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Operation of the Covaris LE220

I. Purpose

This document describes operation of the Covaris LE220 for shearing cDNA/gDNA.

II. Scope

These procedures are applicable to all BCGSC Laboratory Groups.

III. Policy

This procedure will be controlled under the policies of the Genome Sciences Centre, as outlined in the Genome Sciences Centre High Throughput Production Quality Manual (QM.0001). Do not copy or alter this document. To obtain a copy see a QA associate.

IV. Responsibility

It is the responsibility of all personnel performing this procedure to follow the current protocol. It is the responsibility of the Group Leader to ensure personnel are trained in all aspects of this protocol. It is the responsibility of Quality Assurance Management to audit this procedure for compliance and maintain control of this procedure.

V. References

Reference Title	Reference Number
Covaris E and LE-Series USER MANUAL	IM.0291
WCS Water Conditioning System User Manual	IM.0292
Covaris Minitube Blue	IM.0246

VI. Related Documents

Document Title	Document Number
Raindance 3kb Shearing	PR.0132
Care and Maintenance of the Covaris LE220	EQUPR.0021
Manual PCR-enriched Library Construction for Illumina Sequencing	LIBPR.0120
Manual Bisulfite Library Construction for Illumina Sequencing	LIBPR.0091
Manual PCR free TruSeq Illumina gDNA Library Construction	LIBPR.0101
Manual Plate-based FFPE Genome Library Construction for Illumina Sequencing	LIBPR.0109

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Document Title	Document Number
Nimbus-assisted 96-well PCR –enriched Library Construction for Illumina Sequencing	LIBPR.0137
96-well PCR Free Library Construction on NIMBUS for Illumina Sequencing	LIBPR.0133

VII. Safety

All Laboratory Safety procedures will be complied with during this procedure. The required personal protective equipment includes a laboratory coat and gloves. See the material safety data sheet (MSDS) for additional information.

VIII. Materials and Equipment

Name	Supplier	Number	Model or Catalogue #	
Covaris LE220 with WCS and Chiller	Covaris	LE220	✓	N/A
Deionized water	In house	N/A	N/A	
microTUBE AFA Fiber Crimp-Cap	Covaris	520052	✓	
96 microTUBE Plate with foil tape	Covaris	520078	✓	
8-strip microTUBE strips	Covaris	520053	✓	
Aluminum Rack 96 Place microTUBE	Covaris	500282	✓	
8 microTUBE Strip Sealing Foil (12)	Covaris	520108	✓	
8 microTUBE Strip (120)	Covaris	520109	✓	
Eppendorf/Gilson P200 Pipette	Eppendorf	P200	✓	
P200 Barrier Tip Diamond TIPACK	Gilson/Mandel	DF200ST	✓	
Screw-Cap microTUBE Fiber (red)	Covaris	520096	✓	
Snap-Cap microTUBE (blue)	Covaris	520045	✓	

IX. Procedure

1. Equipment preparation

- 1.1. The following steps refer to what the GST should typically follow when they run shearing programs. Information on maintenance and preparation which is typically performed by the designated start of day person can be found in Appendix B.
- 1.2. Ensure the transducer is already submerged and the required temperature for your program is achieved (the temperature readout as shown Fig 1 should be blue). Treatments cannot be run until the set point is reached.

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- 1.3. You should see the program main window displayed. Confirm the “DEGAS ON” button is greyed out and the bar under it is lit green. You should see the degasser producing bubbles if on. If degassing is not on, click the “DEGAS ON” button so it looks like the image below. See Fig 1 below.

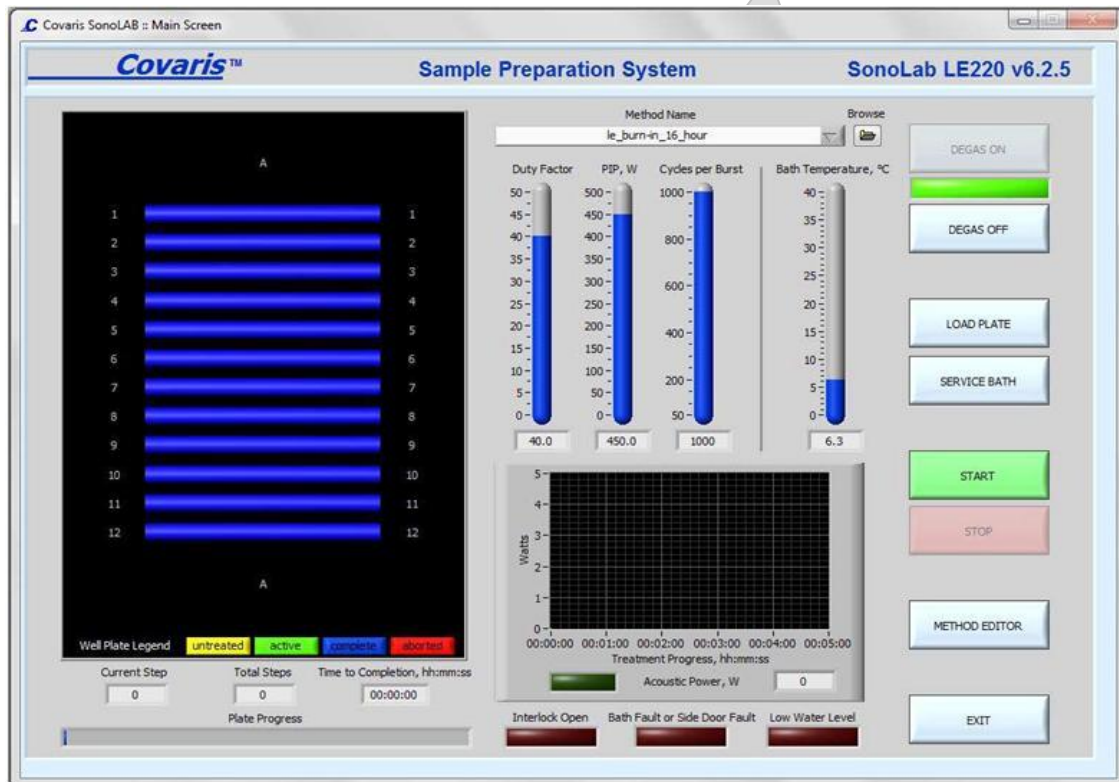


Figure 1: Covaris SonoLAB Main Screen

Note: Covaris shearing can be done in tubes, MicroTUBE Plates or MicroTube 8-Strips depending on the sample size. Refer to step 2-4 accordingly.

2. Loading samples in tubes

- 2.1. All samples should be resuspended in Tris-EDTA, pH 8.0 or EB buffer but NOT in any other non-validated buffer or water.
- 2.2. Use P200 pipettes to load samples.
- 2.3. Use one microTUBE with crimp cap for each sample. Tilt the pipette tip on an angle and pierce the septum of the microTUBE. Dispense the sample slowly into the microTUBE.

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- 2.4. Quick-spin the tubes in a microfuge with tube adapters to ensure the samples are at the bottom and all air bubbles are removed. Otherwise DNA shearing will be incomplete.

*Note: Quick spin in step 2.4 does not apply for the current clinical procedure using miniTube blue sample containers.

- 2.5. Place the microTUBEs in the Covaris **aluminum** tube rack with your first sample in the top left position of the rack (A1), which has an angled corner (see Fig 2 below). Place the remaining tubes in their respective locations.

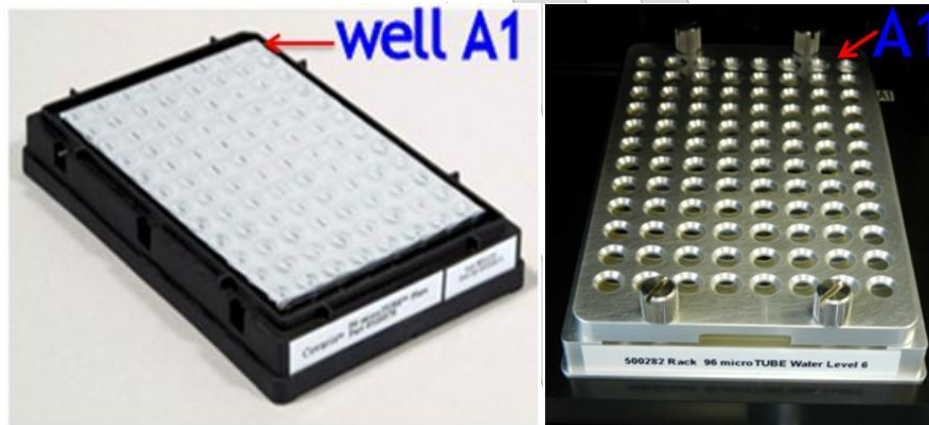


Figure 2: Left: A 96 microTUBE Plate with pierceable foil tape. Right: A microTUBE rack with cover. The well A1 location is shown with respect to the front stickers.

- 2.6. Firmly screw down the Covaris **aluminum** plate cover, ensuring the 4 pins are holding the microTUBEs in place in the tube rack.

3. Loading samples in MicroTUBE Plates

- 3.1. To transfer samples to the Covaris plate for plate based library construction, refer to the appropriate library construction SOP. A1 well on the plate should correspond to the top right position A1 of the Covaris MicroTUBE Plate, which has an angled corner (see Fig 2 above).
- 3.2. Quick-spin the plate to remove air bubbles and ensure all the samples are at the bottom of the wells before running the treatment method. Allow the samples to equilibrate by placing the sample plate on ice or in the 4 degree fridge for 10 minutes.

4. Loading Samples into the Re-usable MicroTube 8-Strip Rack

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- 4.1. The 8-tube strips fit into the microTube 8-strip rack in one orientation only, with the tabs at one end spanning an alignment peg (see Figure 3.) If the strip is in the wrong orientation the lid will not fit onto the rack evenly and securely. When all strips are correctly positioned, screw down the rack cover to barely finger tight.

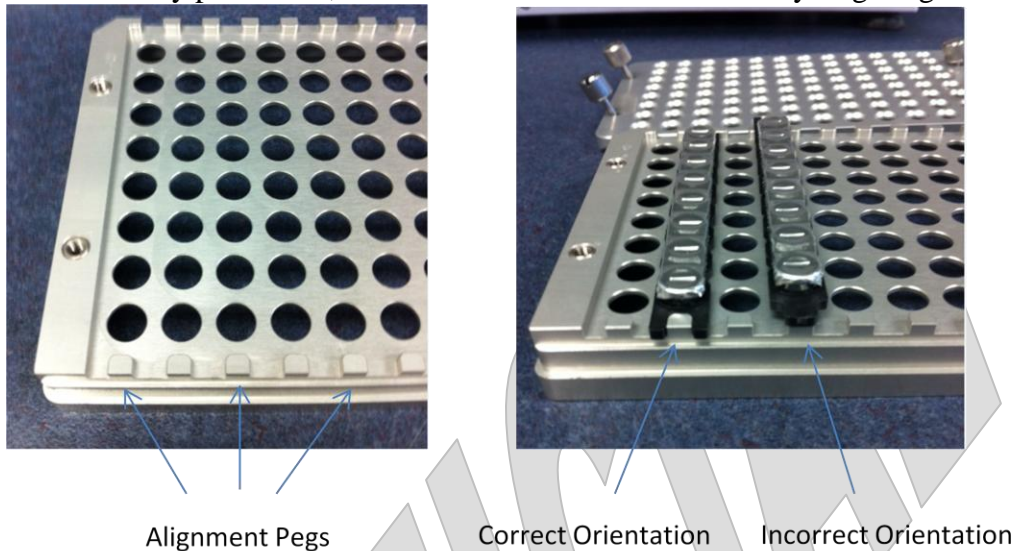


Figure 3: loading samples into the re-usable MicroTube 8-Strip rack

- 4.2. To transfer your samples from your AB1000 plate into the Covaris strip tubes, refer to the appropriate library construction SOP.

5. Selecting a Treatment Method and Adjusting Water Level with a Dummy Plate

- 5.1. The following steps need to be performed to check alignment and adjust water levels. Unlike E210, there is no “Check alignment” button for LE220. Also, an entire column (8 wells) is treated at the same time for LE220 unlike E210 where each well is treated individually.
- 5.2. **Select** the library-specified treatment method by clicking the “**Browse**” button as shown in the upper right corner of **Fig 1** above. Each group has its own folder of methods. See folders: C:\Covaris\Clinical, Libcore, TechD or Archive. See “**Appendix A**” for program names that correspond to your samples and library strategy. Consult with your supervisor if you do not know which method to run.
- 5.3. Click OK once you select the appropriate program.
- 5.4. Select the “LOAD PLATE” button to present the tray. Open the door when the green “DOOR” button lights up by pressing and pulling on the door handle.

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- 5.5. Place the dummy plate or tube rack on the tray. There is no need to have tubes for the latter.
- 5.6. Click “START” in the main window and following the dialog box that appears, confirm that there is water in the water bath, and the system has been degassed. After checking, press “START” on the dialog box.
- 5.7. After the stage moves to the water level and treatment begins on the dummy plate, click the “STOP” button on the main screen. “STOP” halts the operation of the sample positioning system. Then select “ABORT” and subsequently “OK” as you must restart from the beginning after you load the sample plate.

NOTE: The “STOP” button on the instrument and the “STOP” button on the display perform the same function; however methods halted with the instrument “STOP” button cannot be resumed.

- 5.8. Figures 4 and 5 show the correct water levels for the microTUBE plate and microTUBE rack. The microTUBE plate sits higher on the stage than the microTUBE rack, so the tank water level must be higher, near the top edge of the microTUBE plate. The water levels must be adjusted manually to reach these points, which is done by opening the door (press the “DOOR” button and pull the handle) and either removing or adding ddH₂O. Note: no degassing is necessary when removing or adding small amounts of water to bring the tank to the desired level.

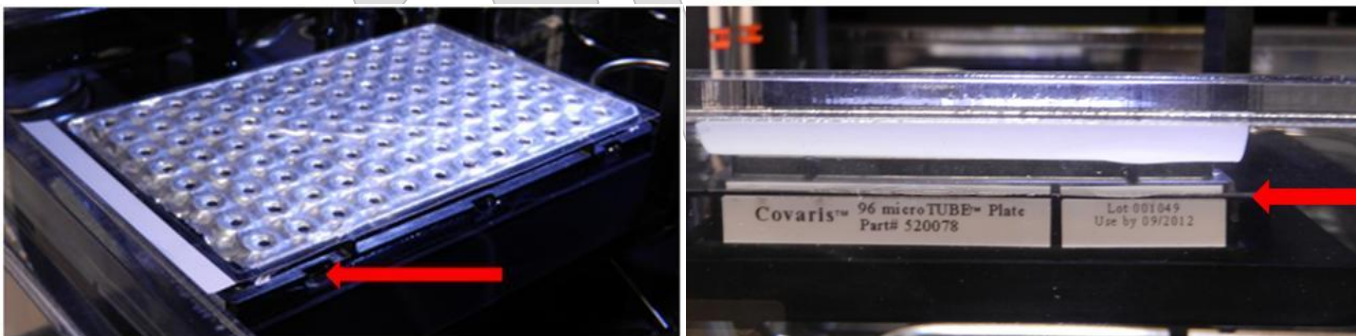


Figure 4: Red arrow indicates the water level for shearing samples in a microTUBE plate. The water level must be at the very TOP of the black plate holder

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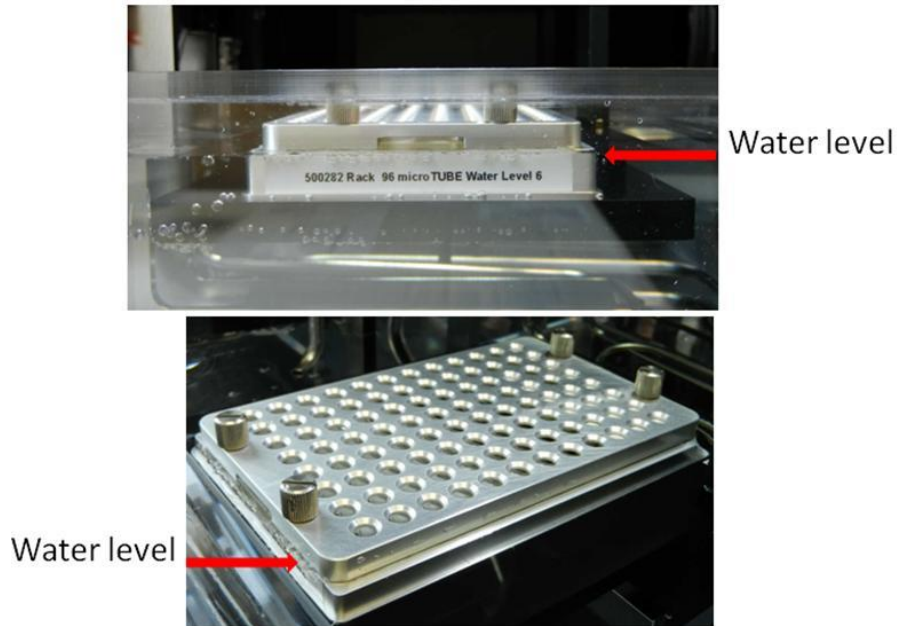


Figure 5: Red arrow indicates the water level for shearing samples in microTUBEs

- 5.9. Once the ddH₂O level is adjusted, close the door and press the “LOAD PLATE” button to present the dummy plate for retrieval.

6. Running the samples

- 6.1. The MicroTUBE plate, MicroTUBE rack can now be loaded. For a partial plate, load the unused positions in that column and the adjacent column with red-marked empty tubes to reduce splashing. See Figure 6 below.



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Figure 6. A partially filled microTUBE rack showing empty tubes, marked in red, surrounding the samples to reduce splashing.

- 6.2. Once the sample plate is loaded, click “**METHOD EDITOR**” button as shown Fig 1. This will result in the window shown in Fig 7 below.

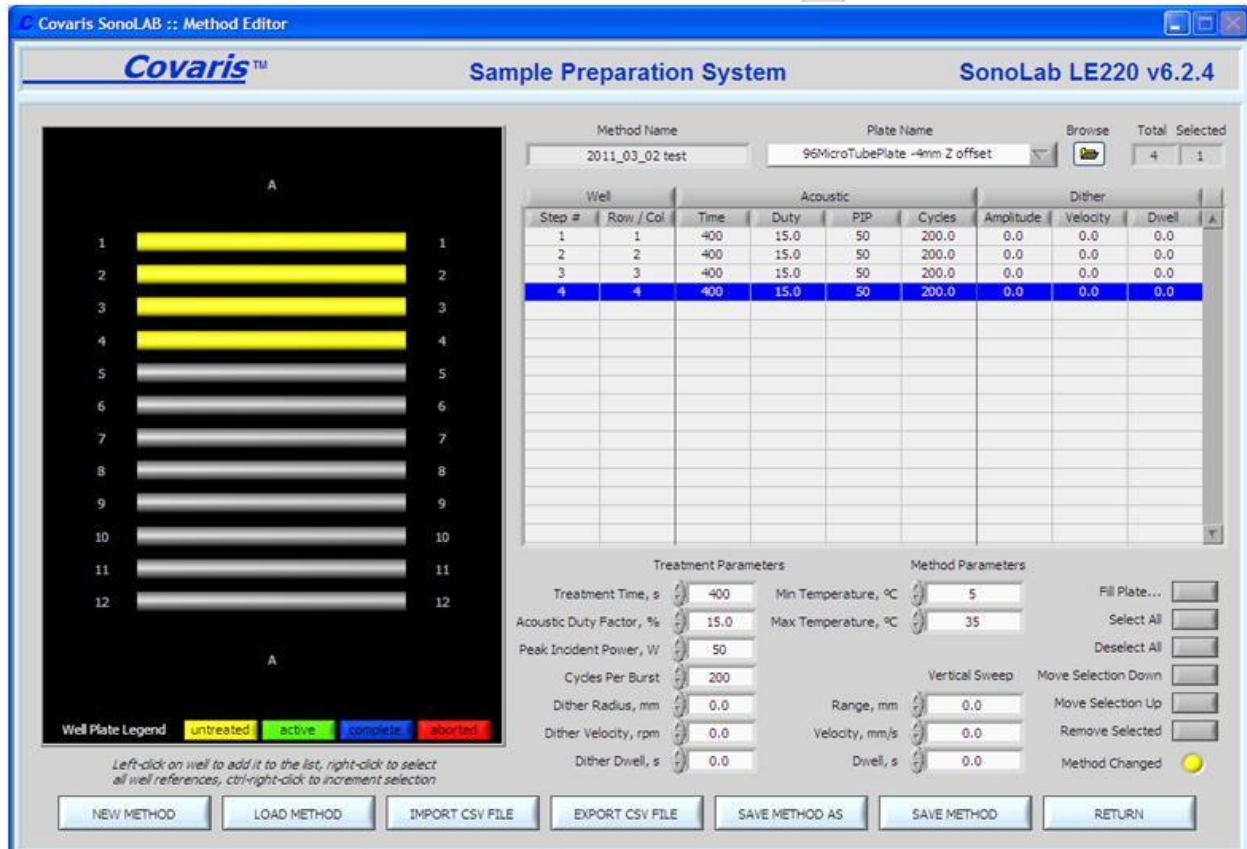


Figure 7. The method editor panel, used for specifying methods and plate types.

- 6.3. Specify the columns you plan to run. To add columns, highlight the last entry in the table as shown in Fig 6 and then click on the column in the plate view on the left (should turn yellow after selection). To remove columns, highlight the column in the table and click on the “Remove Selected” at the lower right corner of the window.
- 6.4. After ensuring that you are under the correct method name, the plate name and correct plate layout, click on the “SAVE METHOD” at the lower right corner of the window.
- 6.5. Click the “RETURN” button at the lower right corner of the window. This brings you back to the main page (see Figure 1).

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- 6.6. After double-checking your selections, Click “START” in the main window and following the dialog box that appears, press “START” again on the dialog box.
- 6.7. When the treatment is complete, press the “LOAD PLATE” button to present the sample plate. Press the green “DOOR” light to open the door and retrieve the samples.
- 6.8. Close the LE220 front door.
- 6.9. Spin briefly in the Eppendorf bench top centrifuge (700g, 1 min; Program 1) to collect all the samples at the bottom for more uniform and optimal shearing.
- 6.10. If a second round of treatment is required, load the plate again, click on “METHOD EDITOR” to refresh plate view and repeat step 6.5-6.9 to complete the second half of the shearing program. Otherwise, proceed to step 6.11.
- 6.11. When the run is completed, do NOT close the software. Leave the transducer submerged.

7. Removal of samples from Covaris tubes

- 7.1. For plate based library construction or when using the 8-strip microTUBE strips, refer to the appropriate library construction SOP for details of the transfer back to plates.
- 7.2. For samples in Covaris microTUBEs, the samples can be withdrawn from the tubes using P200 pipette.
 - 7.2.1. Spin down the Covaris microTUBE in a minifuge.
 - 7.2.2. To withdraw the samples, angle the pipette tip while perforating the septum. Sample withdrawal can be difficult. Repeat pipetting out until the entire sample is withdrawn. If a vacuum persists, insert a new P2 tip alongside the P200 tip so air can enter the microTUBE. Do not use the same P2 or P200 tip on more than one tube or the samples will be cross contaminated.

8. Shut Down Procedures

- 8.1. The end of day shut-down will be performed by a designated employee. Please refer to Appendix B section 8 for proper procedures.

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Appendix A: Treatment Parameters for Library and Labware Types:

Note: Use Covaris E210 for amplicon samples

Labware Type: 96 microTUBE Plate:

Library Types	Target Size	Sample Type	Max Amount	Volume
1. Plate based PCR Free Genome	425bp	gDNA	1ug	62.5uL

LE220 Protocol: Plate_90sec_PCR_Free_Genome_Large_Gap.e1proc

Procedure: Run Protocol twice, quick-spin 96 microTUBE Plate before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: 96 microTUBE Plate
	20	450	200	2x 45sec	P/N 520078

Library Types	Target Size	Sample Type	Max Amount	Volume
2. Plate based Small Gap	250bp	N/A	1ug	62.5uL
3. Plate based Multiplex Exome	250bp	gDNA	1ug	62.5uL
4. Plate based Custom Capture	250bp	gDNA	1ug	62.5uL

LE220 Protocol: Plate_135sec_Small_Gap_Multiplex_Capture.e1proc

Procedure: Run Protocol twice, quick-spin 96 microTUBE Plate before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: 96 microTUBE Plate
	30	450	200	2x 68sec	P/N 520078

Library Type	Target Size	Sample Type	Amount	Volume
5. Plate based Strand-Specific RNA	250bp	cDNA	N/A	40uL

LE220 Protocol: Plate_130sec_cDNA.e1proc

Procedure: Run Protocol twice, quick-spin 96 microTUBE Plate before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: 96 microTUBE Plate
	30	450	200	2x 65sec	P/N 520078

Library Type	Target Size	Sample Type	Amount	Volume
6. Plate based FFPE	300-350bp	gDNA	0.1ug	62uL

LE220 Protocol: Plate_120sec_FFPE_Modified_gap.e1proc

Procedure: Run Protocol once only

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: 96 microTUBE Plate
	20	450	200	2x60sec	P/N 520078

Library Types	Target Size	Sample Type	MaxAmount	Volume
7. Bisulfite	300bp	gDNA	1ug	62.5uL

LE220 Protocol: Plate_100sec_Bisulfite.e1proc

Procedure: Run Protocol twice, quick-spin aluminum tube rack before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: 96 microTUBE Plate
	30	450	200	2x 50sec	P/N 520078

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Labware Type: 8 microTUBE Strips:

Library Types	Target Size	Sample Type	Max Amount	Volume
8. Plate based PCR Free Genome	425bp	gDNA	1ug	62.5uL

LE220 Protocol: 8 microTUBE strip protocol: Strip_76sec_PCR_Free.elproc

Procedure: Run Protocol twice, quick-spin microTUBE strips in aluminum tube rack before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: Covaris 8 microTUBE strip
	15	450	200	2x 38sec	P/N 520053

Library Types	Target Size	Sample Type	Max Amount	Volume
9. Plate based Small Gap	250bp	N/A	1ug	62.5uL
10. Plate based Multiplex Exome	250bp	gDNA	1ug	62.5uL
11. Plate based Custom Capture	250bp	gDNA	1ug	62.5uL

LE220 Protocol: 8 microTUBE strip protocol: Strip_80sec_Small_Gap.elproc

Procedure: Run Protocol twice, quick-spin Crimp-Cap microTUBE strips in Aluminum Rack before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: Covaris 8 microTUBE strip
	30	450	200	2x 40 sec	P/N 520053

Library Type	Target Size	Sample Type	Amount	Volume
12. Plate based Strand-Specific RNA	250bp	cDNA	N/A	40uL

LE220 Protocol: 8 microTUBE strip protocol: Strip_100sec_cDNA.elproc

Procedure: Run Protocol twice, quick-spin Crimp-Cap microTUBE strips in Aluminum Rack before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: Covaris 8 microTUBE strip
	30	450	200	2x 50sec	P/N 520053

Library Type	Target Size	Sample Type	Max Amount	Volume
13. Plate based FFPE	250bp	gDNA	1ug	62uL

LE220 Protocol: 8 microTUBE strip protocol: Strip_76sec_FFPE.elproc

Procedure: Run Protocol once only

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: Covaris 8 microTUBE strip
	30	450	200	2x 38sec	P/N 520053

Library Types	Target Size	Sample Type	Amount	Volume
14. Bisulfite	300bp	gDNA	1ug	62.5uL

LE220 Protocol: 8 microTUBE strip protocol: Strip_110sec_Bisulfite.elproc

Procedure: Run Protocol twice, quick-spin microTUBE strips in aluminum tube rack before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: Covaris 8 microTUBE strip
	15	450	200	2x 55sec	P/N 520053

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Labware Type: Crimp-Cap microTUBEs:

Library Types	Target Size	Sample Type	Amount	Volume
15. Plate based PCR Free Genome	425bp	gDNA	1ug	60uL

LE220 Protocol: **Tube_76sec_Manual_PCR_Free.e1proc**

Procedure: Run Protocol twice, quick-spin aluminum tube rack before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: microTUBE Crimp-Cap
	15	450	200	2x 38sec	P/N 500282

Library Types	Target Size	Sample Type	Amount	Volume
16. Plate based Small Gap	250bp	N/A	1ug	60uL
17. Plate based Multiplex Exome	250bp	gDNA	1ug	60uL
18. Plate based Custom Capture	250bp	gDNA	1ug	60uL

LE220 Protocol: **Tube_80sec_Manual_Small_Gap_Multiplex_Capture.e1proc**

Procedure: Run Protocol twice, quick-spin Crimp-Cap microTUBEs in Aluminum Rack before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: microTUBE Crimp-Cap
	30	450	200	2x 40 sec	P/N 500282

Library Type	Target Size	Sample Type	Amount	Volume
19. Plate based Strand-Specific RNA	250bp	cDNA	N/A	40uL

LE220 Protocol: **Tube_100sec_Manual_cDNA.e1proc**

Procedure: Run Protocol twice, quick-spin Crimp-Cap microTUBEs in Aluminum Rack before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: microTUBE Crimp-Cap
	30	450	200	2x 50sec	P/N 500282

Library Type	Target Size	Sample Type	Amount	Volume
20. Plate based FFPE	250bp	gDNA	1ug	40uL

LE220 Protocol: **Tube_90sec_Manual_FFPE.e1proc**

Procedure: Run Protocol once only

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: microTUBE Crimp-Cap
	30	450	200	1x90s	

Library Types	Target Size	Sample Type	Amount	Volume
21. Bisulfite	300bp	gDNA	1ug	60uL

LE220 Protocol: **Tube_110sec_Bisulfite.e1proc**

Procedure: Run Protocol twice, quick-spin aluminum tube rack before & after each treatment

Parameters	Duty	PIP	Cycles/Burst	Treatment Time	Labware: microTUBE Crimp-Cap
	15	450	200	2x 55sec	P/N 500282

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Appendix B: Equipment maintenance and preparation:

1. The WCS water conditioner located to the right of the instrument and the PolyScience circulating ethylene glycol/water (50:50) bath on the floor should always be left on.
2. If the LE220 is powered off, power it on by flipping the switch located on the right of the instrument to the “1” position. See Fig 7.

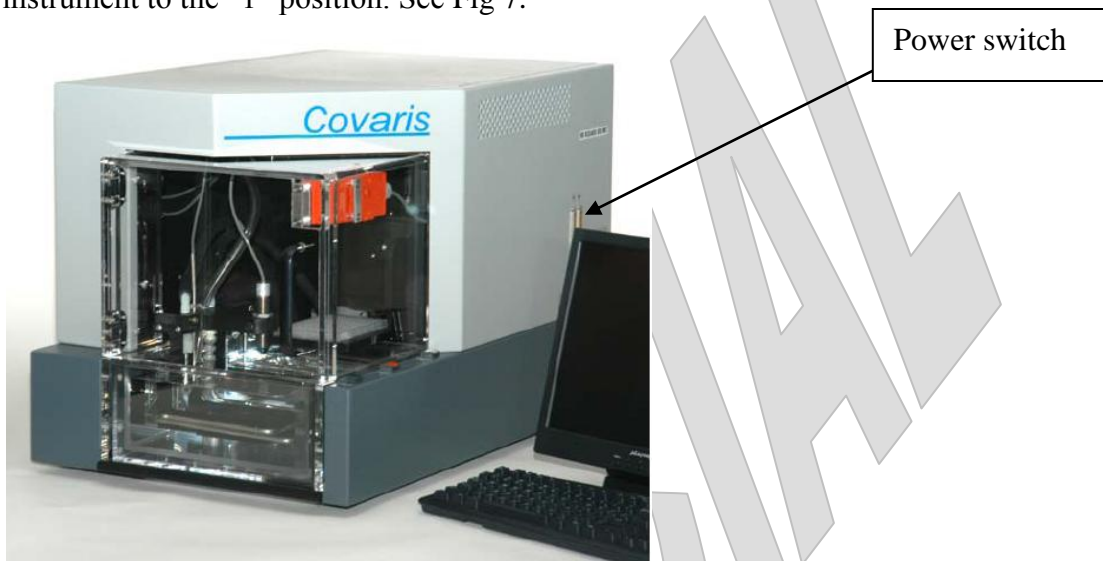


Figure 7: Covaris LE-series

3. The LE220 should always have ddH₂O water in the tank circulating through the attached water conditioner. Only distilled deionized water should be used to fill the water bath or adjust the level of the water bath (tap water or any other solution will void the warranty).
4. The ethylene glycol/water chiller located under the bench should always be left on. Make sure the temperature of the chiller is set to 3°C.
5. **Initialization:**
 - 5.1. The laptop should always be left on. If the laptop is logged off, log into the desktop with user “Covaris-PC” and password “covaris”. Log off the desktop only on Fridays.
 - 5.2. Click on the SonoLAB icon to open the software and, when prompted, click START HOMING button or press ENTER.

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- 5.3. The transducer will submerge and resume degassing and chilling the tank water. Treatments cannot be run until the set point is reached.

6. After the homing sequence is completed, the program main window is displayed. Confirm the “DEGAS ON” button is greyed out and the bar under it is lit green. If degassing is not on, click the “DEGAS ON” button so it looks like the image below. See Fig 1 above.

7. The transducer should be submerged and the water degassed and chilled during the day.

8. **Shutdown Steps**
 - 8.1. Leave the Covaris LE-series turned ON and keep the chiller located under the bench turned ON. The WCS (Water conditioning system) should be left turned ON.

 - 8.2. At the end of the day, press EXIT in the lower right corner of the SonoLab main menu. A message will ask you to confirm the status of the shutdown. Make sure that all of the listed steps in that window are completed. This will raise the transducer out of the water.

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Appendix C: Creating and Modifying a Method in the Method Editor Panel

- To open an existing method to run or to modify, click the “METHOD EDITOR” button. Figure 1 below for the Method Editor.

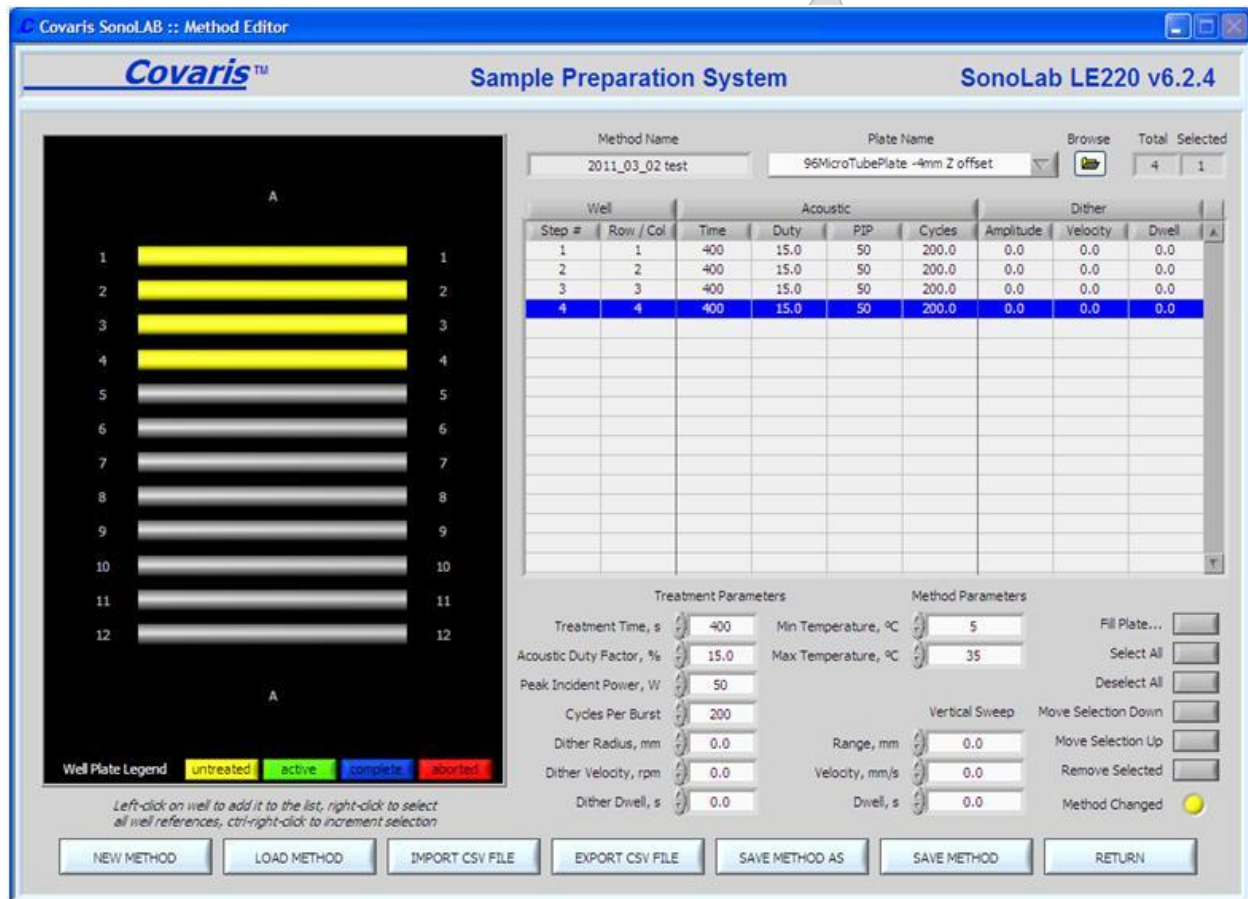


Figure 1: Method Editor Panel with Plate Map, Method Name and Plate Name (labware type).

- Click on “LOAD METHOD” and navigate to the designated process folder. Choose the Treatment Method for your sample type from the list of previously saved methods.
- To create a new method, select the “NEW METHOD” button.
- Select the Well Plate Configuration from the Plate Name menu.
- In the following example, 7 columns (shown sideways as horizontal bars) of samples receive different Treatment times (sec). Column 1 and 2 receive a lower Duty (15%). See Figure 2 below.

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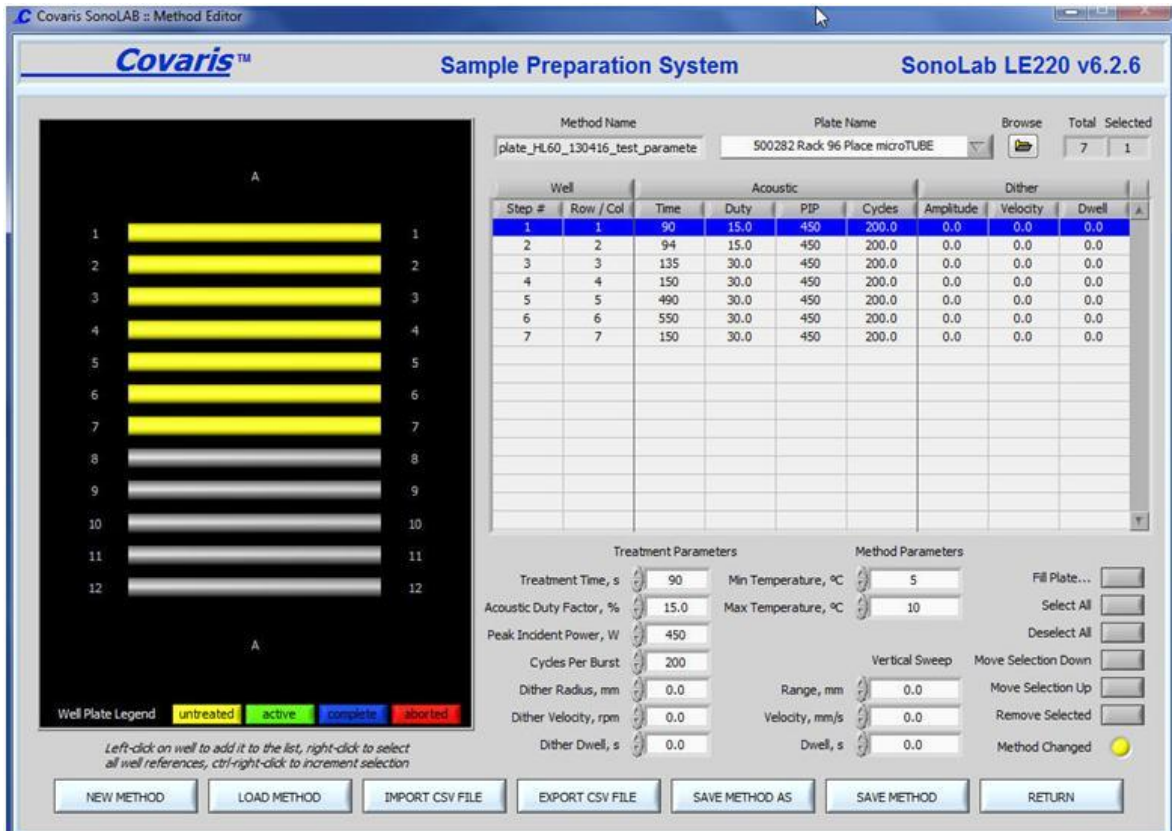


Figure 2 Method Editor showing different treatment parameters for the specified Method Name.

- Parameters may now be selected in the Treatment Parameters area. Click on a Step # in the table representing a column of samples. Adjust parameters as instructed by clicking the up/down radio buttons or entering new values in the boxes under Treatment Parameters. Normally, only Treatment Time and Acoustic Duty Factor should need changing.
- Treatment parameters for additional wells may be programmed by clicking on those wells and using the “Treatment Parameters” controls. Well treatments may be copied across an entire plate by employing the “FILL PLATE” button on the right. This feature also allows several choices for the order in which wells are listed in the method. Wells will always be treated in order as displayed in the treatment entry list.
- Click “SAVE METHOD AS” button when done to save it with a new name, or click “RETURN” to run the revised method but not save it.

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