# 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). Protocols were optimized using purified control DNA >40kb. Covaris recommends setting up a time course experiment for determining appropriate treatment times for the target DNA source (e.g. FFPE, blood, saliva, DBS, purified DNA, etc.). Refer to Appendix B for time course set-up.
- **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
- The ML230 must use a pulsing program that includes a repeated treatment and delay protocol. Refer to Appendix B for instructions on pulsing method creation.

> ***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.covarisinc.com/wp-content/uploads/pn_010522 for updates to this document.

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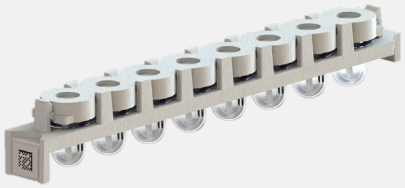
## Table of Contents

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

| | 8 microTUBE-50 AFA Fiber Strip V2 (PN 520174)* | 8 microTUBE-50 AFA Fiber H Slit Strip V2 (PN 520240)* |
|---|---|---|
| Vessel |  | |
| 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 |

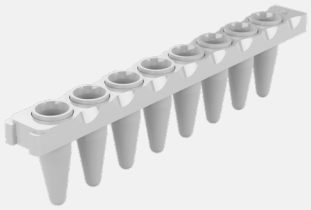
*Both 520174 and 520240 may be used for either the 150 or 350 bp Protocol.

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.

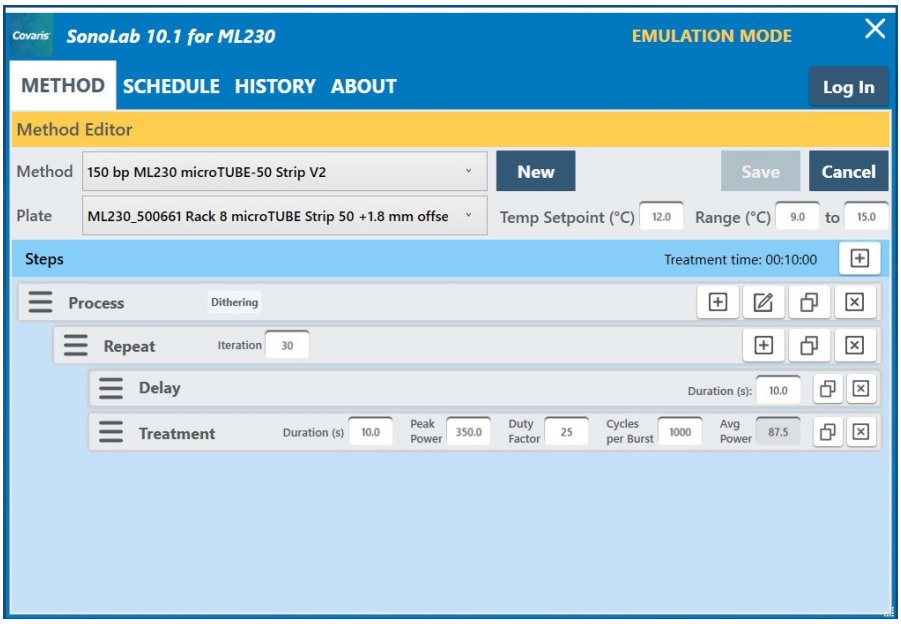
See ***Appendix A*** for screenshots of a programmed protocol in SonoLab 10.1.0. The 8 AFA-TUBE TPX Strip will follow the protocol order for the 8 microTUBE-50: Process, Repeat, Delay, and Treatment. See ***Appendix B*** for how to set up a time course for protocol optimization.

## Appendix A: Protocol on SonoLab 10.1.0 or Higher

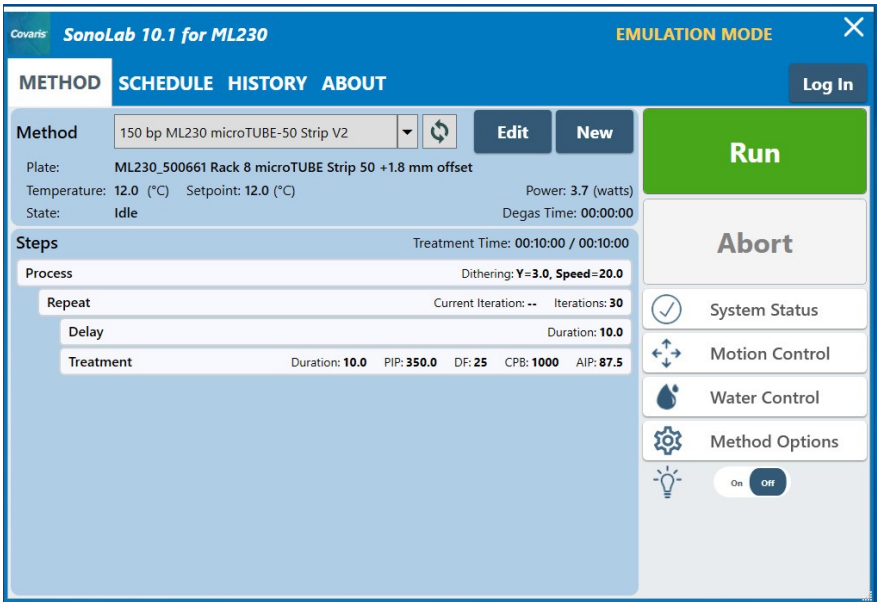
Following are some example screenshots of the 150 bp protocol for the microTUBE-50 in the Method Editor and Method Screen.

### 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

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 | 360 |
| Total Run Duration (sec) | 520 | 560 | 600 | 640 | 680 | 720 |
| 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

## Revision History

| Part Number | Revision | Date | Description of Change |
|---|---|---|---|
| 010522 | A | 7/2020 | Initial release |
| 010522 | B | 10/2020 | Pulsing protocol requirement and typo updates |