

For research use only

# ***E-series Robotic***

## **USER MANUAL**

an automated, batch system for  
high performance sample preparation  
using adaptive focused acoustic energy

Product Name: E-series

Product Number: E110R, E210R



### **UNIVERSAL PRECAUTIONS**

Universal Precautions should be followed on all specimen samples, regardless of whether a sample is known to contain an infectious agent. Laboratories handling specimen samples are advised to comply with applicable parts of the following governmental and clinical standards, or their equivalent in the country of use:

- Centers for Disease Control (CDC), Universal Precautions for Prevention of Transmission of HIV and Other Bloodborne Infections, published 1987, updated 1996
- Clinical and Laboratory Standards Institute (CLSI), GP17-A2 Clinical Laboratory Safety; Approved Guideline - Second Edition, published 2004, ISBN 1-56238-530-5
- Clinical and Laboratory Standards Institute (CLSI), M29-A3 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Third Edition, published 2005, ISBN 1-56238-5674
- Occupational Safety and Health Administration (OSHA), 29 CFR 1910.1030 Bloodborne Pathogens
- International Standards Organization (ISO) 15190:2003, Medical Laboratories – Requirements for Safety

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## Warnings

### For safety of operating personnel:

1. Make sure that the equipment is properly grounded. DO NOT operate if it is not properly grounded.
2. The unit is equipped with a power plug appropriate for the destination country. DO NOT, under any circumstances, remove the grounding prong from the power cord.
3. DO NOT attempt to operate the equipment without the safety door closed. When the safety door is open, the mechanical positioning system and the acoustic wave system will not run. If there is any indication that the safety system is not functioning properly, DO NOT operate the equipment and contact Covaris immediately.
4. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

### To prevent damage to the equipment:

1. The instrument is designed to operate in ambient laboratory conditions e.g., 15 to 32°C (60 to 90°F). DO NOT operate the instrument in a cold room environment; the system is designed to operate with a water bath and re-circulating heater/chiller apparatus to control sample temperatures.
2. NEVER run a method without a water bath; this could damage the transducer. The instrument is equipped with a water level sensor to protect the transducer. The system will not allow the acoustic wave treatment to start unless an adequate volume of water is present.
3. ONLY distilled or deionized water should be used to fill the water bath.
4. Empty the water bath and wipe dry EVERY day. DO NOT leave water in the tank for extended time as there is no water filtration or water cleaning system with the apparatus. Drain the degassing lines (see section 4.5 Shutdown Procedure).
5. Establish a standard of operation and periodically test equipment, as described in the Maintenance section of this manual.
6. Be advised that loading third party software onto the computer may interfere with the performance of the Covaris SonoLab™ software. Please consult Covaris.

## Warranty

When used in accordance with written instruction and under normal operating conditions, the Covaris instruments are guaranteed to be free of defects in MATERIAL and WORKMANSHIP for one (1) year from the date of original delivery. Any component which proves defective during the stated period will be repaired free of charge or replaced at the sole discretion of Covaris, F.O.B., Woburn, Massachusetts, provided the defective component is returned properly packaged with all transportation charges prepaid. The customer is expected to perform basic diagnostics and component replacement with telephone support from Covaris personnel. If Covaris personnel are required to perform on-site repair, all travel costs are paid by the customer. A limited warranty as specified may apply to certain components of the equipment.

### Warranty Exceptions

This warranty is void if failure of the software or hardware has resulted from accidents, abuse, improper maintenance, or repair, or misapplication by the customer. It is also void if damage is caused by any unauthorized attachments or if modifications are made to the equipment. Removing or tampering with the Safety Enclosure will void the warranty, and the customer will assume all liabilities.

This warranty is limited to the original purchaser and is not transferable.

The software will perform according to the accompanying written materials and the medium on which the software is delivered is free of defects in materials under normal use and service. The warranty is void if damage has resulted from third party software not intended for use with the system.

The high power focused transducer is designed to give maximal mechanical energy output in water. Permanent damage to the transducer and electronic circuits could result if the transducer is operated without water. Operation of the system without water in the water bath voids the warranty.

CONTACT COVARIS, INC. SHOULD YOU HAVE ANY QUESTIONS CONCERNING EQUIPMENT.

### Warranty Services

The purchased equipment is covered by a twelve (12) month warranty which includes all the service and support necessary so that the customer can operate the equipment successfully. Extended warranties are available at the end of the original 12 month warranty period.

Services included with the original purchase of the system are:

**Installation and Training** – Setup and installation of the equipment and operator training can be purchased at time of initial acquisition of the equipment. Both will be performed by a qualified Covaris service representative. One half day will be scheduled to perform the installation and on-site training.

- The operators' training will include valuable hands-on time with the equipment.
- Preventative maintenance and troubleshooting tips will also be covered.

**Technical Support** – On-going assistance with the operation or application of the equipment and/or troubleshooting is provided via:

- Telephone (+1 781 932 3959) during the hours of 9:00am to 4:00pm, Monday through Friday,
  - United States, Eastern Standard Time (EST).
  - Greenwich Mean Time (GMT-05:00)
- E-mail queries to [techsupport@covarisinc.com](mailto:techsupport@covarisinc.com)

**Parts Replacement** – Replacement of parts (excluding consumables and cables) for normal operation of equipment are provided on a priority basis. Shipping charges are included. Failure due to accident, abuse, or improper operation is not covered.

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## 1.0 INTRODUCTION

### 1.1 Overview of the Manual

This manual contains operation and maintenance instructions for the *Covaris E-series* instruments (the E110R and E210R). It contains background information essential to the proper use and care of this equipment.

The following definitions apply in this manual:

**NOTE:** Inconvenience only if disregarded – no damage or personal injury

**CAUTION:** Equipment damage may occur, but not personal injury.

**WARNING:** Personal injury may occur – DO NOT DISREGARD.

There is also a PDF version of this manual on the system computer (if a computer was supplied with your order). Open the manual by double clicking the manual icon on the desktop.

### 1.2 Purpose of the Equipment

The E Series instruments are used for high-throughput, sample preparation and extraction. The technology uses controlled and highly focused nonlinear acoustic waves that are delivered to each sample. The systems are designed to work with a variety of closed or open sample vessels including vials, tubes, and multi-well plates. In general, the E110R is best for samples less than 50 mg and volumes less than 0.5 ml and the E210R is most appropriate for samples less than 500 mg and volumes less than 5 ml. These ranges may be increased or decreased depending upon buffer composition and sample/buffer ratios (contact Technical Support for more information).

**CAUTION:** Methods developed and optimized in the E110R system are not necessarily transferable to the E210R system. Contact Covaris Technical Support for help.

The instruments are not intended for use in diagnostic procedures; for research use only.

**CAUTION:** The system is designed to work primarily with closed sample holders. Open vessels may be used; however, the energy should be dramatically reduced as there is potential for leakage and spilling into the water tank. Please contact Covaris Technical Support for guidance in this area if open vessel processing is required.

### 1.3 Main Components

The main components of the E Series instruments include:

- acoustic transducer
- temperature sensing device
- sample positioning system
- robot interface
- degassing pump
- water bath
- user manual

External components include a notebook computer.

The user is expected to provide a recirculating chiller/heater. Covaris can provide specifications for recommended recirculators.



Figure 1

## 1.4 Principle of Operation

**The Covaris Process** generates acoustic shock waves to cause controlled cavitation and acoustic streaming at the focal point within the sample treatment vessel. Acoustic energy has been utilized for many years for a variety of diagnostic, therapeutic and research purposes. Well-known uses of sonic or acoustic energy in materials processing include "sonication", an uncontrolled process of mechanical disruption involving the direct immersion of an unfocused kHz range (e.g., 15,000 cycles per second) ultrasound source into a fluid suspension of the material being treated. There are also specific clinical examples of the utilization of therapeutic ultrasound (e.g. lithotripsy) and of diagnostic ultrasound (e.g., fetal imaging).

Typically, when ultrasound is applied to a bulk biological sample solution (as for the extraction of intracellular constituents from tissue), the treatment causes a complex, heterogeneous, mixture of sub-events that vary during the course of a treatment dose. In other words, the ultrasonic energy may be partitioned between various states. For example, the energy may directly treat a sample; the energy may displace the target and shift the target out of the optimal energy zone; the energy may result in interference, (i.e., a "bubble shield"), that reflects the acoustic energy; or the large particles may move to low energy nodes thereby leaving the smaller particles with more dwell-time in the high energy nodes. In addition, the sample viscosity, temperature, and uniformity will vary during the ultrasonic process, resulting in gradients during the process. The current low frequency, unfocused sonicator-type processes are random and non-uniform. This limits the use of ultrasound in high throughput applications where treatment standardization is required.

In contrast, focused, nonlinear acoustic waves are computer generated and delivered to individually-addressable samples with either of the *E* series systems: E110R and E210R. The mechanical energy imparted on the sample results in a controlled series of compression, rarefaction events. The *E-series* instruments were designed to provide the researcher with methods and systems to control the delivery of mechanical energy into a biological sample for high-throughput sample preparation applications.

## 2.0 INSTALLATION

### 2.1 Unpacking and System Setup

The Covaris system is shipped in one crate, containing the instrument, the instrument supplies, optionally purchased notebook computer, and the selected accessory kit. Remove all wrappings, taking care not to inadvertently discard any parts, accessories, or documents.

Normal precautions in unpacking and reasonable care in handling should be exercised to avoid possible damage to the unit. Visually inspect all external controls, indicators and surfaces to detect any damage that may have occurred during shipment.

The shipping company is responsible for damage to equipment during shipment. If damage has occurred, notify the shipping company immediately to establish proper basis for a claim.

**NOTE:** In case of damage do not discard packing material until Covaris has inspected for cause of damage.

**WARNING:** Risk of back injury. The instrument weighs approximately 110 pounds (50 kilograms).

### 2.2 Placement of Equipment

The area required for the instrument (see Figure 1) is approximately 23" wide by 30" deep by 19" high (59 cm x 76 cm x 48 cm). Space on or adjacent to the bench is required for a notebook computer. Additional space may be required for a user-supplied recirculator.

Two fans maintain a safe operating temperature in the instrument by circulating air over the internal components. Therefore, place the instrument so that there is a minimum of 1" (3cm) between the rear panel and any wall or obstruction for airflow.

### 2.3 Power Requirements

The instrument draws a maximum of 500 VA. The Covaris E-Series instrument is configured with a universal power supply. Refer to label on left side of instrument for specific power requirements

In case of a power interruption, the system will halt. The equipment will then need to be restarted. As files or data not saved prior to the power interruption will be lost, the use of an UPS (Uninterrupted Power Supply) is recommended with this system if your site has frequent power surges or loss of power. The UPS should be capable of supplying full 5 amperes at the voltage used.

**WARNING:** To prevent the possibility of electrical shock, always plug the system into a grounded power source.

### 2.4 Set-up Procedures

#### 2.4.1 Powering Up the System

Power up the Covaris system by moving the power switch to the "1" position. If the computer is supplied by Covaris, turn it on. Open the SonoLab software by double clicking the SonoLab icon on the desktop.

#### 2.4.2 Connections

Ensure that the user-supplied chiller is connected to the CHILLER IN and OUT ports on the left side of the instrument using the Covaris supplied hoses. The hoses are setup with quick disconnect fitting to be attached to the instrument and hose clamps for the user supplied chiller

Ensure there is a USB cable connected from a USB port on the computer to the USB port on the instrument (located above the power switch on the right side, near the back of the instrument).

### 2.4.3 Control Buttons

There are two control buttons located on the right side of the instrument as shown in Figure 2. The red STOP button can be depressed at any time to halt operation. The green DOOR button, when lit, can be depressed to allow the operator to open the safety enclosure door (See section 3.4).



Figure 2

### 2.4.4 Water Bath Configuration

The water bath tank needs to be oriented properly when inserted into the system. The bath should be oriented with the label "Insert this end" at the back of the tank (see Figure 3). The "waterline" label will be displayed on the front side of the tank.



Figure 3

**CAUTION:** The magnetic safety interlock will not allow the instrument to operate if the water tank is not oriented properly and fully inserted.

Fill the water bath to the water line on the side of the tank, as the water level will rise when the transducer is submerged. Only distilled or deionized water should be used to fill the water bath.

**CAUTION:** Never run a method without a water bath. This will permanently damage the transducer.

**CAUTION:** Using anything besides distilled or deionized water in the water bath is a misapplication of the equipment and will void the warranty.

The system includes a water level sensor. If the water level is too low, a warning message will be issued by the software. In addition, the degas pump will not operate for more than 10 seconds if a low water level is detected. This is to protect the pump from running without water.

Prior to running a method, the water bath should be degassed for 1/2 hour (30 minutes). Degas the system by lowering the transducer (click the START HOMING button in the Initiation Sequence or click the LOAD PLATE button on the MAIN PANEL of SonoLAB). Then click the DEGAS ON button.

**NOTE:** The DEGAS ON button will not engage the degassing pump if the transducer is in the SERVICE BATH position

**NOTE:** The degas pump will turn off after 10 seconds if the water tank is not filled with water to the fill line.

## 3.0 SYSTEM DESCRIPTION

### 3.1 General Overview

The *Covaris E110R* or *E210R*, as shown in Figure 1, is a custom instrument for high-throughput sample preparation and compound management. The system is comprised of the Treatment System which delivers the energy to the sample, the Safety System which provides intelligent interruption during the “treatment”, and the Computer and the Application Software which provide the user interface to the entire system.

This instrument is a very powerful, high-energy system. It is designed to disrupt and homogenize biological tissue, extract biochemicals, and accelerate reagent dissolution.

### 3.2 Treatment System

#### 3.2.1 Acoustic Transducer

The acoustic transducer delivers a high intensity, focused, computer-controlled acoustic shock wave to a sample. This is called the “treatment”. The treatment of all samples in a tube rack or well plate is called a method.

**WARNING:** Objects at the focal point of the transducer will be exposed to high energy when the system is activated.

#### 3.2.2 Temperature Sensors and Temperature Control

The water bath sensor, a 5cm metal probe, monitors the temperature of the water bath. The water bath temperature limit can be set to a value between 5° C and 40° C. The water bath temperature is controlled by connecting an external recirculating chiller/heater to the ports in the back of the system. The recirculator will pump a temperature-controlled fluid through a stainless steel loop immersed in the water bath.

**NOTE:** Although the E-series instrument reports the bath temperature via the Temperature Sensor, it does not control the recirculating chiller. Typically, for most applications (e.g., gene expression, proteomics, compound dissolution, and drug metabolism studies) the temperature of the recirculating chiller is set to 15 degree C with the temperature of the water bath equilibrating at 18 degree C.

**CAUTION:** A recirculating chiller is required for efficient and controlled operation of the E-series instrument.

### 3.2.3 Sample Positioning System

The major components of the system include:

- The sample positioning system (x, y, z axes)
- The transducer positioning system (z' axis)
- The sample holder plate
- The robotic interface

Figure 4 shows the sample holder plate with the A1 position identified.

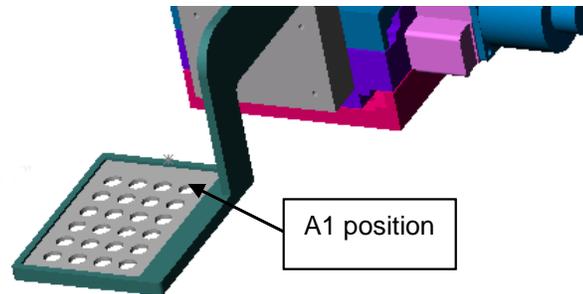


Figure 4

### 3.2.4 Water Bath and Degassing System

The water bath holds approximately 1.3 gallons (4.92 liters) of distilled or deionized water. The water line is marked on the front wall of the water bath. The water level should be within +/- 3mm of the waterline when the transducer is submerged. The water bath has double walls for insulation.

**WARNING:** The water bath is heavy when filled. Use caution when moving it.

The degassed, temperature controlled water provides an efficient energy couplant from the transducer to the sample to be processed. The water bath must be degassed for proper operation of the instrument. The degassing system is engaged by depressing the DEGAS PUMP button on the right side of the instrument when the transducer is in a lowered position. The degassing system circulates the water past the sample vessel being processed.

The degassing system consists of a dip tube with six small holes, a positive displacement pump, and an outlet nozzle. Dissolved gasses and vapors are reduced in the water by pulling the water through the small holes in the dip tube and pumping the resulting coalesced bubbles out the outlet nozzle before they can re-dissolve. During normal operation, there should be an intermittent stream of bubbles coming from the outlet nozzle. If no bubbles are visible for several minutes, the six small holes in the dip tube may be plugged. If this occurs, the dip tube may be removed and the holes cleared with a fine wire (e.g., 26 Gauge needle).

### 3.3 Computer

The computer uses the Microsoft™ Windows® XP Operating System with SonoLab™ application software.

### 3.4 Safety System

The safety system protects the user from hazards associated with the acoustic treatment system and the moving parts in the sample positioning system.

The safety system prevents operation of the instrument if the front door is open, the water tank is not in place, or the robotic interface is not enclosed. The front door can only be opened when the green light on the DOOR button is lit. Once lit, pressing the button will allow the door to be opened. There is also a red STOP button on the right of the safety enclosure (see Figure 2). Depressing the STOP button halts all operations and the Stop Button Dialog Box comes up which allows the user to either Resume or Abort the current operation as shown in Figure 5.



Figure 5

NOTE: The door may be opened in the event of a power failure by inserting a special tool provided with the instrument into the hole in the enclosure above the safety interlock and rotating clockwise 90 degrees. The instrument will not operate if the safety interlock is disabled. The tool is stored in a clip on the back of the instrument.

### 3.5 Water Bath Temperature

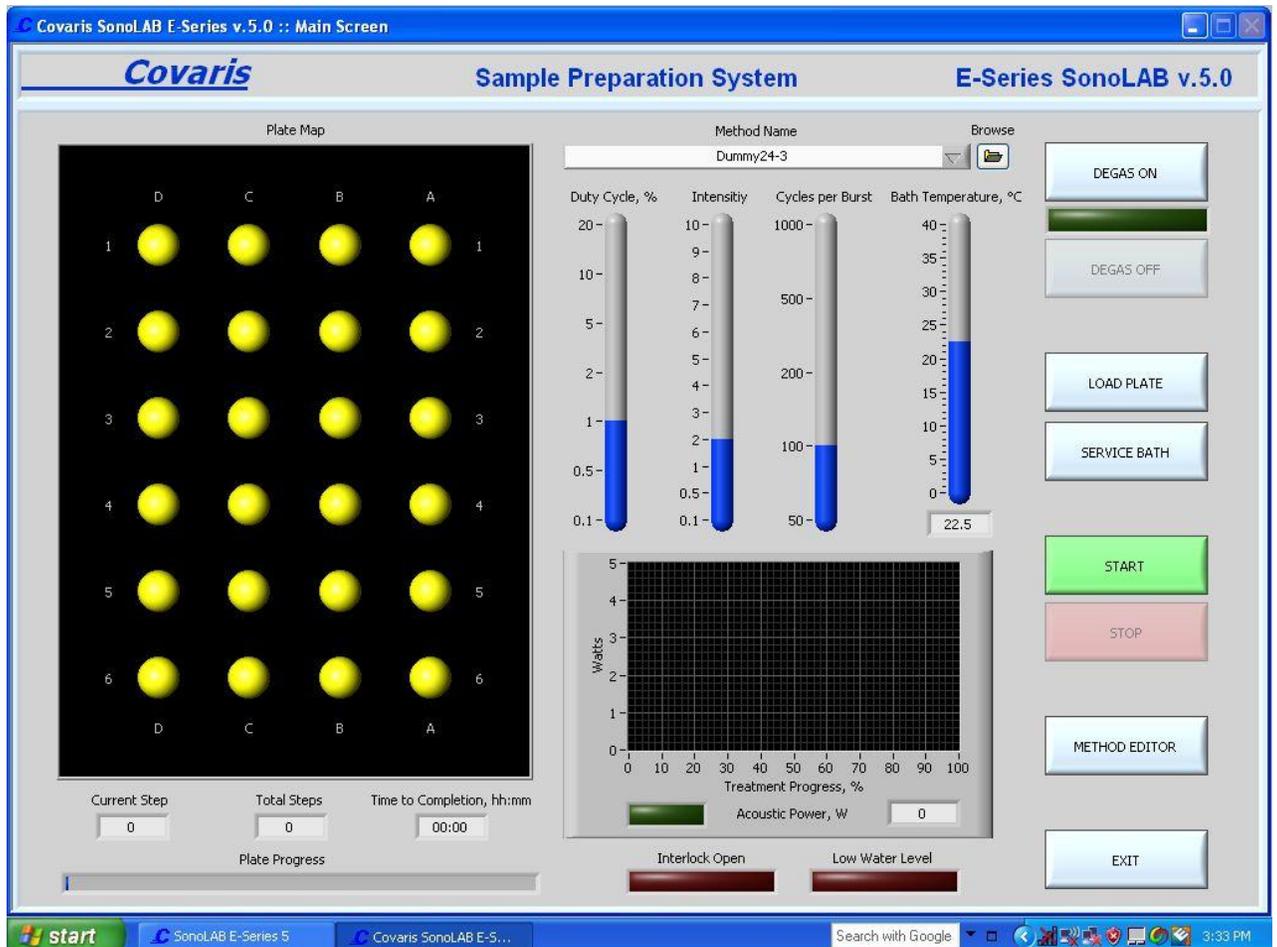
The software monitors water bath temperature. If the bath temperature is higher than a user defined limit, while a method is running, the method is halted and the following dialog box will appear.



Figure 6

### 3.6 Application Software

#### 3.6.1 Main Panel



**Software Panel 1: MAIN PANEL**

#### ***Motion Buttons***

The motion buttons move the sample holder and the transducer. They operate as follows:

DEGAS ON starts the degas pump

DEGAS OFF turns the degas pump off

LOAD PLATE lowers the transducer from a raised position (SERVICE BATH position) and moves the sample holder to the manual load position.

SERVICE BATH raises the transducer and sample holder out of the water bath

METHOD EDITOR button will take the operator to the *Method Editor Panel* which allows the user to create and/or modify a method.

START button starts running a method. After selecting the button, a warning dialog box opens to ensure the system is ready to proceed. The warning reminds the user to check that there is

water in the water bath, that the system has been degassed, and that there are samples in the sample holder. See the warning screen below:



**Figure 7**

If the user is ready to proceed, click the START button.

STOP halts the operation of the sample positioning system.

**NOTE:** The STOP button on the instrument and the STOP button on the display perform the same function.

The EXIT button will exit the program.

### ***The Status Indicator Lights***

ACOUSTIC POWER indicates that the transducer is active.

LOW WATER LEVEL indicates that the water sensor and transducer are not immersed.

INTERLOCK OPEN indicates the software senses that the mechanical safety interlock is in the open position.

DEGAS indicates the degassing pump is on. (Located below the DEGAS ON button.)

While a method is running, the following indicators display current treatment parameters:

BATH TEMPERATURE displays the temperature of the water bath. The thermometer graphic, normally blue, will turn red if bath temperature exceeds the temperature limit set by the user in the method.

DUTY CYCLE, INTENSITY, and CYCLES/BURST show the parameters for the current well being processed. (Refer to section 3.6.2)

ACOUSTIC POWER GRAPH shows the acoustic power being delivered to the sample for each treatment.

### ***Plate Map Graphic***

Each well position is represented in the plate map. Untreated wells are yellow. A well undergoing treatment is green and once completed the color changes to blue.

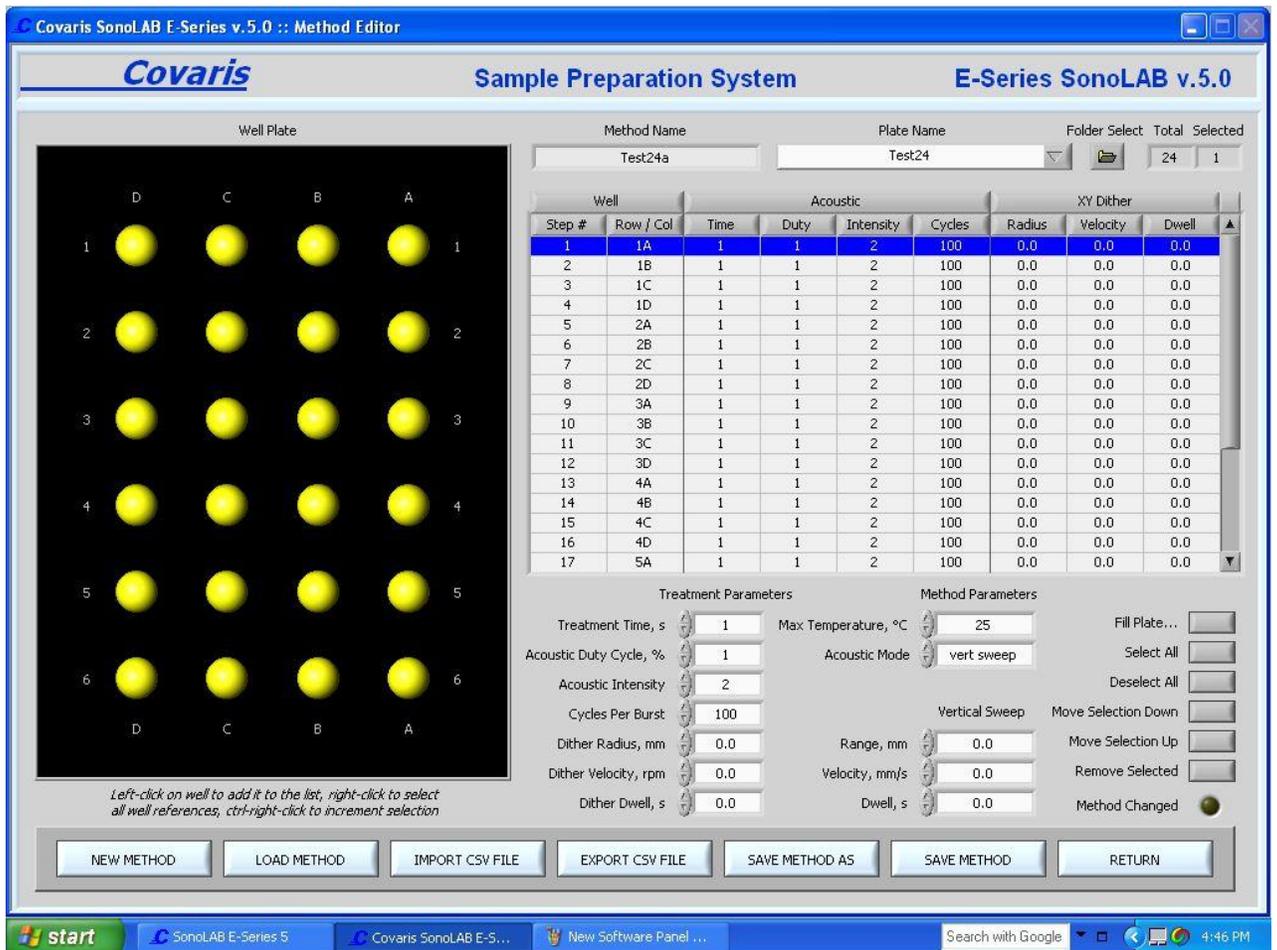
### ***Acoustic Treatment Graph***

The DUTY CYCLE, INTENSITY, and CYCLES/BURST indicators display the treatment parameters being applied to each well during a method run. These parameters are programmed into each method by use of the Method Editor Panel (see section 3.6.2).

### Progress Bar

Indicates the percent completed of the currently running method (as a percent of the total calculated time).

### 3.6.2 Method Editor Panel



#### Software Panel 2: METHOD EDITOR PANEL

This screen allows the user to select either a previously created method (and its associated parameters) or to create a new method. On the left side of the screen are the controls to set-up the well plate.

Left clicking on a table entry highlights that entry. The shift and control keys may be employed to highlight multiple entries. The Treatment Parameters may be edited for all highlighted table entries.

SELECT ALL and DESELECT ALL manipulate highlighting of steps in the Method Table. The MOVE Buttons move highlighted entries within the table.

The FILL PLATE button creates multiple sequences per well by applying the treatments highlighted in one or more rows in the table using the ordering of the selected sequence.

SELECT ALL will highlight all entries in the list of treatments. Edits made to acoustic parameters will apply to all highlighted entries.

DESELECT ALL will remove highlighting from all entries in the list of treatments.

MOVE SELECTION DOWN will move the highlighted entries down one position in the list.

MOVE SELECTION UP will move the highlighted entries up one position in the list.

REMOVE SELECTED will delete highlighted entries from the list.

Note that edits to method parameters will cause the METHOD CHANGED indicator to illuminate until these edits are either undone or saved.

### ***Treatment Parameters***

For a new or an existing method, the user may define and edit the individual well parameters or sequence in the control panel.

ROW/COL is the current treatment position in the well plate. The set of parameters defined for the well is a treatment.

TIME is the amount of time (sec) to apply the parameters or to treat a sample.

Acoustic energy is produced in bursts and the E-series system allows the user to modify this energy to maximize the effects on the treatment of samples for any particular application. Contact Covaris Technical Support for recommendations regarding these settings.

DUTY CYCLE is defined as a percentage. For example, within a timeframe it is the percentage of the time that the transducer is creating acoustic waves; the “on” cycle. Typically, the higher the DC the higher the acoustic waves per unit time is generated by the transducer. Minimum is 0.1% and Maximum is 20%.

INTENSITY: The amplitude of the pressure waves created by the acoustic transducer. The larger the number, the more energy each acoustic wave has. Minimum is 0.1 and maximum is 10.

CYCLES PER BURST: The number of waves generated by the transducer in a burst. For example, 100cpb at 10% duty cycle is a wavetrain of 100 “on” energy cycles followed by 900 “off” energy cycles. Minimum is 50 and Maximum is 1,000.

Dithering is the controlled horizontal circular movement of the treatment vessel around a center point during processing to mix the sample. For horizontal dithering, the following parameters can be set:

RADIUS is the radius (in mm) the vessel moves in a circular motion.

VELOCITY is the speed (in rpm) of this movement of the vessel.

DWELL is the time the vessel remains at the center point between dithering operations.

### ***Method Parameters***

The Acoustic Mode Menu has three modes:

1. **FREQUENCY SWEEPING:** The acoustic frequency sweeps through a range during processing. This is the default setting for the E-series instruments. As plastic readily absorbs acoustic energy, this lower power setting is more appropriate for method development with plastic vessels. Frequency sweeping is both preferred and recommended for plastic sample vessels.
2. **POWER TRACKING:** In this mode, the instrument optimizes the operating frequency to achieve peak power in the treatment vessel for the treatment chosen. This mode delivers the most effective power and the highest level of mixing to the sample. It is

also the best for most applications, especially biologically active samples (e.g., RNA extraction from tumor tissues samples or proteomic preparation) where rapid sample processing is required. This setting may also be appropriate for chemical-based samples (e.g., small molecule drug extraction from slow release tablets, compound dissolution, or metabolite extraction from plant tissue). Power Tracking is both preferred and recommended for high-power treatments in glass sample vessels.

**CAUTION:** When used with some high power treatments and some vessels (e.g., plastic) Power Tracking Mode may result in heating of the sample (e.g., viscous, fibrous samples).

3. VERTICAL SWEEP moves the sample up/down during processing. The menu is active when Vertical Sweep is selected.

VERTICAL +/- is the vertical movement in mm.

VERTICAL RATE is the frequency in cpm (cycles per minute).

**NOTE:** The ability to monitor water bath quality is not active in this mode.

MAX TEMPERATURE allows the user to select a water bath temperature limit for the treatment. The temperature limit can be set to a value in the range of 5° C and 40° C. If the water temperature is over the specified limit, the BATH thermometer on the main panel will turn red and processing will be halted. It can be restarted by selecting OVERRIDE in the temperature warning dialog box.

### **General Operation Buttons**

The following buttons are employed for creating, importing, exporting and saving methods. Note that edits to method parameters will cause the Method Changed indicator to illuminate until these edits are either undone or saved.

NEW METHOD opens a new method with no treatments yet defined for any wells.

LOAD METHOD opens an existing method (\*.pro file) and displays it on the Method Editor panel.

IMPORT CSV FILE takes treatment data stored in a file of Comma Separated Variables (\*.csv file) and displays them as a new method. The method parameters may be edited as desired and saved as a method (\*.pro) file.

EXPORT CSV FILE creates a Comma Separated Variables file containing the method parameters displayed on the Method Editor panel.

SAVE METHOD AS saves the currently displayed method parameters to a new or existing method (\*.pro) file.

SAVE METHOD saves the currently displayed method parameters to the method (\*.pro) file name displayed in Method Name.

RETURN closes the Main Panel. Note that you may be prompted to save or discard any unsaved edits before returning to the Main Panel.

## **4.0 SYSTEM OPERATION**

### **4.1 Start-Up Procedures**

#### **Chiller Connection**

The recirculating chiller (required) should be connected to the treatment system using the hoses supplied. The chiller inlet is connected to the fitting labeled "IN" and the chiller outlet is connected to the fitting labeled "OUT" located on the left side of the instrument.

#### **Water Bath**

The water bath level needs to be checked once the samples are loaded using the water line on the front of the tank. The water level should be within +/-3mm of the waterline when the transducer is submerged. Only distilled or deionized water is used in the water tank.

Special care needs to be taken when changing the water bath. First, select the CHANGE BATH button to raise the transducer. Then carefully pull out the acrylic water tank from the instrument base plate and empty it. Fill the water tank and place it back into its position, ensuring that it is oriented properly in the instrument (see section 2.4.3).

**WARNING:** The water bath is heavy when filled. Use caution when moving it.

**CAUTION:** Never run a method without a water bath; this will permanently damage the transducer.

### ***Degassing***

The Covaris system requires that the water bath be degassed to maintain repeatable acoustic energy coupling, therefore, the pump should be running whenever samples are being treated. This is especially critical for high intensity applications such as solid tissue homogenization. At room temperature, the water bath should be degassed for at least 1/2 hour prior to running a treatment method. Select the DEGAS PUMP button on the Main Panel. Ensure the water bath is filled to the water level before turning on the pump; otherwise, damage to the pump may occur.

**NOTE:** The degassing pump will turn off after 10 seconds if the water tank is not filled with water.

### ***Manual Loading of the Multi – Well Plate Holder***

Select the LOAD PLATE button on the Main Panel to raise the multi-well plate holder. The system will position the stage above the water bath.

Fill the tube rack with the sample vessels to be run. If required, place the hold-down plate over the vessels. The hold-down plate ensures that the vessels stay in place for correct processing.

The plate should be loaded on the multi-well plate holder with the A-1 position in the upper right corner. See Figure 4.

If not all the wells are filled with samples to be treated and the method is high intensity, load the unused positions with empty vessels to reduce splashing.

**NOTE:** Ensure the well-plate is seated flush onto the well-plate holder to insure the samples are correctly aligned with the focal point of the transducer.

## **4.2 Creating and Modifying a Method in the Method Editor Panel**

To open an existing method to run or to modify, select METHOD EDITOR, click on LOAD METHOD and choose from the list of previously saved set-ups.

To create a new method, select the METHOD button.

Select the Well Plate Configuration from the PLATE NAME menu.

*Parameters may now be selected in the Treatment Parameters area.*

### **Select the Current Well Parameters**

Click on any well; a treatment can be configured using the Treatment Parameters.

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. 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.

Save the method by selecting the SAVE METHOD button. The system will prompt the user to name the METHOD.

### 4.3 Running a Method Manually in the Main Panel

Load the samples first by selecting the LOAD PLATE button. The motion system will move the sample holder tray to the front of the enclosure and the green DOOR button will illuminate. Press the DOOR button to open the door and position the samples on the tray.

Click the pull-down menu button under the Method Name. Select from the list of defined methods.

Click the START button. The system will run the treatment displayed in the Method Name box.

**CAUTION:** If the computer loses connection with the instrument during processing, the method will stop.

The following Main Panel indicators provide system status information:

The green indicator between the DEGAS buttons will illuminate when the degas pump is on. This pump should be run for at least 30 minutes prior to running a method.

On the WELL PLATE MAP, each well will appear yellow prior to treatment, green during treatment and blue following treatment.

The CURRENT STEP, TOTAL STEPS and ESTIMATED TIME TO COMPLETION displays monitor progress through the method.

The ACOUSTIC POWER indicator and graph indicate when acoustic treatment is being applied, and its approximate power.

The DUTY CYCLE, INTENSITY, AND CYCLES PER BURST graphics show the treatment parameters being applied to a well when the Acoustic Power indicator is illuminated.

INTERLOCK OPEN will illuminate when human access is occurring in the acoustic treatment area. Acoustic power and internal motion will be suppressed whenever an INTERLOCK OPEN condition is detected. Specifically, this will occur either when the front door is open, and/or when the side robot interface door is open with no robot interface in place.

### 4.4 Manual Overrides

There is a STOP Button on the Computer screen. This will halt the current method. The method may also be stopped by pressing the red STOP button on the instrument. The green DOOR button will light once the method has stopped.

To restart, the system will prompt the user with a message to either RESUME or ABORT the method. If the door is not closed, selecting RESUME will prompt the user again to RESUME or ABORT. Selecting RESUME with the safety system closed will continue the method where it was interrupted. Selecting ABORT terminates the method.

#### 4.5 Shut-Down Procedures

Raise the transducer out of the water bath by selecting the SERVICE BATH button. Sponge out the “bowl” of the transducer to remove water. Empty the water bath. If the instrument will be idle for an extended time, lower the transducer and run the degassing pump with an empty water bath for 10 seconds to drain the degassing lines.

**CAUTION:** Leaving water in the water bath or degassing lines can cause algae growth in the tank and lines which may interfere with sample processing.

Exit the SonoLab software by selecting EXIT on the main panel.

Select Shutdown from the START menu on the Windows XP screen and select the option “Shut down the computer”. The computer will power down. The instrument can be powered down by moving the power switch to the “0” position. The power switch is located at the rear of the instrument on the right side.

**CAUTION:** Failure to raise the transducer from the water bath when not in use may result in permanent damage to the system.

#### 4.6 Robot Interface

The E110R and E210R systems may be employed in a manual mode, or operated as part of a larger automated plate handling system. For this purpose the instrument is equipped with a robot interface portal on its right side. This interface is designed to provide a safe enclosure for robotic loading and unloading of plates.

If the instrument is employed in a non-robotic, manual mode, in which a robot enclosure is not present, the door of the robot interface must be slid shut to ensure no access to the acoustic treatment area.

In automated applications, the plate holder arm will rotate to accept plates through the robot interface. The door of the robot interface must in this case be fully open. The enclosures of the mating robot should mate appropriately with the safety enclosures of the E instrument to ensure product safety. Methods developed in the Method Editor may be run automatically via an automated software command structure.

### 5.0 SYSTEM SPECIFICATIONS

<b>Model</b>	E Series
<b>Treatment System</b>	Bench-top: high intensity acoustic transducer, sample positioning system, temperature monitoring device, circulation pump, and water bath with safety enclosure.  Dimensions: 23" W x 30" D x 19" H (59cm x 76cm x 48cm)  Weight: approximately 110 lbs (50 Kg)
<b>Computer</b>	Notebook computer (may be supplied by Covaris or the user)
<b>Operating System</b>	Microsoft Windows XP Professional
<b>Application Software</b>	Covaris SonoLAB

<b>Data Input</b>	Keyboard, mouse
<b>Chiller</b>	Chiller/heater re-circulating system - not included. Connect with the 3/8 inch I.D. hoses and quick connect fittings supplied
<b>Power Requirements</b>	100-240 V~ 50-60Hz
<b>Water Bath</b>	Range of Temperature limit (water only):+4.0°C to +50.0°C

## 6.0 MAINTENANCE

### 6.1 Water Bath

#### 6.1.1 Recommendations for Daily Maintenance

Only distilled or deionized water should be used to fill the water bath. The water bath should be emptied every day to avoid algae growth. Do not use an algaecide.

The water level is dependent upon the sample vessel type. There is a water line in the tank to help determine the depth required. Refer to the technical notes appropriate to the sample vessel to determine water depth.

#### 6.1.2 Recommendations for Monthly Maintenance

Periodically, the water bath and degassing lines may be rinsed with a 10% solution of bleach (or equivalent). The resultant sodium hypochlorite solution is ~ 0.5% NaOCl. With this solution in the water bath, lower the transducer and run the degassing pump for a few minutes. Repeat with fresh water in the water bath.

### 6.2 Transducer

When not in use, the transducer should be raised out of the water bath and the transducer “bowl” should be sponged out with a soft lint-free cloth or sponge to remove any residual water. Keeping the transducer dry between applications will help prevent corrosion and is strongly encouraged to ensure optimal performance.

Use extreme care when handling the transducer. Permanent damage could result if anything is dropped onto the face of the transducer or if the face of the transducer strikes something.

Permanent damage could also result if the transducer is overheated, (i.e. running the transducer without a water bath).

**WARNING: Do not attempt to remove the transducer as irreparable damage may occur.**

### 6.3 Safety System

Test the system periodically. Make sure that the operation is halted when the STOP button is depressed.

**WARNING: Any failure of the Safety System must be reported immediately to Covaris. DO NOT attempt operation if the Safety System is malfunctioning.**

### 6.4 Degassing the System

The degassing pump will shut down after 10 seconds without a water bath because running the pump without water will damage the pump.

Check that there are bubbles coming from the end of the degassing tube. Remove the dip tube (inlet tube) if necessary and examine it under a microscope to clear any clogged holes.

For long-term storage, all water should be removed from the degassing pump lines. This can be done by running the degassing pump for 5 to 10 seconds with an empty water bath.

## **6.5 Relocation of the System**

Should it be necessary to move the instrument, for whatever reason, the system needs to be properly prepared. This procedure describes the steps necessary to relocate Covaris E-Series instrument. If the instrument is to be moved off site, a crate may be obtained from Covaris.

### **6.5.1 Prepare the System**

- Raise the transducer (“Service Bath” position) and sponge out the bowl of the transducer. Empty the water tank and reinstall it in the instrument
- Lower the transducer and run the degas pump. Run the pump for 10 seconds, enough to drain the water from the pump lines.
- Raise the transducer (“Service Bath” position) and empty the residual water from the tank
- Leave the transducer in the up position (“Service Bath” position).
- Save the current method and close SonoLab software by selecting EXIT on the main panel.
- Shut down the computer from the START menu.
- Power down the instrument by moving the power switch to the “0” position. The power switch is located at the rear of the instrument on the right side.
- Disconnect the instrument power cable, the USB cable, the mouse and the computer power supply. Gather these items and keep with the instrument.
- Disconnect the Chiller from the instrument. Drain the water in the chiller into a bucket through the drain fitting. The hoses can be drained by depressing the valve in the center of each quick-connect fitting and holding over a bucket.
- Protect the chiller quick-connect fittings on the lower left side of the instrument from damage by taping foam or bubble wrap over them.

### **6.5.2 Move of the System**

- If the instrument is to be moved off-site, procure a crate from Covaris. Pack the instrument in the crate using the provided foam inserts.
- Protect the system and computer with a light cloth or plastic to prevent dust contamination.
- Be sure all the components are accounted for, including tube holders and user manual.
- Transport the instrument and accessories. At least two capable people are required to lift and place the instrument. The instrument weighs approximately 50kg (110 lb). The chiller is also heavy.

### **6.5.3 Reinstall the System**

- Instructions for reinstalling the instrument are contained in Section 2 of the User Manual. If the manual is missing, obtain a new copy from Covaris.

## 7.0 TROUBLESHOOTING

### System Trouble Analysis Chart

SYMPTOM	PROBABLE CAUSE	POSSIBLE SOLUTION
Degassing system not generating bubbles.	<ol style="list-style-type: none"> <li>1. Degassing dip tube may be clogged</li> <li>2. Pump not working</li> </ol>	<ol style="list-style-type: none"> <li>1. Remove and backflush under running water</li> <li>2. Examine the six holes (e.g., using a stereo microscope) to see if clogged and use a pin to unclog them</li> <li>3. If pump does not run, contact Covaris.</li> </ol>
Incorrect positioning	Home position lost	Home system by pressing selecting "initialize system from the pull-down menu on the main panel.
Safety interrupt	<ol style="list-style-type: none"> <li>1. Water tank is misaligned</li> <li>2. Door is open.</li> <li>3. STOP button is depressed</li> <li>4. Safety interlock is bypassed</li> </ol>	<ol style="list-style-type: none"> <li>1. Check tank alignment</li> <li>2. Check door</li> <li>3. Check safety interlock</li> </ol>
Sample vessel hits the water jet nozzle	<ol style="list-style-type: none"> <li>1. System has lost the home position.</li> <li>2. Incorrect nozzle position</li> </ol>	<ol style="list-style-type: none"> <li>1. Home the system</li> <li>2. Reposition water jet manually</li> </ol>
Error message that system not shut down correctly	SHUT Down command in Windows XP not used before rebooting	<ol style="list-style-type: none"> <li>1. Follow instructions on screen</li> <li>2. Use SHUT DOWN command</li> </ol>
Delay in powering degassing pump on/off	If a method is running, there is a delay in powering the degassing pump on/off	Wait until method completes before powering the degassing pump on/off
Values for Dithering and Vertical Sweep are different from the values that were entered	Values entered are larger than the limits for a particular Well Plate	Use lower values

## APPENDIX A: Sample Preparation Requirements

Although sample preparation requirements are diverse, for most samples the following ranges apply:

Model E110R -	volume	100 – 1,000 $\mu$ l
	mass	< 100 mg
	tube	13 x 100 mm

Model E210R -	volume	1,000 – 10,000 $\mu$ l
	mass	< 1,000 mg
	tube	16 x 100 mm

The system may also be configured for other tubes and vials, temperatures, and robotic systems.

Please contact Covaris for any additional questions or comments regarding sample processing.

## APPENDIX B: Procedure to install USB drivers and Covaris SonoLAB 5.x E-series software on a Windows XP computer

**NOTE:** Follow the instructions in sequence.

1. Begin with the USB cable disconnected and the instrument powered off.
2. Load the INSTALL CD onto the Laptop's CD-Drive. The SonoLAB installer will launch in about 30 seconds. If the SonoLAB installer does not launch automatically, navigate to the CD drive, open the Volume folder and double-click on the Setup application file to start the installation process.
3. Simply click Next to proceed through the installation process. When queried, accept any software license agreement and click Next to continue with the installation.
4. When installation is complete, click Finish and leave the CD in the CD-Drive. Restart the computer as requested by the installer software.
5. When the computer restart is complete, power on the E instrument and plug the USB cable into a USB port on the computer.
6. The Windows hardware INSTALL Wizard will start. Choose "No not this time" when queried about connecting to Windows Update. If Hardware Installation warnings appear, click "Continue Anyway" to proceed with the installation. The wizard will run four times.
7. When the driver installation is complete, the system should be ready to use. A shortcut to SonoLAB 5.x should appear on the computer desktop.
8. Double click on the shortcut to start SonoLAB E-series software.

**NOTE:** Laptop standby and hibernate modes should be avoided while operating the SonoLAB E-series software. To ensure this, from the start menu, open Control Panel, then Power Options. Select a Power Scheme that sets System standby and System hibernate times to Never. If standby and hibernate modes remain enabled, then the SonoLAB E-series software should be closed and the system powered down whenever the system is not in use.

**NOTE:** SonoLab E-series software was not tested with other application software and therefore we recommend that no additional application software is loaded. Wireless Network Connections on the Laptop should be disabled to avoid loading of any unwanted software. Go to Network Connections in the Control Panel. Highlight the Wireless Network Connection and select "disable this network device".

9. To install the stand-alone Method Editor utility, open the install CD and navigate to the Method Editor Installer folder. Open this folder and double click the Setup utility.
10. Simply click Next to proceed through the installation process. When queried, accept any software license agreement and click Next to continue with the installation.
11. When installation is complete, click Finish and leave the CD in the CD-Drive. You may create a desktop shortcut to the Method Editor if desired.

12. To install the standard set of plate definitions, open the install CD and navigate to the Plates folder. Open this folder. In this folder are located several standard plate definitions that can be employed to create treatment processes in the SonoLAB Method Editor.
13. Highlight the plate definitions and copy the files (not the folder) to the following location on the computer: C:\Covaris\SonoLAB v5.1\Plates
14. To install an example of a treatment process, open the install CD and navigate to the Processes folder. Open this folder. In this folder is located a treatment process named Demonstration. It is a simple low power method. Copy this file to the following location on the computer:

C:\Covaris\SonoLAB v5.1\Processes

This method may be loaded from the Method Editor screen in SonoLAB and run as an example.

### Procedure to Install API drivers and API Test Utility

1. Open the install CD and navigate to the API Installer. Open the Volume folder and double-click on the Setup utility. This utility will install the software needed to remotely communicate with the E Robotic Instrument, using the API commands. The following files should appear in the Program folder under C:\Covaris\SonoLAB v5.1:
  - SONOLABESERIES.DLL – a dynamic link library containing functions accessible by the customer's program;
  - SONOLABESERIES.LIB – a static link library containing functions accessible by the customer's program;
  - SONOLABESERIES.H – a header file containing function prototypes;
2. Return to the API Installer folder and open the API Test Applet folder. Copy the three files named "E-Series API Tester v5.1" to the following location: C:\Covaris\SonoLAB v5.1\Program
3. You may create a desktop shortcut to the E-Series API Tester utility. You may run the API Tester to demonstrate various remote commands. Please refer to the API User Manual before running the robotic system. Commands must be given in a strict sequence in order to be successful.

**NOTE:** The following components used by the API are installed during installation of the stand-alone application SonoLAB E-Series 5.1:

- LabVIEW 8.2 Run-Time Engine (not directly accessible to the customer);
- NI-VISA National Instruments serial communications support
- SONOLAB E-SERIES 5.INI – an initialization file required for instrument operation (should not be modified by the customer);
- MS\_SETTINGS.BIN – an initialization file required for instrument operation (should not be modified by the customer).

The default installation folder is C:\Covaris\E-Series v5.1\Program. It is important that the files SONOLAB E-SERIES 5.INI and MS\_SETTINGS.BIN be located in the same folder as the SONOLABESERIES.DLL.

APPENDIX C: Nominal dimensions for Robotic Interface

