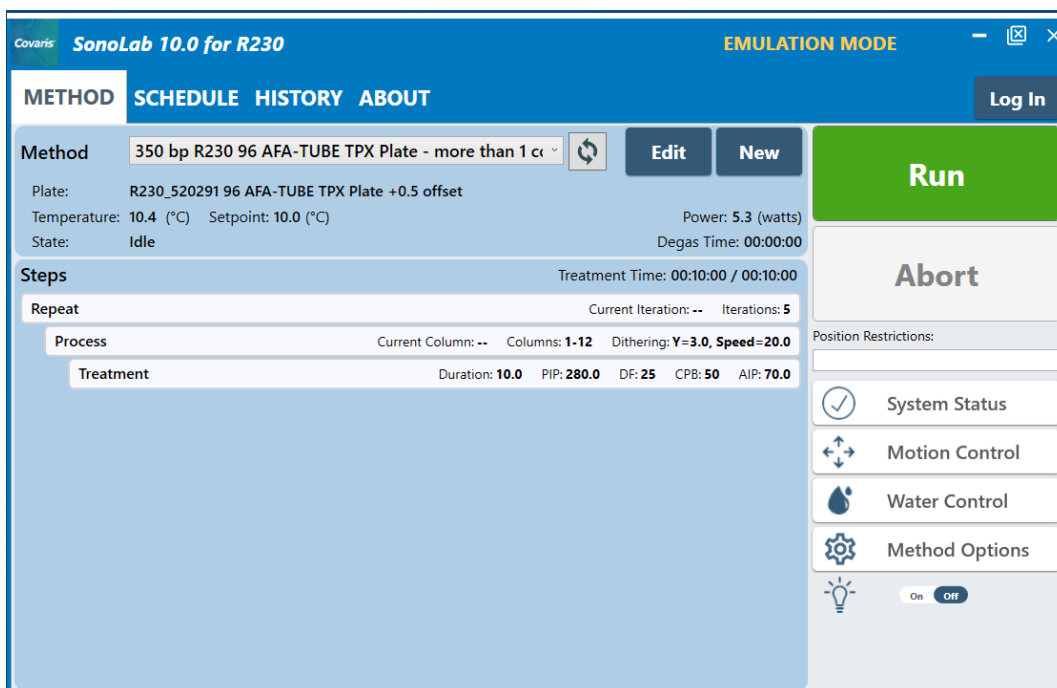
Appendix D: 350 bp Protocol on SonoLab 10.0.0 or Higher for R230 – more than 1 column

Below are some example screenshots for processing of 350 bp protocol with more than 1 column of the AFA-TUBE TPX Plate in the Method Editor and Method Screen. Please note that a Delay step is not required when processing more than 1 column.

Shearing Protocol in Method Editor



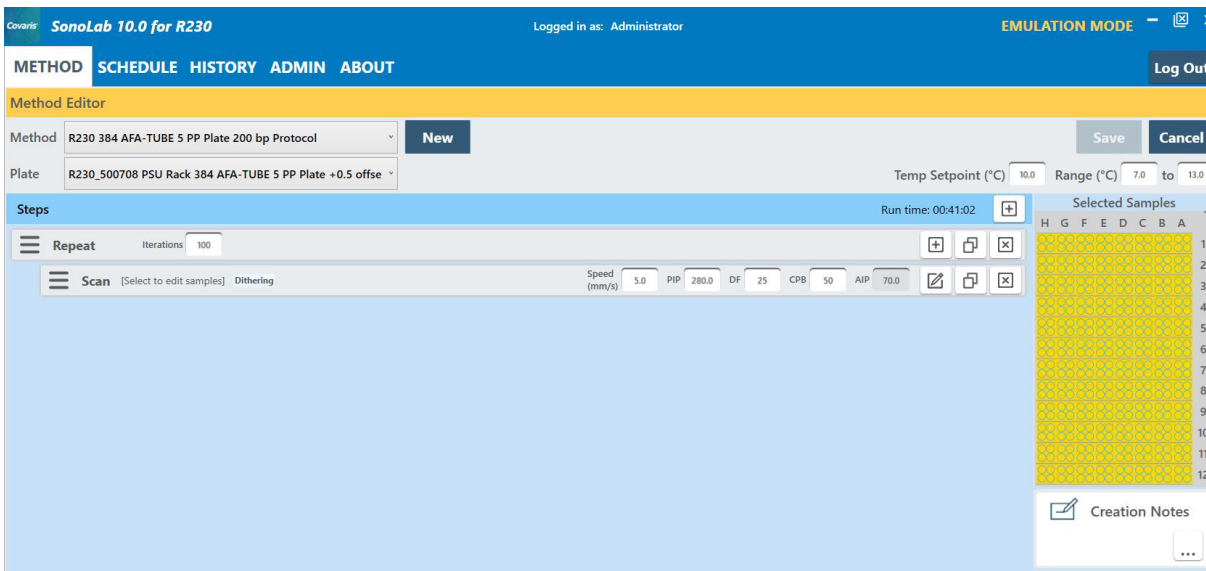
Shearing Protocol in Method Screen



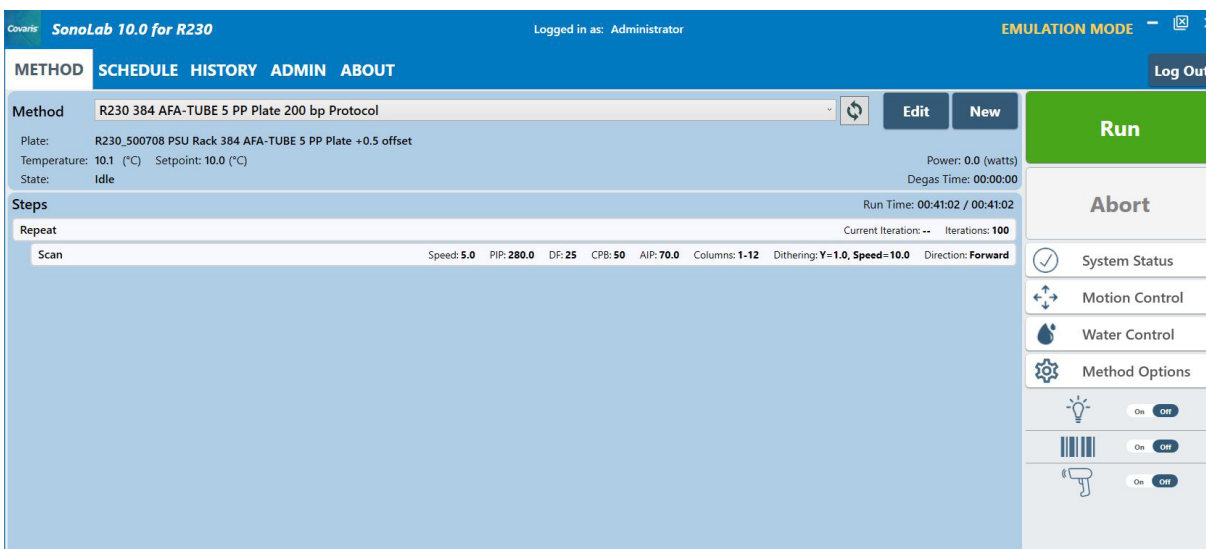
Appendix E: Example Scanning Protocol on SonoLab 10.0.1 or Higher on R230

Below are some example screenshots for processing of 200 bp protocol with 384 AFA-TUBE 5 PP Plate in the Method Editor and Method Screen using the Scan function.

Shearing Protocol in Method Editor



Shearing Protocol in Method Screen



Support and Technical Assistance

Tech Support: Ongoing assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- **Telephone:**

- US & APAC: +1 781.932.3959, during the hours of 8:30 a.m. to 5:00 p.m. (EST), Monday through Friday
- EU: +44 (0)845 872 0100, during the hours of 9:00 a.m. to 5:00 p.m. (GMT), Monday through Friday

- **E-mail:**

- US Customer Service: customerservice@covaris.com
- EU/UK Customer Service: emeacustomerservice@covaris.com
- APAC Customer Service: APACcustomerservice.@covaris.com
- Service and Instrumentation: techsupport@covaris.com
- Solutions: applicationsupport@covaris.com

References

- R230 Focused-ultrasonicator Setup & Instruction Manual: https://www.covaris.com/wp/wp-content/uploads/resources_pdf/pn_010480.pdf
- Variability in DNA Fragment Size and Distribution Analysis Across Various Fragment Analyzers: https://www.covaris.com/wp/wp-content/uploads/resources_pdf/M020131.pdf

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