User Manual

Covaris DNA Shearing Verification Kit (PN 520120)

REVISION HISTORY

PART NUMBER	REVISION	DATE	DESCRIPTION OF CHANGE
010184	D	09/17	Remove obsolete holders, incorporate new instruments and consumables

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, or employed in troubleshooting when applications perform differently than expected. The kit contains a Reference Sample of genomic DNA, pre-fragmented, and a volume of un-fragmented Test genomic DNA sufficient for five performance tests. Simply shear the Test DNA with your Covaris instrument and compare the results to the Reference, using the Agilent® Bioanalyzer 2100 (or equivalent).

KIT CONTENTS

The kit contains sufficient material to perform up to 5 verification tests. This kit includes:

- Reference Sample (Blue Cap): 40 µl of pre-fragmented DNA with an average fragment size distribution between 150 and 250 bp.
- Test Sample (Red Cap): Two tubes each containing 1100 µl of genomic DNA.

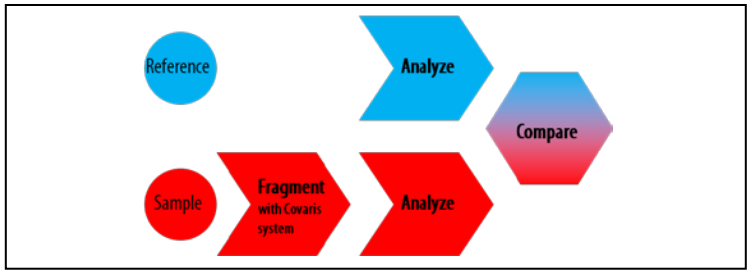
SDS information is available at: http://covarisinc.com/wp-content/uploads/pn_010379.pdf

STORAGE

- 1 year at 4 °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 Table 1. For E- and LE-Series instruments, please position the tubes following Table 2.
  The Reference Sample is already fragmented and does not need to be further processed.
- Analyze the fragment size distribution of both Reference and Processed Test samples on the same chip.
- Compare fragment size distributions to verify that your Covaris Focused-ultrasonicator is performing correctly.



INSTRUMENT PARAMETERS / SETTINGS

This kit is compatible with all microTUBEs and associated holders / racks. Please follow the settings carefully for your Covaris Focused-ultrasonicator and the Covaris 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.

Instrument		microTUBE	Holder / Rack / Insert	Temp	Water Level	Sample Volume	PIP / Intensity	Duty Cycle/ Factor	Time	Cycles per Burst
M-Series	M220	microTUBE-50 Screw-Cap (PN520166)	#500414 + #500488	20°C	N/A	50 µl	75 W	10 %	195 s	200
		microTUBE Snap-Cap (PN520045)	#500414 + #500489	20°C	N/A	130 µl	50 W	20 %	150 s	200
		microTUBE-500 Screw-Cap (PN520185)	#500414 + #500471	20°C	N/A	500 µl	75 W	20 %	210 s	200
	ME220	microTUBE-15 Screw-Cap (PN520145)	#500534 + #500522	20°C	9.5	15 µl	50 W	30 %	70 s	50
		8 microTUBE-15 Strip V2 (PN520159 / 520241)	#500526 + #500518	20°C	9.5	15 µl	50 W	30 %	70 s	50
		microTUBE-50 Screw-Cap (PN520166)	#500534 + #500522	20°C	5.5	55 µl	75 W	25 %	90 s	1000
		8 microTUBE-50 Strip V2 (PN520174 / 520240)	#500526 + #500518	20°C	5.5	55 µl	50 W	30 %	125 s	1000
		microTUBE-130 Screw-Cap (PN520216)	#500534 + #500522	20°C	9	130 µl	70 W	20 %	140 s	1000
		8 microTUBE-130 Strip V2 (PN520217 / 520239)	#500526 + #500518	20°C	9	130 µl	70 W	20 %	130 s	1000
		microTUBE Pre-slit Snap-Cap (PN520045)	#500526 + #500514	20°C	6	130 µl	70 W	20 %	130 s	1000
		microTUBE Crimp-Cap (PN520052)	#500526 + #500514	20°C	7	130 µl	70 W	20 %	140 s	1000
		8 microTUBE Strip V1 (PN520053)	#500526 + #500514	20°C	6	130 µl	70 W	20 %	130 s	1000
S-Series	S2	microTUBE Snap-Cap (PN520045)	#500114	7°C	12	130 µl	I = 5	10 %	180 s	200
	S220	microTUBE-15 Screw-Cap (PN500145)	#500427	20°C	15	15 µl	18 W	20 %	120 s	50
		microTUBE-50 Screw-Cap (PN520166)	#500492	7°C	10	55 µl	75 W	25 %	95 s	1000
		microTUBE Snap-Cap (PN520045) or Crimp-Cap (PN520052)	#500114	7°C	12	130 µl	175 W	10 %	180 s	200
		microTUBE-500 Screw-Cap (PN520185)	#500449	7°C	15	500 µl	175 W	20 %	180 s	200
E-Series	E220 / E220e	microTUBE-15 Screw-Cap (PN520145)	#500308 / #500432	20°C	10	15 µl	18 W	20 %	120 s	50

		8 microTUBE-15 Strip V2 (PN520159 / 520241)	#500444 / #500437	20°C	6	15 µl	18 W	20 %	120 s	50
		microTUBE-50 Screw-Cap (PN520166)	#500308 / #500432	7°C	6	55 µl	75 W	20 %	95 s	1000
		8 microTUBE-50 Strip V2 (PN520174 / 520240)	#500444 / #500437	7°C	-2	55 µl	75 W	15 %	155 s	500
		96 microTUBE-50 Plate (PN520168 / 520232) *	N/A	7°C	0	55 µl	100 W	30 %	90 s	1000
		microTUBE Snap-Cap (PN520045)	#500111 / #500433	7°C	6	130 µl	175 W	10 %	180 s	200
		microTUBE Crimp-Cap (PN520052)	#500282 / #500433	7°C	6	130 µl	175 W	10 %	180 s	200
		8 microTUBE Strip V1 (PN520053)	#500191 / #500430	7°C	6	130 µl	175 W	10 %	180 s	200
		96 microTUBE Plate (PN520078 / 520230)	N/A	7°C	6	130 µl	175 W	10 %	180 s	200
		microTUBE-500 Screw-Cap (PN520185)	#500452 / #500484	7°C	6	500 µl	175 W	20 %	180 s	200
L-Series	LE220 and LE220-plus	8 microTUBE-15 Strip V2 (PN520159 / 520241) **	#500445	20°C	4	15 µl	180 W	30 %	120 s	50
		8 microTUBE-50 Strip V2 (PN520174 / 520240)	#500485	7°C	-2	55 µl	450 W	20 %	160 s	1000
		96 microTUBE-50 Plate (PN520168 / 520232) ***	N/A	7°C	-2	55 µl	450 W	20 %	200 s	1000
		microTUBE Crimp-Cap (PN520052)	#500282	7°C	6	130 µl	450 W	30 %	175 s	200
		8 microTUBE Strip V1 (PN520053)	#500191	7°C	6	130 µl	450 W	30 %	175 s	200
		96 microTUBE Plate (PN520078 / 520230)	NA	7°C	6	130 µl	450 W	30 %	190 s	200

Please note while using the E220 and E220evolution, the intensifier (PN500141) must remain in place for all microTUBES with the exception of the microTUBE-15. *Y-dithering function (0.5mm Y-dither at 10mm/s) required. **Y-dithering function (5mm Y-dither at 20mm/s) required. * X and Y-dithering function (0.5mm X-dither & 0.5mm Y-dither at 10mm/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.

Table 1 – Covaris Instrument DNA Shearing Settings

	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 2 – Test samples position in an E or LE-Series Covaris instrument

INTERPRETATION

For analysis, employ the available analysis device (Agilent Bioanalyzer 2100, Caliper® LabChip, Agilent® 2200 TapeStation, Bio-Rad® Experion, Agarose gel, etc.). 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 three Processed Test Samples, calculate the average and the Coefficient of Variation. Compare the peak size of the Reference and Processed Test Samples using Table 3.

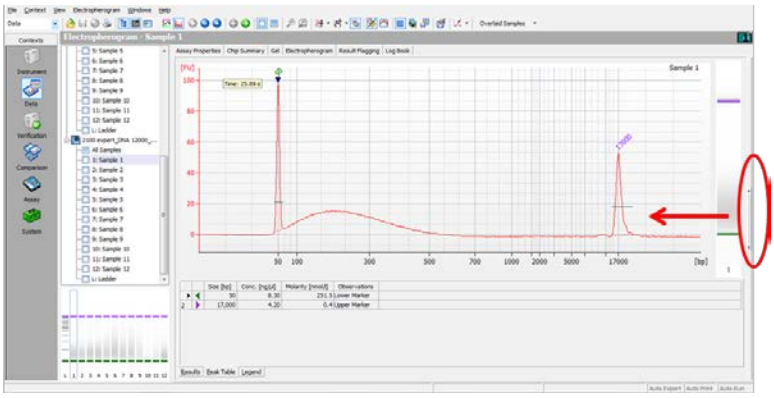
	Average of Processed Test Samples within +/- 15% of Reference Sample	**Average of Processed Test Samples more than 15% different from Reference Sample**
Coefficient of Variation of Processed Samples < 10%	Covaris system OK	Contact Covaris
Coefficient of Variation of Processed Samples > 10%	Contact Covaris	Contact Covaris
Reference Sample in the 150-250 bp range	Covaris system OK	Contact Covaris
Reference Sample out of the 150-250 bp range	Problem with fragment size distribution analysis	Contact Covaris

Table 3 – Covaris Performance Verification Kit interpretation

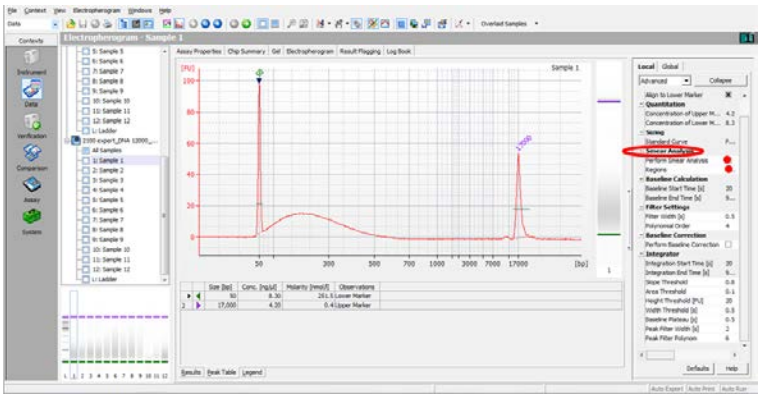
Covaris Contact: **Applicationsupport@covaris.com**

DETAILED INSTRUCTIONS FOR USING THE AGILENT® BIOANALYZER 2100

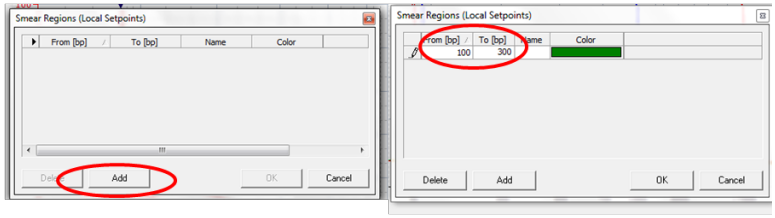
- Load both Reference and Processed Test Samples on an Agilent Chip following the manufacturer's instructions.
  - 12k, 7.5k and 1k Chip: load 1 µl of both the Reference and Processed Test DNA Samples.
  - High Sensitivity: dilute both Reference and Processed Test DNA Samples 1:10 and load 1 µl on the Chip.
- Analyze the results.
  - Open the Analysis tab on the right of the 2100 Expert Software by clicking on the dots and sliding your mouse on the left.



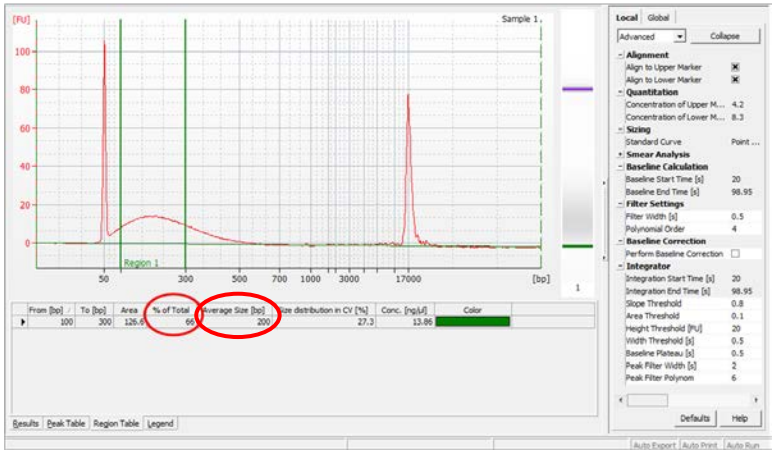
  - In the "Global" tab, go in the "Smear Analysis" section and click on "Perform Smear Analysis", then click on the dots following "Regions".



- In the “Regions” window, click on “Add” to add a new region starting at 100 bp and finishing at 300 bp. Click “OK”.



- In the main window, click on “Region Table” at the bottom and note the value for “% of Total” and “Average Size”.



- Repeat the smear analysis for the Reference Sample and each one of the Processed Test Samples.
  ⚠ A spike in the fragment distribution or bump in the baseline may happen in some Agilent Bioanalyzer runs and will significantly compromise the accuracy of the “% of Total” value. In this case, please re-run the samples on a new Chip.
- “% of Total” for the Reference Sample should be > 50%. If < 50%, this indicates a problem with the fragment size distribution analysis. Please check that the Bioanalyzer is functioning correctly then repeat with a new chip.
- As an alternate to a peak size analysis, calculate the “% of Total” of the three Processed Test Samples, average these results, calculate the Coefficient of Variation, and use Table 3 to determine the status of your Covaris Instrument.
- If the Coefficient of Variation of the three Processed Test Samples is > 10% or if the average is > 15% different from the Test 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.