

# Quick Guide

## DNA Shearing with ML230 Focused-ultrasonicator

---

This Quick Guide provides DNA Shearing protocols for the Covaris 8 microTUBE-50 V2 Strip and 8 AFA-TUBE TPX Strip consumables using a Covaris ML230 Focused-ultrasonicator instrument.

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

- **Temperature:** +/- 3 °C
- **Sample Volume:**
  - microTUBE-50: 55 µL, ± 2.5 µL
  - 8 AFA-TUBE TPX Strip for ≥ 300 bp: 50 µL, ± 2.5 µL
  - 8 AFA-TUBE TPX Strip for ≤ 300 bp: 20 µL, ± 2.5 µL
- **Water Level:** +/- 0.5

### Sample Guidelines

- **DNA Input:** For both microTUBE-50 Strips and 8 AFA-TUBE TPX Strips - up to 5 µg purified DNA
- **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 treatment times.
- **WARNING: DO NOT** use the microTUBE-50 Strips or AFA-TUBE TPX Strips for long term sample storage. Samples should be transferred after processing.

### Instrument Setup

- Refer to the instrument manual for complete setup
- DNA Shearing vessels have specific racks associated with them

### Instrument Settings

- Recommended settings are subject to change without notice

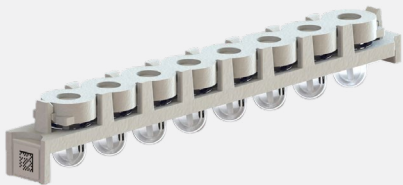
**NOTE:** DNA fragment representation will vary with analytical systems. Please carry out a time course experiment based on the settings provided in this document to reach the desired fragment size distribution (**Appendix B**).

See [www.covaris.com/wp-content/uploads/pn\\_010522](http://www.covaris.com/wp-content/uploads/pn_010522) for updates to this document.

## Table of Contents

8 microTUBE-50 Strip with SonoLab™ 10.1.0 or Higher .....	3
Additional Accessories.....	3
8 AFA-TUBE TPX Strip with SonoLab 10.1.0 or Higher .....	4
Appendix A: Protocol on SonoLab 10.1.0 or Higher.....	5
Shearing Protocol in Method Editor.....	5
Shearing Protocol in Method Screen .....	5
Appendix B: Time Course – Create Pulsing Method using SonoLab 10.1.0 or Higher.....	6
DNA Shearing Time Course Examples.....	7
8 microTUBE-50 AFA Fiber Strip V2 150 bp Protocol Time course example setup .....	7
8 microTUBE-50 AFA Fiber Strip V2 350 bp Protocol Time course example setup .....	7
Technical Assistance .....	8
Revision History .....	8

## 8 microTUBE-50 Strip with SonoLab 10.1.0 or Higher

Vessel	8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)*	8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)*
		
Sample Volume	55 µl	
Rack	ML230 Rack 8 microTUBE Strip 50 (PN 500661)	
Plate Definition	ML230_500661 Rack 8 microTUBE Strip 50 +1.8 mm offset	
Dithering	3 mm Y @ 20 mm/s	
Temperature (°C)	12	
Analytical System	Agilent High Sensitivity NGS Fragment Analyzer Kit DNF-474	
Base Pair Mode (bp)	150	350
Repeat/Iterations (#)	30	7
Repeat Process Treatment Duration (sec)	10	10
Peak Incident Power (W)	350	350
Duty Factor (%)	25	20
Cycles per Burst (#)	1000	1000
Delay Duration (sec)	10	10
Total Treatment Time per Sample(s)	300	70
Total Run Duration (sec)	600	140

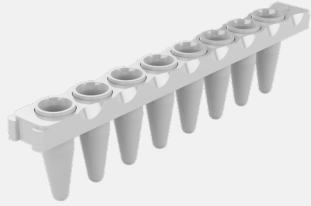
\*Both 520174 and 520240 may be used for either the 150 or 350 bp Protocol.

See **Appendix A** for screenshots of protocols in SonoLab 10.1.0 or Higher. See **Appendix B** for how to set up a time course for protocol optimization.

## Additional Accessories

Part Number	Product	Product Description
500541	Centrifuge 8 microTUBE-50 Strip V2 Adapter	Fits the 8 microTUBE-50 Strip into a Thermo Scientific™ mySPIN™ 12 mini centrifuge

## 8 AFA-TUBE TPX Strip with SonoLab 10.1.0 or Higher

Vessel	8 AFA-TUBE TPX Strip (PN 520292)	
		
Supported Sample Volume*	5 to 50 µl	
Rack	ML230 Rack 8 AFA-TUBE TPX Strip (PN 500660)	
Plate Definition	ML230_500660 Rack 8 AFA-TUBE TPX Strip +12.7 mm offset	
Dithering	3 mm Y @ 20 mm/s	
Temperature (°C)	12	
Analytical System	Agilent High Sensitivity NGS Fragment Analyzer Kit DNF-474	
Base Pair Mode (bp)	175	350
Repeat/Iterations (#)	16	7
Repeat Process Treatment Duration (sec)	10	10
Peak Incident Power (W)	210	210
Duty Factor (%)	25	25
Cycles per Burst (#)	50	50
Delay Duration (sec)	10	10
Total Treatment Duration (sec)	160	70
Total Run Duration (sec)	320	140
Sample Volume (µl)	20	50

\*Current protocols support 20 µl sample volume for 175 bp and 50 µl sample volume for 350 bp.

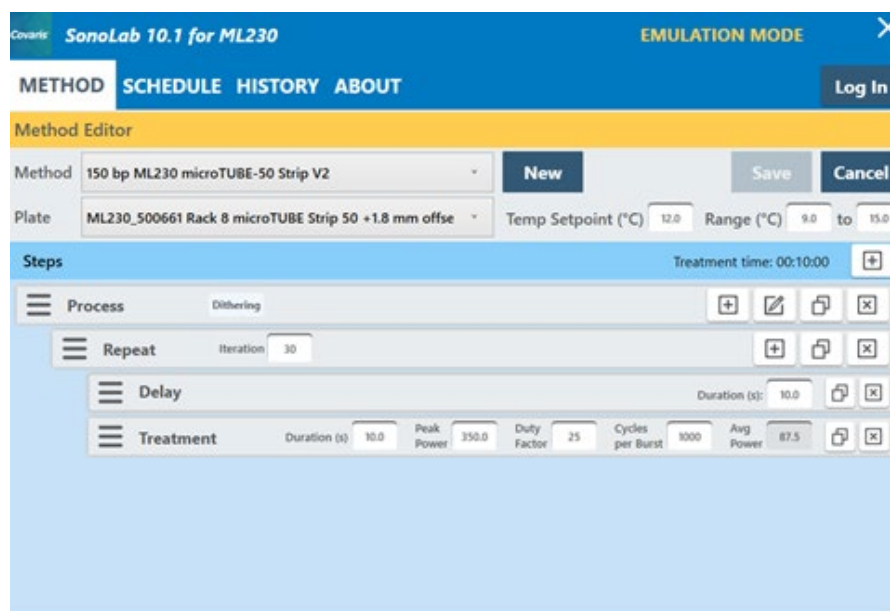
See **Appendix A** for screenshots of a programmed protocol in SonoLab 10.1.0. The 8 AFA-TUBE TPX Strip will follow the protocol order for the 8 microTUBE-50: Process, Repeat, Delay, and Treatment. See **Appendix B** for how to set up a time course for protocol optimization.

## Appendix A: Protocol on SonoLab 10.1.0 or Higher

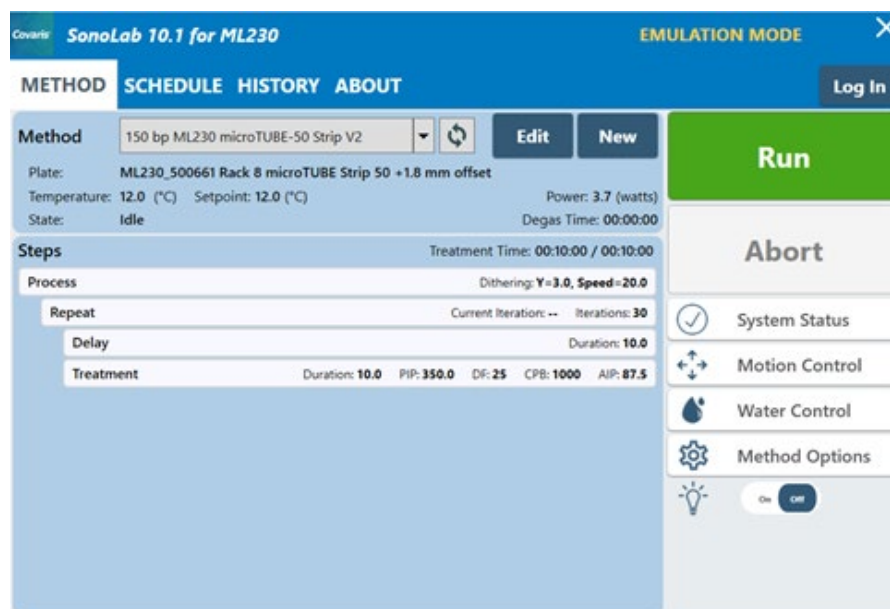
Following are some example screenshots of the 150 bp protocol for the microTUBE-50 in the Method Editor and Method Screen.

### Shearing Protocol in Method Editor

**NOTE:** Repeat ("Iterations") is programmed after the Process.



### Shearing Protocol in Method Screen



## Appendix B: Time Course – Create Pulsing Method using SonoLab 10.1.0 or Higher

Refer to the ML230 User Manual for detailed instructions on pulsing method creation.

[www.covaris.com/wp-content/uploads/pn\\_010516.pdf](http://www.covaris.com/wp-content/uploads/pn_010516.pdf)

1. **Time Course:** A time course is necessary to optimize shearing protocols based on user needs. Covaris recommends a total of 6 time points for any target size, using the maximum number of samples (N = 8 for strip consumables) for each time point in the time course. The time course shall be performed by starting with the nearest recommended protocol (containing “Z” iterations) and increasing or decreasing the total number of iterations used to adjust total treatment time.
2. **Time Course Set-Up:**
  - a) Fill Covaris Consumable with appropriate volume of lambda or genomic DNA in a desired buffer at a concentration of 10 ng/μL .
    - Recommended buffer: Tris-EDTA
  - b) Input protocol settings into SonoLab with a value of Z-2 or Z-4 iterations to begin: Z value is as published in the shearing protocols. The choice between Z-2 or Z-4 iterations for the first time point is determined based on target size.
    - I.e. For methods shearing to above 200 bp, # iterations for first time point should be Z-2.
    - I.e. For methods shearing to below 200 bp, # iterations for first time point should be Z-4.
  - c) Insert tubes into the ML230 and run protocol.
  - d) Once the treatment is complete, remove samples from the Instrument.
  - e) Transfer samples to a LoBind™ tube for sizing analysis.
  - f) Repeat Steps ‘a’ through ‘e’ until a total of 6 time points have been tested. For methods shearing to above 200 bp, the iterations should change in ‘+1’ increments, whereas for methods shearing to below 200 bp, the iterations should change in ‘+2’ increments.
    - I.e. For methods shearing to above 200 bp, # iterations for 6 runs should be Z-2, Z-1, Z, Z+1, Z+2, Z+3.
    - I.e. For methods shearing to below 200 bp, # iterations for 6 runs should be Z-4, Z-2, Z, Z+2, Z+4, Z+6.
  - g) If extra time points are necessary, additional runs can be performed by adjusting the # iterations.
  - h) Retain total iterations or duration(s) used to obtain desired fragment sizes for programming into SonoLab and future use.

**NOTE:** Do not alter settings when running a time course. Only iterations should be altered.

## DNA Shearing Time Course Examples

Note:

- Sample for each time point - Appropriate volume of lambda or genomic DNA (10 ng/μL) in Tris-EDTA Buffer.
- Only settings highlighted in '*red*' should change.
- Final setting to be determined based on time course results.

## 8 microTUBE-50 AFA Fiber Strip V2 150 bp Protocol Time course example setup

Timepoint	1	2	3	4	5	6
Test Strip	1	2	3	4	5	6
# Iterations	Z - 4	Z - 2	Z	Z + 2	Z + 4	Z + 6
Instrument Settings on ML230						
Repeat / Iterations (#)	26	28	30	32	34	36
Repeat Process Treatment Duration (sec)	10	10	10	10	10	10
Peak Incident Power (W)	350	350	350	350	350	350
Duty Factor (%)	25	25	25	25	25	25
Cycles per Burst (#)	1000	1000	1000	1000	1000	1000
Delay Duration (sec)	10	10	10	10	10	10
Total Treatment Duration (sec)	260	280	300	320	340	340
Total Run Duration (sec)	520	560	600	640	680	680
Volume (μl)	55	55	55	55	55	55

## 8 microTUBE-50 AFA Fiber Strip V2 350 bp Protocol Time course example setup

Timepoint	1	2	3	4	5	6
Test Strip	1	2	3	4	5	6
# Iterations	Z - 2	Z - 1	Z	Z + 1	Z + 2	Z + 3
Instrument Settings on ML230						
Repeat / Iterations (#)	5	6	7	8	9	10
Repeat Process Treatment Duration (sec)	10	10	10	10	10	10
Peak Incident Power (W)	350	350	350	350	350	350
Duty Factor (%)	20	20	20	20	20	20
Cycles per Burst (#)	1000	1000	1000	1000	1000	1000
Delay Duration (sec)	10	10	10	10	10	10
Total Treatment Duration (sec)	50	60	70	80	90	100
Total Run Duration (sec)	100	120	140	160	180	200
Volume (μl)	55	55	55	55	55	55

### 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](mailto:ApplicationSupport@covaris.com)

### Revision History

Part Number	Revision	Date	Description of Change
010522	A	7/2020	Initial release