User Manual

Covaris DNA Shearing Verification Kit

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 genomic DNA sufficient for five performance tests. Simply shear the DNA with your Covaris instrument and compare results to the Reference, employing your analyzer (Covaris recommends the Agilent® Bioanalyzer 2100).

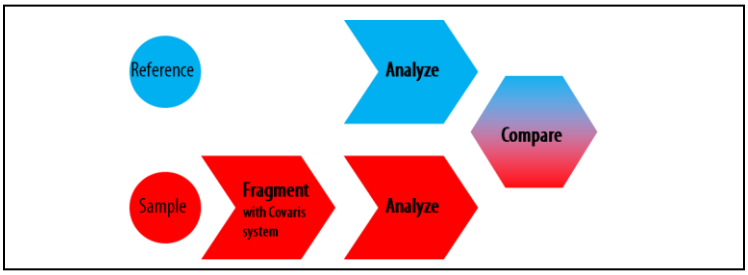
Kit Contents

The kit is sufficient to perform 5 tests.

- 🔵 Reference Sample. 40 µl of pre-fragmented DNA with a mean fragment size distribution between 150 and 250 bp.
- 🔴 Test Sample. Two tubes with 1100 µl of genomic DNA each.

Workflow

- Load three microTUBE with Test Sample (red cap tube 🔴).
- Process these three samples following instrument settings given in Tables 1 and 2. For E- and LE-Series, please follow Table 3 to position the Test Samples. Note: The Reference Sample (blue cap tube 🔵) is already fragmented and does not need to be further processed.
- Analyze the fragment size distribution of both Reference and Processed Test samples.
- Compare fragment size distributions to verify that your Covaris Focused-ultrasonicator is performing properly.



Storage

- 1 year at 4 °C

Instrument Parameters / Settings

It is important to properly follow the settings in Table 1 and 2, as well as to use the right Covaris consumables. This kit is compatible with all microTUBE and associated holders / racks. Settings will depend on the Covaris Focused-ultrasonicator and the Covaris microTUBE that you're using. Please be careful to load the correct volume of sample, to use the correct rack/ holder and intensifier if applicable.

Instrument		microTUBE	Holder or rack	Intensifier #500141	Sample Volume	PIP or Intensity	Duty Cycle/ Factor	Time	Cycles per Burst
M-Series	M220	**microTUBE** Snap-Cap (PN520045)	#500414 and #500489	NA	130 µl	50 W	20 %	150 s	200
		microTUBE-50 Screw-Cap (PN520166)	#500414 and #500488	NA	50 µl	75 W	10 %	195 s	200
		microTUBE Snap-Cap (PN520045)	#500301	NA	130 µl	50 W	20 %	160 s	200
S-Series	S2	**microTUBE** Snap-Cap (PN520045) or Crimp-Cap (PN520052)	#500114	NA	130 µl	I = 5	10 %	180 s	200
	S220	**microTUBE** Snap-Cap (PN520045) or Crimp-Cap (PN520052)	#500114	NA	130 µl	175 W	10 %	180 s	200
E-Series	E210	**microTUBE** Snap-Cap (PN520045)	#500111	Yes	130 µl	I = 5	10 %	180 s	200
	E210	**microTUBE** Crimp-Cap (PN520052)	#500182	Yes	130 µl	I = 5	10 %	180 s	200
	E210	**microTUBE** Crimp-Cap (PN520052)	#500143	Yes	130 µl	I = 5	10 %	180 s	200
	E210	96 **microTUBE** Plate (PN520078)	NA	Yes	130 µl	I = 5	10 %	180 s	200
	E220	**microTUBE** Snap-Cap (PN520045)	#500111	Yes	130 µl	175 W	10 %	180 s	200
	E220	**microTUBE** Crimp-Cap (PN520052)	#500182	Yes	130 µl	175 W	10 %	180 s	200
	E220	**microTUBE** Crimp-Cap (PN520052)	#500143	Yes	130 µl	175 W	10 %	180 s	200
	E220	96 **microTUBE** Plate (PN520078)	NA	Yes	130 µl	175 W	10 %	180 s	200
L-Series	LE220	**microTUBE** Crimp-Cap (PN520052)	#500282	NA	130 µl	450 W	30 %	175 s	200
	LE220	8 **microTUBE** Strip (PN520053)	#500191	NA	130 µl	450 W	30 %	175 s	200
	LE220	96 **microTUBE** Plate (PN520078)	NA	NA	130 µl	450 W	30 %	190 s	200

Table 1 – Covaris Instrument DNA Shearing Settings

Instrument	Intensifier #500141	Water Bath Temperature	Water Level
M-Series	NA	20 °C	NA
S-Series	NA	7 °C	12
E-Series	Yes	7 °C	6
L-Series	NA	7 °C	6

Table 2 – Covaris Instruments Setup

	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 3 – 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.). In all cases, it is important to run both the Reference and Processed Test Samples on the same chip or gel to normalize your 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.

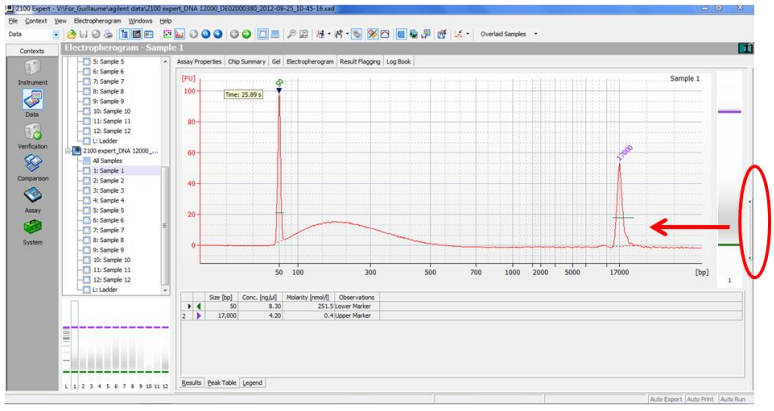
	Average of Processed Samples within +/- 15% of Reference Sample	**Average of Processed Samples more than 15% different from Reference Sample**
Coefficient of Variation of Processed Samples < 10%	Covaris system OK	Contact Covaris
Coefficient of Variation of Processed Samples > 10%	Contact Covaris	Contact 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

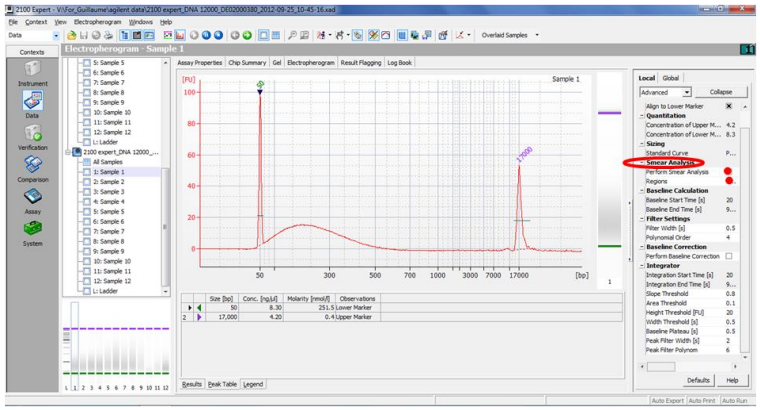
Covaris Contact: TechSupport@covarisinc.com

DETAILED INSTRUCTIONS FOR AGILENT® BIOANALYZER 2100

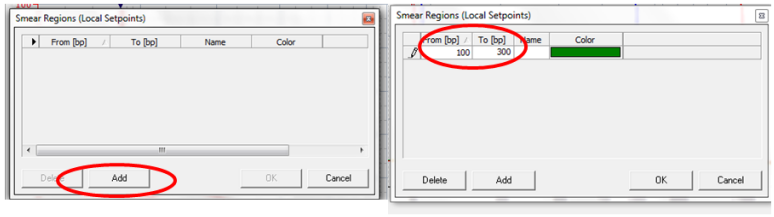
- Load both Reference and Processed Test Samples on an Agilent Chip following manufacturer's instructions
  - 12k, 7.5k and 1k chip: load 1 µl of both the Reference and Processed Test DNA Samples
  - HiSensitivity: dilute both Reference and Processed Test DNA Samples 1:10 and load 1 µl on the Chip
- 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



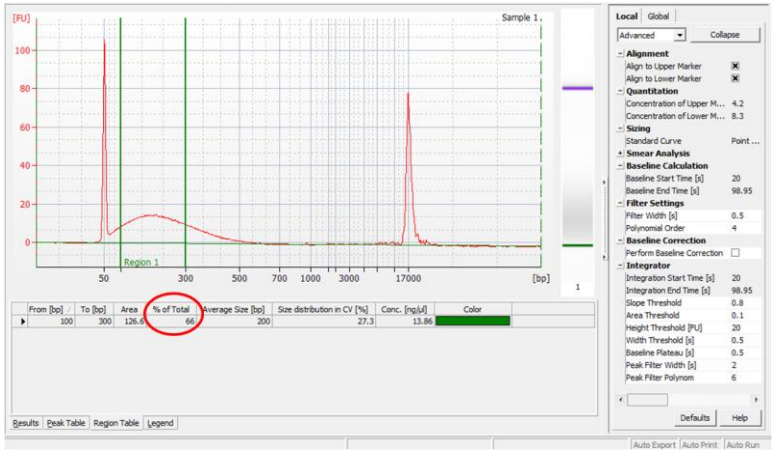
  - In the "Global" tab, go in the "Smear Analysis" section and click on "Perform Smear Analysis", then click on the dots following "Regions"



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



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



- Repeat this step for the Reference Sample and each one of your processed Test Samples
  Note: Spike in the fragment distribution or bump in the baseline may happen in some Agilent Bioanalyzer run 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 only should be > 50%. If not, it means a problem with the fragment size distribution analysis. Please double check that you’re Bioanalyzer is running properly and run a new chip.
- As an alternate to a peak size analysis, you may calculate the “% of Total” of your 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 your three processed Test Samples is > 10% or if their average is > 15% different from the Test Sample, contact Covaris at **TechSupport@covarisinc.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.