User Manual

Covaris DNA Shearing Verification Kit for the AFA-TUBE TPX (PN 520275 and PN 520272) on the LE220-plus (PN 520120)

REVISION HISTORY

PART NUMBER	REVISION	DATE	DESCRIPTION OF CHANGE
010474	A	12/2018	New document
010474	B	4/2019	Updating name of oneTUBE to AFA-TUBE
010474	C	5/2019	Add AFA-TUBE plate. Update part numbers. Update AFA Settings

INTRODUCTION

This kit allows users to verify the performance of their LE220-plus 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 Reference Sample of Lambda-DNA, pre-fragmented, as well as un-fragmented Test Sample of Lambda-DNA sufficient for 1 performance test. Covaris recommends diluting both the Test Sample and the Reference Sample 1:3 in TE buffer and performing the analysis on a high sensitivity DNA analysis kit. Alternatively, the undiluted solutions can be used in combination with a standard sensitivity DNA analysis kit. 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 250 bp.
- Test Sample (Red Cap): One tube containing 1100 µl of Lambda DNA.

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

Note: Please check the lowest and highest allowed DNA concentration of your DNA analyzer prior to run shearing and perform DNA distribution analysis.

- The kit contains enough material to perform one verification test (4 strips, n=32) of 20 µl samples of the Test Sample DNA solution at 30 ng/µl.
- We recommend diluting the Test Sample 1:3 with TE Buffer for a DNA concentration of 10 ng/µl. This kit may be used to perform up to four verification tests (18 strips, n=144) with the 1:3 DNA solution at 10 ng/µl.

CUSTOMER SUPPLIED MATERIALS

- Fragment Analysis Reagents (Agilent Bioanalyzer High Sensitivity DNA Kit PN 5067-4626 or equivalent)
- 8 AFA-TUBE TPX Strips (PN 520275) or 96 AFA-TUBE TPX Plates (PN 520272)
- 1x Tris-EDTA, pH 8.0 (TE buffer, optional for dilution of samples)

STORAGE

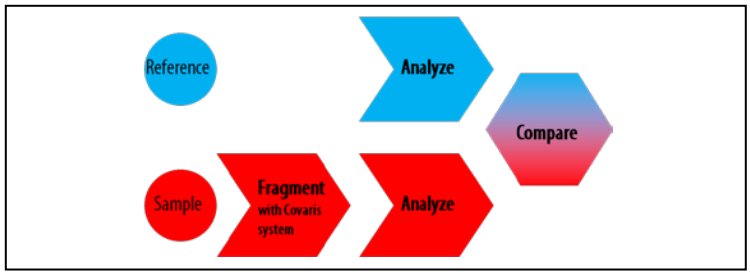
- 1 year at 2-8 °C.

WORKFLOW

- It is recommended to dilute the Test Sample (Red Cap) 1:3 in TE buffer by transferring the entire contents of the vial to an appropriately sized vial (holds >3.3mL) containing 2200 µl TE buffer. Mix well.
    - If the Test Sample is diluted, also dilute the Reference Sample (Blue Cap) 1:3 in TE Buffer. Quick spin the tube and transfer the entire contents of the vial to an appropriately sized vial (holds >1.2mL) containing 80 µl TE buffer. Mix well.
- Fill 50 µl of either the stock Test Sample solution (Red Cap) or the 1:3 diluted Test Sample into:
    - **AFA-TUBE TPX Strip:** two strips (PN 520275) placed in position 1 and 6 of the LE220-plus Rack 8 AFA-TUBE TPX Strip (PN 500608)
    - **AFA-TUBE TPX Plate:** one plate (PN 520272) placed in the Rack 96 AFA-TUBE TPX Plate (PN 500637)
- Process these samples following instrument settings given in Table 1.

⚠ **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. Note, two Bioanalyzer chips will be required per verification test. Load the Reference standard in the first and last position used of each chip and the verification samples in the remaining positions between the Reference standards. If running undiluted samples on a High Sensitivity assay, dilute all samples 1:3 before loading on the chip.
- Compare fragment size distributions to verify that your Covaris Focused-ultrasonicator is performing correctly.



INSTRUMENT PARAMETERS / SETTINGS

This kit is compatible with the 8 AFA-TUBE TPX Strip (PN 520225). Please follow the settings carefully for your LE220-plus Focused-ultrasonicator and the AFA-TUBE TPX consumable. Ensure you are using the matching rack for your consumable.

Instrument	AFA-TUBE	Rack	Plate Definition	Temp	Sample Volume	PIP	Duty Factor	Cycles per Burst	Time
LE220-plus	520275	500608	"LE220plus_500608 8 AFA-TUBE TPX Strip	10 °C	20 µl	200 W	25 %	50	290 s

			-2.2 offset"						
LE220-plus	520272	500637	"LE220plus_500637 96 AFA-TUBE Plate Strip -2.2 offset"	10 °C	20 µl	200 W	25 %	50	290 s

The Y-dithering function (1mm Y-dither at 20mm/s) is required. This function is only available on SonoLab version 8.3 and up. Please refer to the DNA Shearing Quick Guide for detailed instructions.

Table 1 – Covaris Instrument DNA Shearing Settings

INTERPRETATION

For analysis, employ the available analysis device (Agilent Bioanalyzer 2100, Fragment Analyzer AATI, Caliper® 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 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 2.

	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

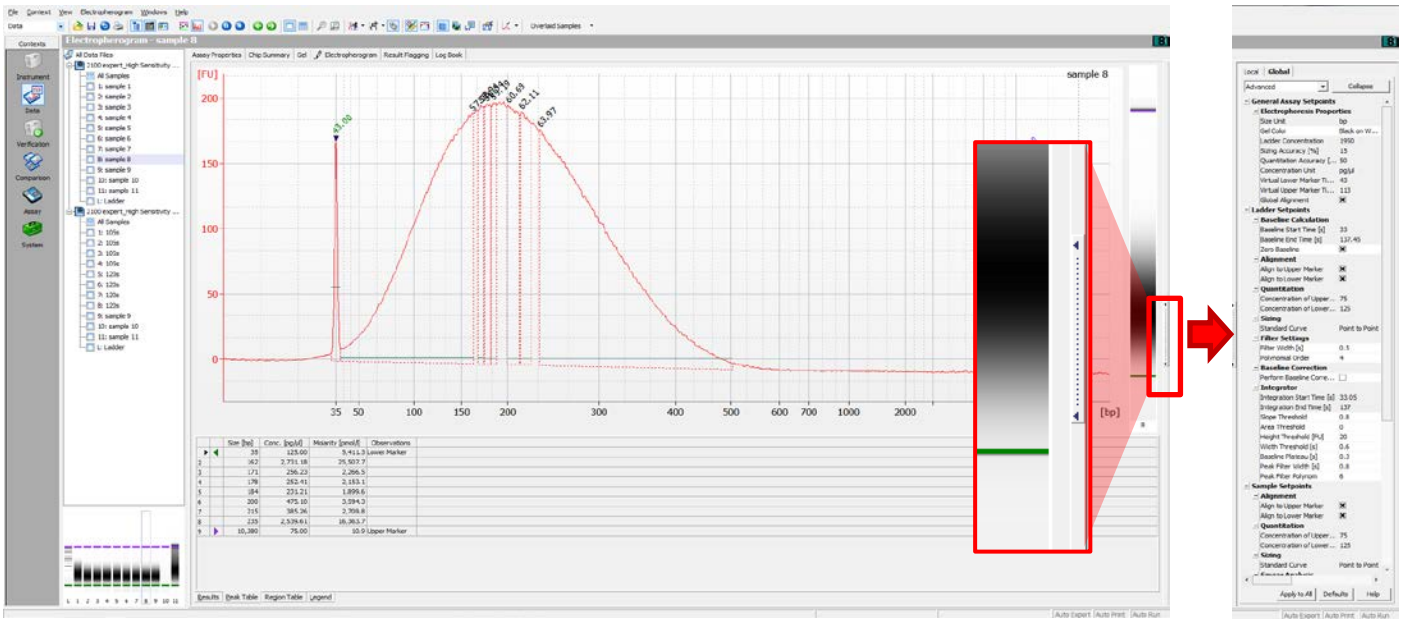
Table 2 – Covaris Performance Verification Kit interpretation

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

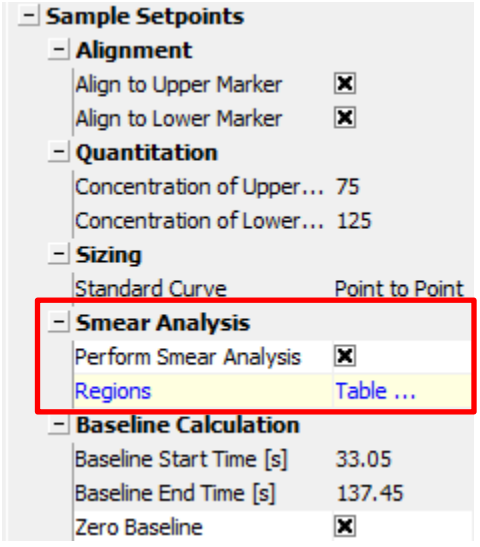
DETAILED INSTRUCTIONS FOR USING THE AGILENT® BIOANALYZER 2100

To perform smear analysis using the Agilent Bioanalyzer 2100, follow the steps provided below:

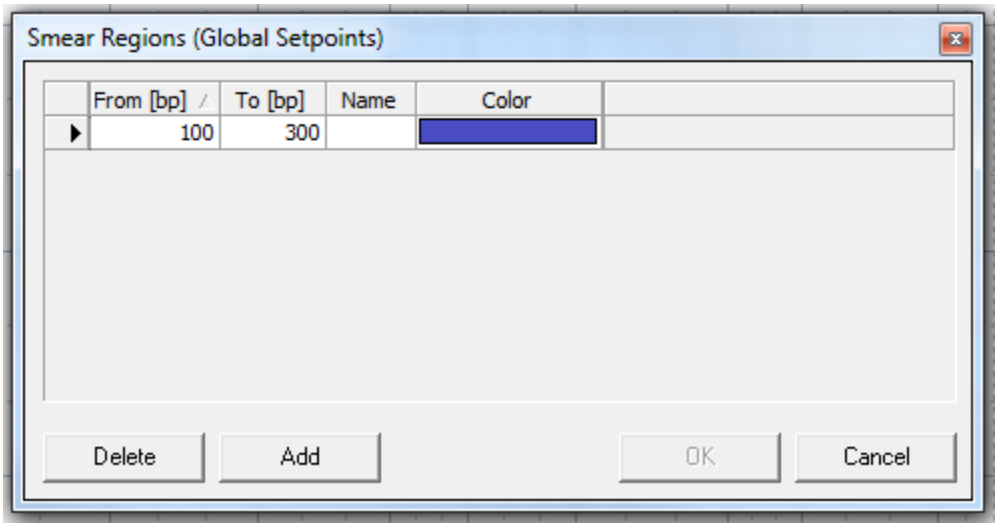
1. Select the "Global" tab on the right side of the screen and click "Advanced" on the drop-down menu.
    a. If you cannot see the "……" to the right side of the screen.



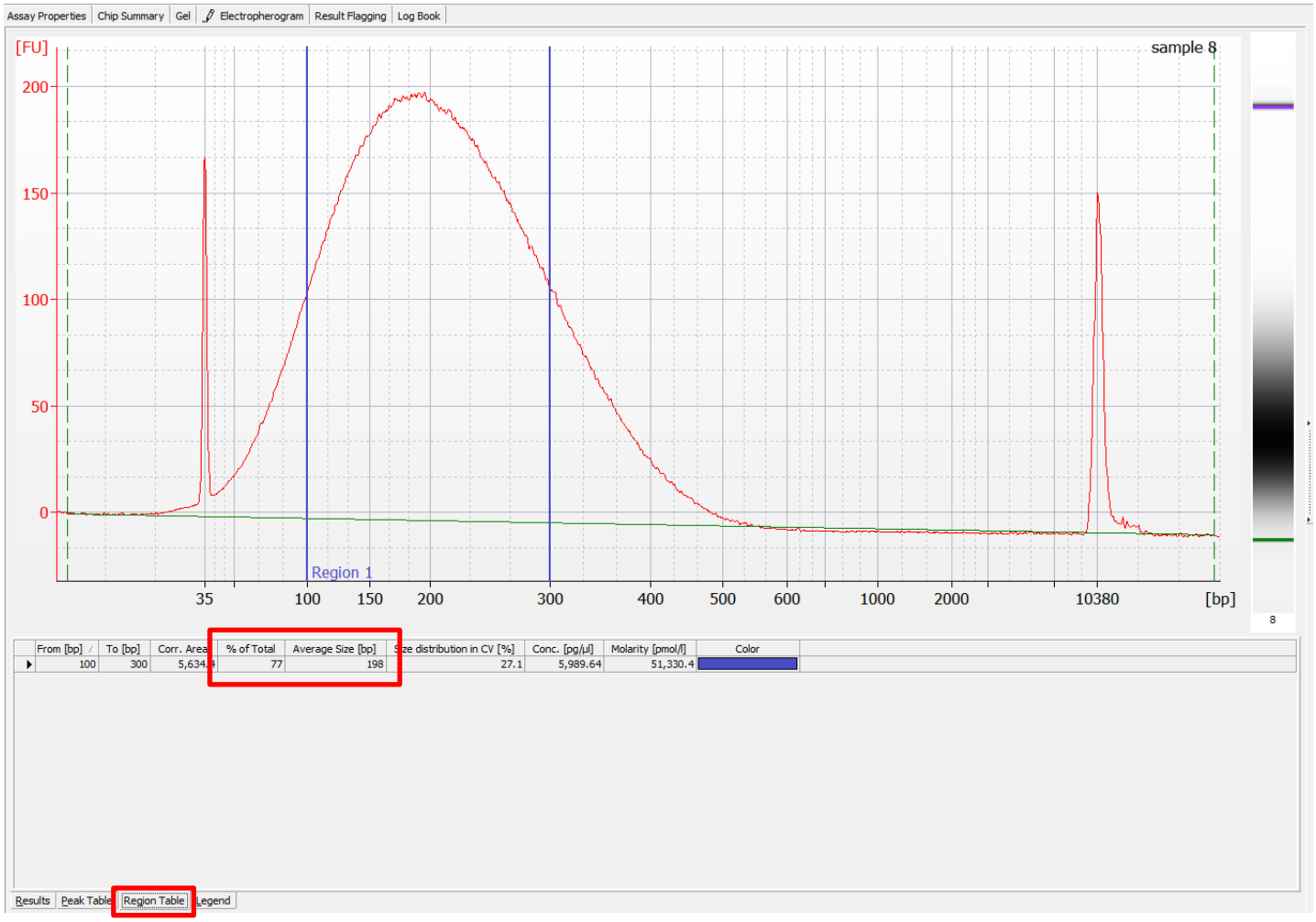
2. Scroll down to "Smear Analysis" under "Sample Setpoints".



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.

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.