

User Manual

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

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

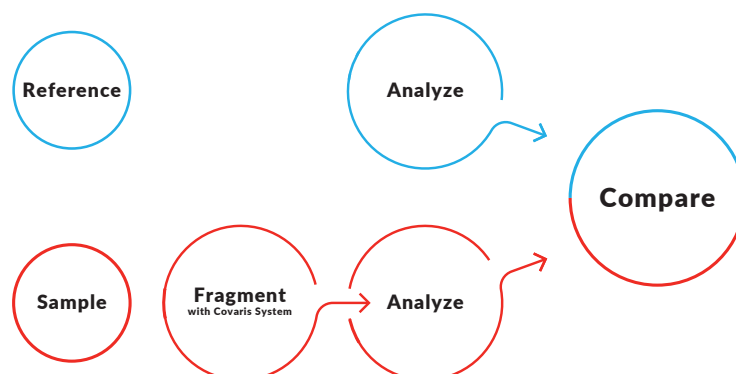
Storage

- 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 10**.

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 are using. Please be careful to load the correct volume of sample, to use the matching rack/holder, and the intensifier, if applicable.

M220

microTUBE	Holder & Insert	Temperature	Sample Volume	Peak Incident Power (PIP)	Duty Factor	Cycles per Burst	Time
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

microTUBE	Holder & Insert	Rack Definition	Temp.	Sample Volume	Peak Incident Power (PIP)	Duty Factor	Cycles per Burst	Time
microTUBE-15 Screw-Cap (PN 520145)	500534 & 500522	4 microTUBE-15 PN 520145.2	20 °C	15 µl	50 W	30 %	50	70 s
8 microTUBE-15 Strip V2 (PN 520159/520241)	500526 & 500518	8 microTUBE-15 Strip V2 PN 520159.2/ 8 microTUBE-15 H-slit Strip V2 PN 520241.2	20 °C	15 µl	50 W	30 %	50	70 s
microTUBE-50 Screw-Cap (PN 520166)	500534 & 500522	4 microTUBE-50 PN 520166.2	20 °C	55 µl	75 W	25 %	1000	90 s
8 microTUBE-50 Strip V2 (PN 520174/520240)	500526 & 500518	8 microTUBE-50 Strip V2 PN 520174.2/ 8 microTUBE-50 H-slit Strip V2 PN 520240.2	20 °C	55 µl	50 W	30 %	1000	125 s
microTUBE-130 Screw-Cap (PN 520216)	500534 & 500522	4 microTUBE-130 PN 520216.2	20 °C	130 µl	70 W	20 %	1000	140 s
8 microTUBE-130 Strip V2 (PN 520217/520239)	500526 & 500518	8 microTUBE-130 Strip V2 PN 520217.2/ 8 microTUBE-130 H-slit Strip V2 PN 520239.2	20 °C	130 µl	70 W	20 %	1000	130 s
microTUBE Pre-slit Snap-Cap (PN 520045)	500526 & 500514	8 microTUBE-130 Snap-Cap PN 520045.2	20 °C	130 µl	70 W	20 %	1000	130 s
microTUBE Crimp-Cap (PN 520052)	500526 & 500514	8 microTUBE-130 Crimp-Cap PN 520052.2 8 microTUBE-130 V1 PN 520053.2	20 °C	130 µl	70 W	20 %	1000	140 s
8 microTUBE Strip V1 (PN 520053)	500526 & 500514	8 microTUBE-130 V1 PN 520053.2	20 °C	130 µl	70 W	20 %	1000	130 s

Table 2. ME220 DNA Shearing Settings. There are 2 Rack Definitions based on the version of SonoLab installed. SonoLab 8.0.1 or lower may not contain the “.2,” and SonoLab 8.0.2 or higher should contain the “.2.”

ML230

microTUBE	Holder & Insert	Rack Definition	Temp.	Sample Volume	Peak Incident Power (PIP)	Duty Factor	Cycles per Burst	Time	Repeat/Iterations
8 microTUBE-50 Strip V2* (PN 520174/520240)	500661	ML230_500661 Rack 8 microTUBE Strip 50 +1.8mm offset	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

microTUBE	Holder	Temperature	Water Level	Sample Volume	Intensity	Duty Cycle	Cycles per Burst	Time
microTUBE Snap-Cap (PN 520045)	500114	7 °C	12	130 µl	5	10 %	200	180 s

Table 4. S2 DNA Shearing Settings.

S220

microTUBE	Holder	Temperature	Water Level	Sample Volume	Peak Incident Power (PIP)	Duty Factor	Cycles per Burst	Time
microTUBE Snap-Cap (PN 520045)	500114	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 5. S220 DNA Shearing Settings.

E220evolution

microTUBE	Rack	Plate Definition	Temp.	Water Level	Sample Volume	Peak Incident Power (PIP)	Duty Factor	Cycles per Burst	Time
microTUBE-15 Screw-Cap (PN 520145)	500432	500432 E220e 4 microTUBE-15 Screw Cap 0.18mm offset	20 °C	10	15 µl	18 W	20 %	50	120 s
8 microTUBE-15 Strip V2 (PN 520159/520241)	500437	500437 E220e 8 microTUBE-15 Strip V2 -1.58mm offset	20 °C	6	15 µl	18 W	20 %	50	120 s
microTUBE-50 Screw-Cap (PN 520166)	500432	500432 E220e 4 microTUBE-50 Screw Cap -8.32mm offset	7 °C	6	55 µl	75 W	20 %	1000	95 s
8 microTUBE-50 Strip V2 (PN 520174/520240)	500437	500437 E220e 8 microTUBE50 Strip V2 -10mm offset	7 °C	-2	55 µl	75 W	15 %	500	155 s
96 microTUBE-50 Plate (PN 520168/520232)*	N/A	N/A	7 °C	0	55 µl	100 W	30 %	1000	90 s
microTUBE Snap-Cap (PN 520045)	500433	500433 E220e 8 microTUBE Crimp and Snap Cap -3.7mm offset	7 °C	6	130 µl	175 W	10 %	200	180 s
microTUBE Crimp-Cap (PN 520052)	500433	500433 E220e 8 microTUBE Crimp and Snap Cap -3.7mm offset	7 °C	6	130 µl	175 W	10 %	200	180 s
8 microTUBE Strip V1 (PN 520053)	500430	500430 E220e 8 microTUBE Strip -6mm offset	7 °C	6	130 µl	175 W	10 %	200	180 s
96 microTUBE Plate (PN 520078/520230)	N/A	N/A	7 °C	6	130 µl	175 W	10 %	200	180 s
microTUBE-500 Screw-Cap (PN 520185)	500484	500484 E220e 4 microTUBE-500 Screw-Cap -9.9mm offset	7 °C	6	500 µl	175 W	20 %	200	180 s

Table 6. E220evolution DNA Shearing Settings (See Table 10 for positions). Please note while using the E220evolution, the intensifier (PN 500141) must remain in place for all microTUBES except for 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.

E220

microTUBE	Rack	Rack Definition	Temp.	Water Level	Sample Volume	Peak Incident Power (PIP)	Duty Factor	Cycles per Burst	Time
microTUBE-15 Screw-Cap (PN 520145)	500308	E220_500308 Rack 24 Placemicro-TUBE-15 Screw-Cap +15mm offset	20 °C	10	15 µl	18 W	20 %	50	120 s
8 microTUBE-15 Strip V2 (PN 520159/520241)	500444	E220_500444 Rack 12 Place 8 micro-TUBE-15 Strip V2 -1.5mm offset	20 °C	6	15 µl	18 W	20 %	50	120 s
microTUBE-50 Screw-Cap (PN 520166)	500308	E220_500308 Rack 24 Place micro-TUBE-50 Screw-Cap +6.5mm offset	7 °C	6	55 µl	75 W	20 %	1000	95 s
8 microTUBE-50 Strip V2 (PN 520174/520240)	500444	E220_500444 Rack 12 Place 8 micro-TUBE-50 Strip V2 -10mm offset	7 °C	-2	55 µl	75 W	15 %	500	155 s
96 microTUBE-50 Plate (PN 520168/520232)*	N/A	E220_520168 96 microTUBE-50 Plate -10.5mm offset E220_520232 96 microTUBE-50 Plate Thin Foil -10.5mm Offset	7 °C	0	55 µl	100 W	30 %	1000	90 s
microTUBE Snap-Cap (PN 520045)	500111	500111 24 microTUBE snap +4mm offset	7 °C	6	130 µl	175 W	10 %	200	180 s
microTUBE Crimp-Cap (PN 520052)	500282	E220_500282 Rack 96 Place microTUBE -6mm offset	7 °C	6	130 µl	175 W	10 %	200	180 s
8 microTUBE Strip V1 (PN 520053)	500191	E220_500191 8 microTUBE strip Plate -6mm offset	7 °C	6	130 µl	175 W	10 %	200	180 s
96 microTUBE Plate (PN 520078/520230)	N/A	E220_520078 96 microTUBE Plate -6mm offset E220_520230 96 microTUBE Plate Thin Foil -6mm offset	7 °C	6	130 µl	175 W	10 %	200	180 s
microTUBE-500 Screw-Cap (PN 520185)	500452	E220_500452 Rack 24 Place micro-TUBE-500 Screw-Cap +6mm offset	7 °C	6	500 µl	175 W	20 %	200	180 s

Table 7. E220 DNA Shearing Settings (See **Table 10** for positions). Please note while using the E220, the intensifier (PN 500141) must remain in place for all microTUBES except for 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

microTUBE	Rack	Rack Definition	Temp.	Water Level	Sample Volume	Peak Incident Power (PIP)	Duty Factor	Cycles per Burst	Time
8 microTUBE-15 Strip V2 (PN 520159/520241) *	500445	LE220_500445 Rack-LV 12 Place 8 microTUBE-15 Strip V2 -4mm offset	20 °C	4	15 µl	180 W	30 %	50	120 s
8 microTUBE-50 Strip V2 (PN 520174/520240) **	500485	LE220_500485 Rack-XT 12 Place 8 microTUBE-50 Strip V2 -12mm offset	7 °C	-2	55 µl	450 W	20 %	1000	160 s
96 microTUBE-50 Plate (PN 520168/520232) **	N/A	LE220_520168 96 microTUBE-50 Plate -12mm offset LE220_520232 96 microTUBE-50 Plate Thin Foil -12mm offset	7 °C	-2	55 µl	450 W	20 %	1000	200 s
microTUBE Crimp-Cap (PN 520052)	500282	LE220_500282 Rack 96 Place micro-TUBE -4mm offset	7 °C	6	130 µl	450 W	30 %	200	175 s
8 microTUBE Strip V1 (PN 520053)	500191	LE220_500191 Rack 12 Place 8 microTUBE Strip -4mm offset	7 °C	6	130 µl	450 W	30 %	200	175 s
96 microTUBE Plate (PN 520078/520230)	N/A	LE220_520078 96 microTUBE Plate -4mm offset LE220_520230 96 microTUBE Plate Thin Foil -4mm offset	7 °C	6	130 µl	450 W	30 %	200	190 s

Table 8. LE220 DNA Shearing Settings (See **Table 10** 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.

LE220-plus/LE220R-plus/LE220Rsc

microTUBE	Rack	Rack Definition	Temp.	Water Level	Sample Volume	Peak Power	Duty Factor	Cycles per Burst	Time
8 microTUBE-15 Strip V2 (PN 520159/520241) *	500445	LE220plus_500445 Rack-LV 12 Place 8 microTUBE-15 Strip V2 -4mm offset	20 °C	4	15 µl	180 W	30 %	50	120 s
8 microTUBE-50 Strip V2 (PN 520174/520240) **	500485	LE220plus_500485 Rack-XT 12 Place 8 microTUBE-50 Strip V2 -12mm offset	7 °C	-2	55 µl	450 W	20 %	1000	160 s
96 microTUBE-50 Plate (PN 520168/520232) **	N/A	LE220plus_520168 96 microTUBE-50 Plate -12mm offset LE220plus_520232 96 microTUBE-50 Plate Thin Foil -12mm offset	7 °C	-2	55 µl	450 W	20 %	1000	200 s
microTUBE Crimp-Cap (PN 520052)	500282	LE220plus_500282 Rack 96 Place microTUBE -4mm offset	7 °C	6	130 µl	450 W	30 %	200	175 s
8 microTUBE Strip V1 (PN 520053)	500191	LE220plus_500191 Rack 12 place 8 microTUBE Strip -4mm offset	7 °C	6	130 µl	450 W	30 %	200	175 s
96 microTUBE Plate (PN 520078/520230)	N/A	LE220plus_520078 96 microTUBE Plate -4mm offset LE220plus_520230 96 microTUBE Plate Thin Foil -4mm offset	7 °C	6	130 µl	450 W	30 %	200	190 s

Table 9. LE220-plus/LE220R-plus/LE220Rsc DNA Shearing Settings (See **Table 10** 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 10. 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 three 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 11**. Covaris Contact: applicationsupport@covaris.com.

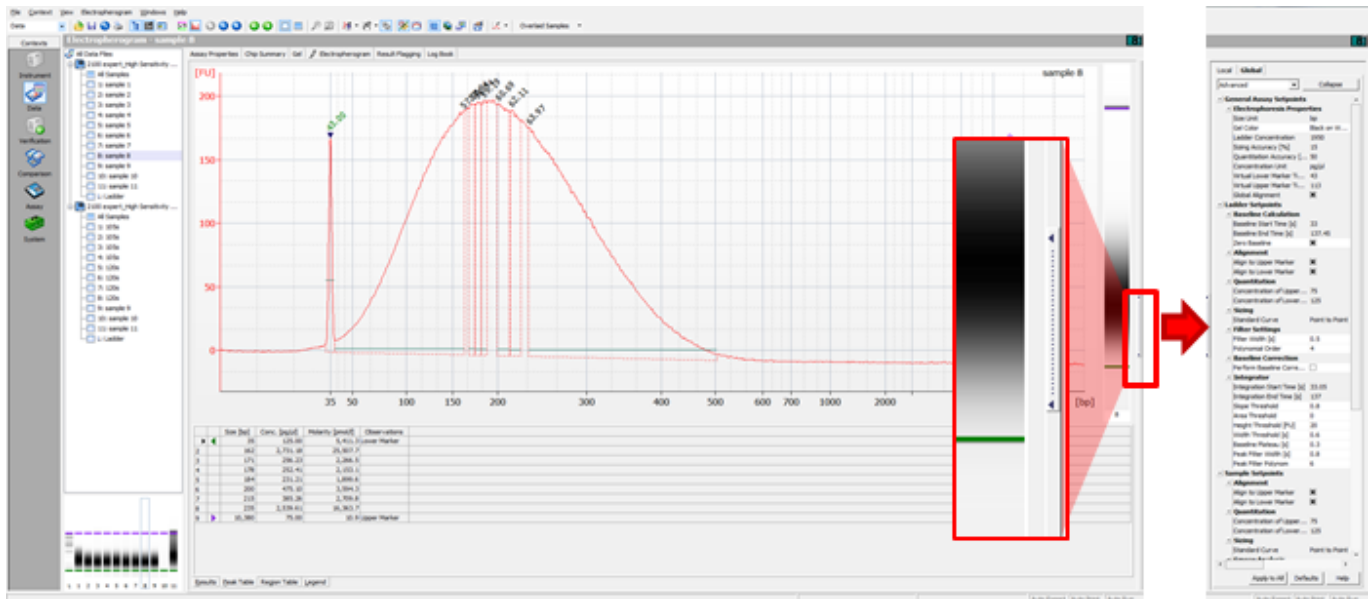
	Average of Processed Test Samples within +/- 15% of Reference Sample	Average of Processed Test Samples more than 15% different from Reference Sample
Coefficient of Variation of Processed Samples < 15%	Covaris system OK	Contact Covaris
Coefficient of Variation of Processed Samples > 15%	Contact Covaris	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 11. Covaris Performance Verification Kit interpretation.

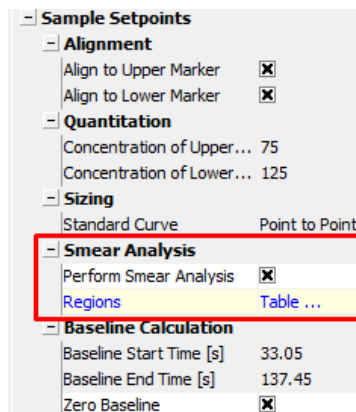
Appendix A: Detailed Instructions for using the Agilent Bioanalyzer 2100

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

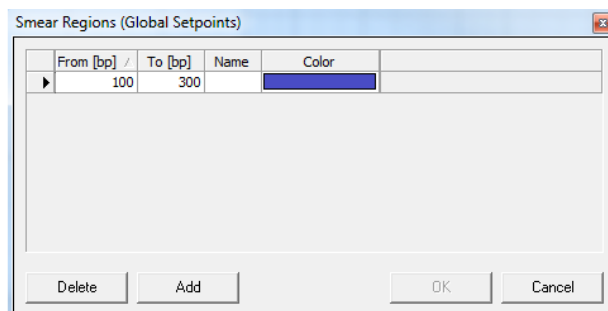
1. Select the “Global” tab on the right side of the screen and click “Advanced” on the drop-down menu.
 - a. If you cannot see the “Global” tab click on the “.....” to the right side of the screen.



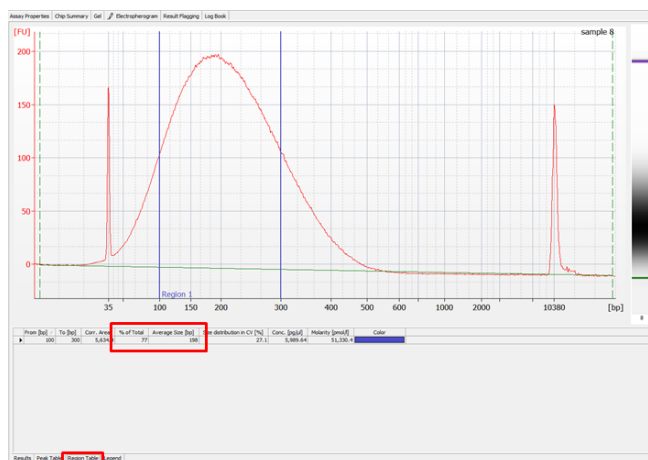
2. Scroll down to “Smear Analysis” under “Sample Setpoints”.



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.



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



- 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.

- 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.
010184	G	12/2020	Addition of rack/plate definition file names.