Covaris®

User Manual

Covaris DNA Shearing Verification Kit for microTUBE Consumables (PN 520120)

Part Number: 010184 Rev F

April 2020

Introduction

This kit allows users to routinely verify the performance of their Covaris Focused-ultrasonicator. The kit may be used for periodic assurance of performance, for instrument QC, or employed in troubleshooting when applications perform differently than expected. The kit contains a pre-fragmented Reference Sample of Lambda DNA, as well as unfragmented Test Sample of Lambda DNA sufficient for performance testing. Simply shear the Test Sample DNA with your Covaris instrument and compare the results to the Reference Sample, using the Agilent® Bioanalyzer 2100 (or equivalent).

Kit Contents

This kit includes:

- Reference Sample (Blue Cap): 40 µl of pre-fragmented DNA with an average fragment size distribution between 150 and 300 bp.
- Test Sample (Red Cap): Two tubes each containing 1100 µl of Lambda DNA. SDS information is available at: http://covaris.com/wp-content/uploads/pn_010379.pdf

> ***NOTE:*** Please check the lowest and highest allowed DNA concentration of your DNA analyzer prior to shearing and performing DNA distribution analysis.

Customer Supplied Materials

- Fragment Analysis Reagents (Agilent Bioanalyzer DNA 12000 Kit PN 5067-1508, High Sensitivity Kit PN 5067-4626, or equivalent)
- Focused-ultrasonicator: see ***Instrument Tables*** for settings
- microTUBE from the respective Instrument Table

> ***NOTE:*** For the AFA-TUBE TPX, please refer to User Manual 010474: https://covaris.com/wp-content/uploads/pn_010474.pdf

- Holder or Rack for the microTUBE

Storage

- 1 year at 2 °C to 8 °C.

Workflow

- Load the recommended volume for a given microTUBE with Test Sample into 3 separate tubes.
- Process these three samples following instrument settings given in each Instrument Table. For E- and LE-Series instruments, please position the tubes following ***Table 7***.

> ***NOTE:*** The Reference Sample is already fragmented and does not need to be further processed.

Instrument Parameters/Settings

This kit is compatible with the stated microTUBEs and associated holders/racks. Please follow the settings carefully for your Covaris Focused-ultrasonicator and microTUBE that you're using. Please be careful to load the correct volume of sample, to use the matching rack/holder, and the intensifier, if applicable.

M220

Instrument	microTUBE	Holder & Insert	Temp	Sample Volume	Peak Incident Power (PIP)	Duty Factor	Cycles per Burst	Time
M220	microTUBE-50 Screw-Cap (PN 520166)	500414 & 500488	20 °C	55 µl	75 W	10 %	200	260 s
	microTUBE Snap-Cap (PN 520045)	500414 & 500489	20 °C	130 µl	50 W	20 %	200	150 s
	microTUBE-500 Screw-Cap (PN 520185)	500414 & 500471	20 °C	500 µl	75 W	20 %	200	210 s

Table 1. M220 DNA Shearing Settings

ME220

Instrument	microTUBE	Holder & Insert	Temp	Sample Volume	Peak Incident Power (PIP)	Duty Factor	Cycles per Burst	Time
ME220	microTUBE-15 Screw-Cap (PN 520145)	500534 & 500522	20 °C	15 µl	50 W	30 %	50	70 s
	8 microTUBE-15 Strip V2 (PN 520159/520241)	500526 & 500518	20 °C	15 µl	50 W	30 %	50	70 s
	microTUBE-50 Screw-Cap (PN 520166)	500534 & 500522	20 °C	55 µl	75 W	25 %	1000	90 s
	8 microTUBE-50 Strip V2 (PN 520174/520240)	500526 & 500518	20 °C	55 µl	50 W	30 %	1000	125 s
	microTUBE-130 Screw-Cap (PN 520216)	500534 & 500522	20 °C	130 µl	70 W	20 %	1000	140 s
	8 microTUBE-130 Strip V2 (PN 520217/520239)	500526 & 500518	20 °C	130 µl	70 W	20 %	1000	130 s
	microTUBE Pre-slit Snap-Cap (PN 520045)	500526 & 500514	20 °C	130 µl	70 W	20 %	1000	130 s
	microTUBE Crimp-Cap (PN 520052)	500526 & 500514	20 °C	130 µl	70 W	20 %	1000	140 s
	8 microTUBE Strip V1 (PN 520053)	500526 & 500514	20 °C	130 µl	70 W	20 %	1000	130 s

Table 2. ME220 DNA Shearing Settings

ML230

Instrument	microTUBE	Rack	Temp	Sample Volume	Peak Power	Duty Factor	Cycles per Burst	Time	Repeat/ Iterations
ML230	8 microTUBE-50 Strip V2* (PN 520174/520240)	500661	12 °C	55 µl	350 W	15 %	1000	10 s treatment / 10 s delay	35**

Table 3. ML230 DNA Shearing Settings (* Y-dithering function (3mm Y-dither at 20mm/s) required. **See ***Appendix C*** for a screenshot of the programmed protocol.)

S2 and S220

Instrument	microTUBE	Holder	Temp	Water Level	Sample Volume	PIP / Intensity	Duty Cycle/ Factor	Cycles per Burst	Time
S2	microTUBE Snap-Cap (PN 520045)	500114	7 °C	12	130 µl	I = 5	10 %	200	180 s
S220	microTUBE-15 Screw-Cap (PN 500145)	500427	20 °C	15	15 µl	18 W	20 %	50	120 s
	microTUBE-50 Screw-Cap (PN 520166)	500492	7 °C	10	55 µl	75 W	25 %	1000	95 s
	microTUBE Snap-Cap (PN 520045) or Crimp-Cap (PN 520052)	500114	7 °C	12	130 µl	175 W	10 %	200	180 s
	microTUBE-500 Screw-Cap (PN 520185)	500449	7 °C	15	500 µl	175 W	20 %	200	180 s

Table 4. S-series DNA Shearing Settings

E220 and E220*evolution*

Instrument	microTUBE	Rack	Temp	Water Level	Sample Volume	PIP	Duty Cycle/ Factor	Cycles per Burst	Time
E220 & E220*evo*	microTUBE-15 Screw-Cap (PN 520145)	500308 & 500432	20 °C	10	15 µl	18 W	20 %	50	120 s
	8 microTUBE-15 Strip V2 (PN 520159/520241)	500444 & 500437	20 °C	6	15 µl	18 W	20 %	50	120 s
	microTUBE-50 Screw-Cap (PN 520166)	500308 & 500432	7 °C	6	55 µl	75 W	20 %	1000	95 s
	8 microTUBE-50 Strip V2 (PN 520174/520240)	500444 & 500437	7 °C	-2	55 µl	75 W	15 %	500	155 s
	96 microTUBE-50 Plate (PN 520168 /520232)*	N/A	7 °C	0	55 µl	100 W	30 %	1000	90 s
	microTUBE Snap-Cap (PN 520045)	500111 & 500433	7 °C	6	130 µl	175 W	10 %	200	180 s
	microTUBE Crimp-Cap (PN 520052)	500282 & 500433	7 °C	6	130 µl	175 W	10 %	200	180 s
	8 microTUBE Strip V1 (PN 520053)	500191 & 500430	7 °C	6	130 µl	175 W	10 %	200	180 s
	96 microTUBE Plate (PN 520078/520230)	N/A	7 °C	6	130 µl	175 W	10 %	200	180 s
	microTUBE-500 Screw-Cap (PN 520185)	500452 & 500484	7 °C	6	500 µl	175 W	20 %	200	180 s

Table 5. E-series DNA Shearing Settings (See ***Table 7*** for positions)

Please note while using the E220 and E220*evolution*, the intensifier (PN 500141) must remain in place for all microTUBES with the exception of the microTUBE-15. *Y-dithering function (0.5 mm Y-dither at 10 mm/s) required. These functions are only available on SonoLab version 7.3 and up. Please refer to the DNA Shearing Quick Guide for detailed instructions.

LE220 and LE220-plus

Instrument	microTUBE	Rack	Temp	Water Level	Sample Volume	PIP	Duty Cycle/ Factor	Cycles per Burst	Time
LE220 & LE220-plus	8 microTUBE-15 Strip V2 (PN 520159/520241) *	500445	20 °C	4	15 µl	180 W	30 %	50	120 s
	8 microTUBE-50 Strip V2 (PN 520174/520240) **	500485	7 °C	-2	55 µl	450 W	20 %	1000	160 s
	96 microTUBE-50 Plate (PN 520168/520232) **	N/A	7 °C	-2	55 µl	450 W	20 %	1000	200 s
	microTUBE Crimp-Cap (PN 520052)	500282	7 °C	6	130 µl	450 W	30 %	200	175 s
	8 microTUBE Strip V1 (PN 520053)	500191	7 °C	6	130 µl	450 W	30 %	200	175 s
	96 microTUBE Plate (PN 520078/520230)	NA	7 °C	6	130 µl	450 W	30 %	200	190 s

Table 6. LE-series DNA Shearing Settings (See ***Table 7*** for positions)

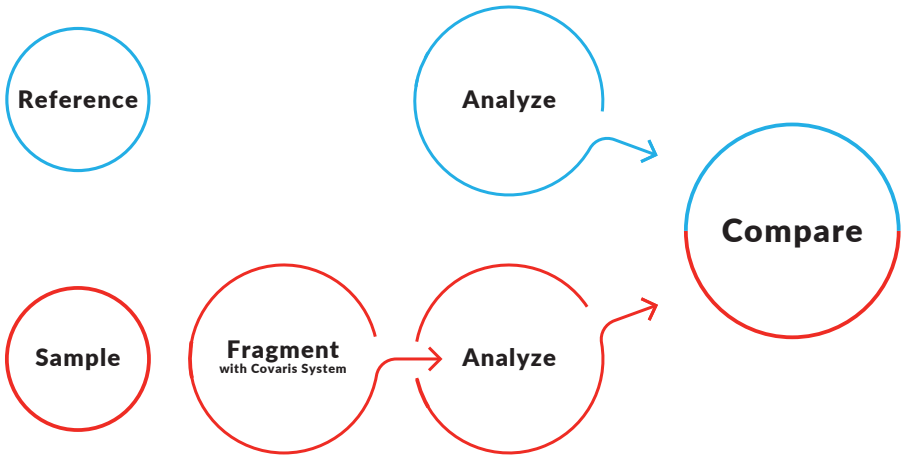
* Y-dithering function (5mm Y-dither at 20mm/s) required. **X and Y-dithering function (0.5 mm X-dither & 0.5 mm Y-dither at 10 mm/s) required. These functions are only available on SonoLab version 7.3 and up. Please refer to the DNA Shearing Quick Guide for detailed instructions.

	Position of Sample #1	Position of Sample #2	Position of Sample #3
24 well rack	A1	B3	D6
96 well rack	A1	D6	H12

Table 7. Test samples position in an E or LE-Series Covaris instrument.

Analysis

- Analyze the fragment size distribution of both Reference and Processed Test Samples on the same chip.
- We recommend running 3 replicates of the Reference Sample and averaging the values to compare to the Test Samples.
- Compare fragment size distributions to verify that your Covaris Focused-ultrasonicator is performing correctly.



Interpretation

For analysis, employ the available analysis device (Agilent Bioanalyzer 2100, Agilent Fragment Analyzer, Perkin Elmer® LabChip, Agilent 2200 TapeStation, Bio-Rad® Experion, Agarose gel, or equivalent). It is important to run both the Reference and Processed Test Samples on the same chip or gel to normalize the results from analytical assay variations.

For each sample, determine the peak size of the fragment distribution. For the Reference Sample replicates, calculate the average and the Coefficient of Variation. For the sixteen Processed Test Samples, calculate the average and the Coefficient of Variation. Compare the peak size and fragment distribution of the Reference and Processed Test Samples using ***Table 8***.

	Average of Processed Test Samples within +/- 15% of Reference Sample	Average of Processed Test Samples more than 15% different from Reference Sample
Coefficient of Variation of Processed Samples < 15%	Covaris system OK	Contact Covaris
Coefficient of Variation of Processed Samples > 15%	Contact Covaris	Contact Covaris
Reference Sample in the 100-300 bp range	Covaris system OK	Contact Covaris
Reference Sample out of the 100-300 bp range	Problem with fragment size distribution analysis	Contact Covaris

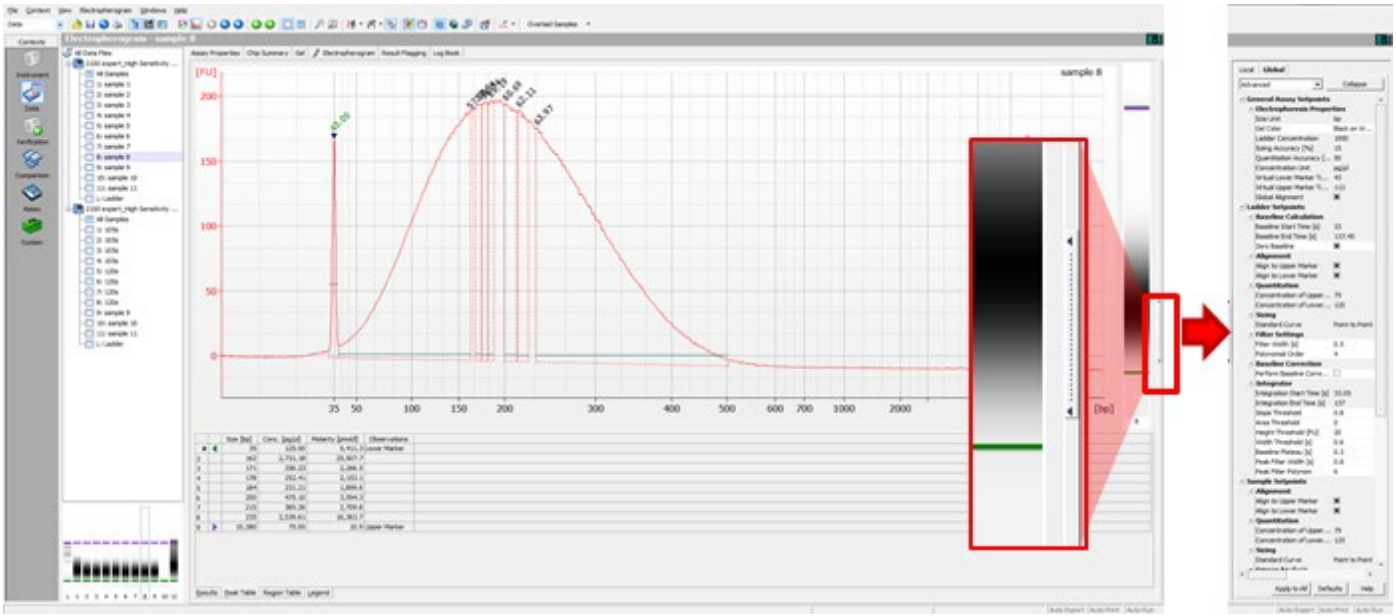
Table 8 Covaris Performance Verification Kit interpretation

Covaris Contact: Applicationsupport@covaris.com

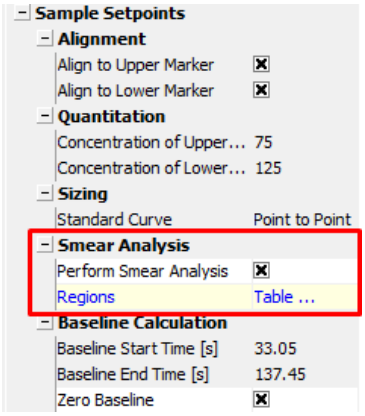
Appendix A: Detailed Instructions for using the Agilent Bioanalyzer 2100

To perform average fragment size (smear) analysis using the Agilent Bioanalyzer 2100, follow the steps provided below:

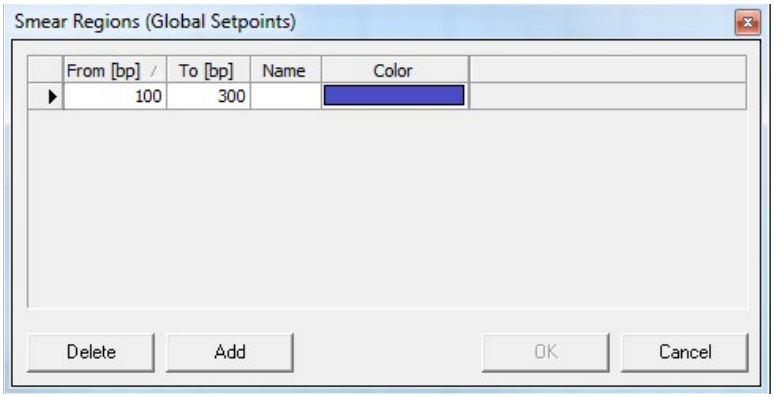
1. Select the “Global” tab on the right side of the screen and click “Advanced” on the drop-down menu.
    a. If you cannot see the “Global” tab click on the “.....” to the right side of the screen.



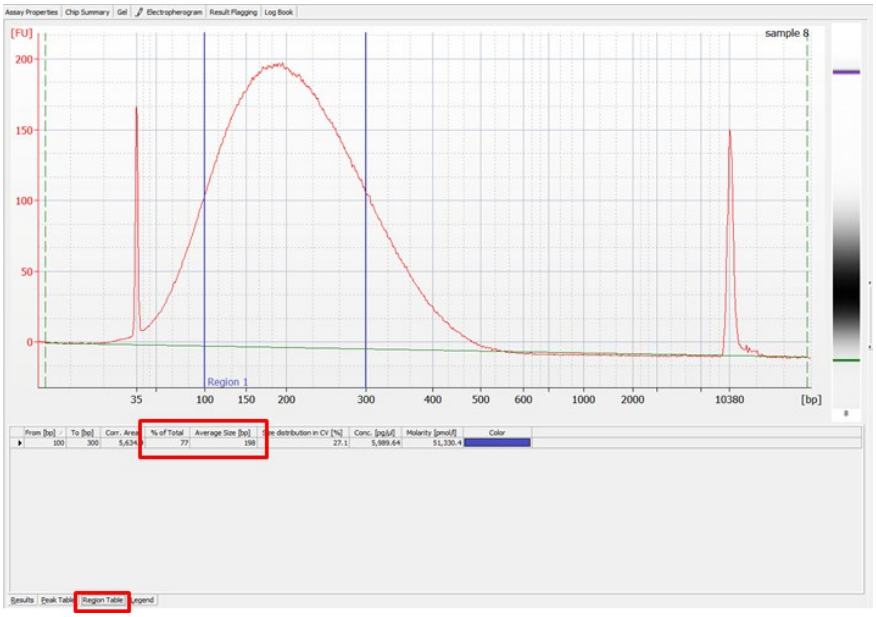
2. Scroll down to “Smear Analysis” under “Sample Setpoints”.



3. Click the box next to “Perform Smear Analysis”.
4. Double click “Table ...” located to the right of “Regions” to open the “Smear Regions” window.



5. Click “Add” to create a new smear region or edit the Smear Region if there is one populated.
6. Double click the values under “From [bp]” and “To [bp]” and enter “100” to “300” then click “OK”.



7. In the main window for each sample, the "Region Table" tab will be populated, and the Region will be marked in the electropherogram.

   Note the "% of Total" and "Average Size [bp]" values in the "Region Table". The "% of Total" for the Reference Standard should be >50%.

> ***CAUTION:*** A spike in the fragment distribution or a bump in the baseline may occur in some Agilent Bioanalyzer runs. If this occurs, the accuracy of "% of Total" value will be compromised. In this case, please re-run samples on a new chip.

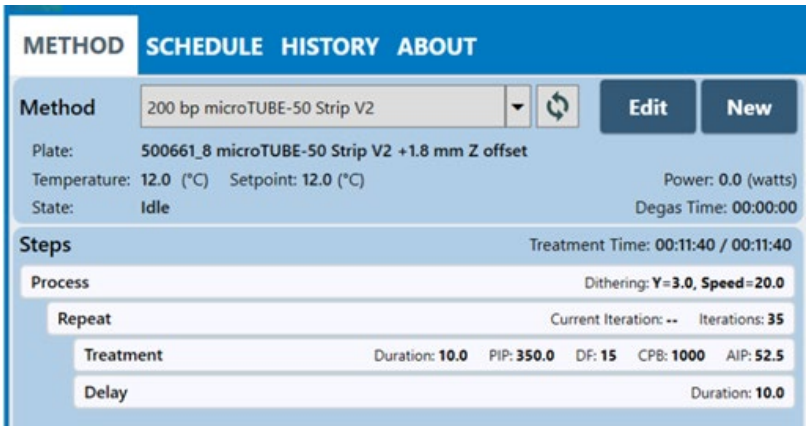
8. Repeat the smear analysis for the Reference Sample and each processed Test Sample.

Appendix B: Troubleshooting

- "% of Total" for the Reference Sample should be > 50%. If it is < 50%, there is a problem with the fragment size distribution analysis. Please check that the Bioanalyzer is functioning correctly then repeat with a new chip.
- If the Coefficient of Variation of the sixteen Processed Test Samples is > 15% or if the average fragment size is > 15% different from the Reference Sample, contact Covaris at Applicationsupport@covaris.com
- The "% of Total" takes into account the area below the upper and lower marker, so the results are dependent on sample concentration and do not reflect the actual area of the fragment distribution in the range of interest. It is therefore critical to load the same volume, and the same concentration of Reference and Processed Test Samples.

Appendix C: ML230 Protocol Screenshot

The Figure below depicts the pulsing protocol and user interface for the ML230.



Revision History

Part Number	Revision	Date	Description of Change
010184	D	09/2017	Remove obsolete holders, incorporate new instruments and consumables
010184	E	07/2019	Updated M220 microTUBE-50 protocol for 55 µL volume
010184	F	04/2020	Addition of ML230. Separate Instrument Tables. Updated format.