Sierra BioSystems

Shasta Oligo Synthesizer

User's Manual

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Introduction

Overview

About This Chapter This chapter provides an overview of the Shasta High Throughput Oligo Synthesizer documentation, safety considerations, and technical support resources available.

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Shasta Synthesizer Documentation

Background Needed

This manual assumes that you are familiar with the following:

- Basic Windows 10 operations, such as using the mouse, selecting commands, working with windows, and using the Windows 10 computer file management system
- The general manipulation of data files
- Good laboratory practices and basic laboratory techniques
- Oligo synthesis chemistry

About the Documentation Set

This three-quarter view shows you the front and the left side of the Shasta instrument. Use the following table to determine which Shasta instrument document you need for the task at hand. All of the documents listed are sent to Shasta instrument customers.

If you want...	Refer to the
• To prepare your laboratory for installation of the instrument • The instrument's electrical, ventilation and space requirements • A Site Preparation Checklist • Explanations of instrument safety alert symbols in several languages	Preinstallation Guide
• Instructions for general instrument setup and run initiation using pre-programmed cycles • Routine maintenance information • Operational safety information	User's Manual

Safety

Documentation User Attention Words

Five user attention words appear in the text of all Sierra BioSystems user documentation. Each word implies a particular level of observation or action as described below.

Note – Calls attention to useful information.

IMPORTANT! – Indicates information that is necessary for proper instrument operation.

DANGER! - Indicates if the danger is not avoided, it will cause death or serious injury.

WARNING! - Indicates if the warning is not heeded, it can cause death or serious injury.

CAUTION! - Indicates if the precaution is not taken, it may cause minor or moderate injury.

Chemical Hazard Warning

WARNING! Chemical Hazard. Some of the chemicals used by the Shasta instrumentation and protocols are potentially flammable.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous material.
- Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., safety glasses, gloves, or protective clothing).
- Do not leave chemical containers open. Use only with adequate ventilation.
- Check regularly for chemical leaks or spills. If leaks or spills occur, follow the manufacturer's cleanup procedures as recommended by the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

WARNING! Chemical Hazard. The Shasta ships with default protocols for oligonucleotide production. Within these protocols, flammable liquids are set to dispense in volumes smaller than 50µl. Alteration of these protocols is not recommended by Sierra BioSystems, Inc.

Instrument Safety Labels

Safety Labels are located on the instrument. Each safety label has three parts:

- A signal word panel, which implies a particular level of observation or action (e.g., CAUTION or WARNING). If a safety label encompasses multiple hazards, the signal word corresponding to the greatest hazard is used.
- A message panel, which explains the hazard and any user action is required.
- A safety alert symbol, which indicates a potential personal safety hazard.

Before Operating the Instrument

Ensure that everyone involved with the operation of the instrument has:

- Received instruction in general safety practices for laboratories.
- Received instruction in specific safety practices for the instrument.
- Read and understand all related MSDSs.

CAUTION! Avoid using this instrument in a manner not specified by Sierra BioSystems, Inc. Although the instrument has been designed to protect the user, this protection can be impaired if the instrument is used improperly.

14		ISO 7000 - 0434B (2004-01)	Caution *

Laboratory Environmental Requirements

Altitude This instrument is for indoor use only and for altitudes not exceeding 2000 m (6500 ft) above sea level.

Temperature and Humidity Laboratory temperature should be between 16-22°C (60-72°F), but the instrument can handle temperatures of between 10°C and 40°C. The instrument can tolerate maximum relative humidity of 99%.

Pollution This instrument may be installed in an environment with nonconductive pollutants only.

Electrical Requirements

Location	Voltage (VAC)	Frequency	Amperage (A)
USA/Canada	120 ± 10%	50/60 Hz ± 1%	10
Europe	240 ± 10%	50/60 Hz ± 1%	10
Australia	240 ± 10%	50/60 Hz ± 1%	10
Japan	110 ± 10%	50/60 Hz ± 1%	10

Power Line The electrical receptacle must have a dedicated 1.5 kVA power line and ground or a 1.5 kVA power line with a line conditioner or uninterruptible power supply (UPS).

Power Rating This instrument is rated for a maximum output of 240 W

Power Cords In the USA, Canada, and Japan, the instrument is supplied with a detachable cord equipped with a standard three-prong plug.

In Europe and Australia, the instrument is supplied with a detachable electrical cord equipped with a standard EC plug.

Grounding Certain types of electrical noise are greatly exaggerated by poor or improper electrical ground connections. To prevent these problems, it is very important to have a dedicated line and ground between the instrument and building main electrical service.

Placing the Instrument

Note: Please refer to the Shasta Preinstallation Guide for a thorough explanation of all site preparation and preinstallation procedures.

Guidelines for Lifting and Carrying the Shasta

The frame of Shasta is made from welded, Teflon-infused metal. As a result, it is a particularly heavy instrument weighing upwards of ~300 lbs. When locating the instrument, the following guidelines must be adhered to at all times.

WARNING! Movement of the instrument should never be attempted without the use of a lift.

Using a hydraulic lift with a minimum carrying capacity of 1,500 lbs, center the Shasta over the lifting forks in both width and depth. The weight of the Shasta is evenly distributed across the machine, so a direct, vertical lift with evenly balanced force is advised. Once elevated, the Shasta can be moved. For the user's safety, the Shasta should be kept near to the ground while in motion.

Once the Shasta has been located, place the instrument so that all four feet are seated flat onto a secure surface.

Installing the Instrument

The Shasta ships with all necessary components for dry operation. This includes:

- The Shasta Synthesizer
- Computer with Windows OS and Shasta Software
- Power Cords (3)
- Ethernet Cord
- Computer Monitor and Keyboard/Mouse

Using these components, following the steps below to install the instrument:

1. Locate the Shasta onto a sturdy table following the guidelines described in "Placing the Instrument".
2. Place the computer either beside or underneath the Shasta, and attach the provided monitor, keyboard, and mouse to the computer.
3. Place the monitor, keyboard, and mouse in a viewable/reachable area.
4. Attach the provided Ethernet cord to the Ethernet port on the Shasta and to any Ethernet port on the computer. Be sure the Ethernet cord sounds an audible "click" as it affixes to the port.
5. Plug the given power cord into a nearby socket and then the back of the instrument. Be sure the socket used provides a grounding source.

Uninstalling the Instrument

When uninstalling the Shasta, the user will must observe all procedures listed in Long-Term Shutdown, and be sure to account for the following:

- The Shasta Synthesizer
- Computer with Windows OS and Shasta Software
- Power Cords (3)
- Ethernet Cord
- Computer Monitor and Keyboard/Mouse

1. Begin by following the steps listed in Long-Term Shutdown
2. Power-off the instrument using the primary power switch located on the back of the instrument.
3. Power-off the computer after saving all necessary data.
4. Disconnect the power cord first from the machine and then from the outlet.
5. Disconnect the ethernet cord first from the machine and then from the computer.
6. Disconnect the computer monitor, keyboard, and mouse.
7. Recollect and bag all cords and computer paraphernalia.

For any questions or concerns, please contact:

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Tour of the Instrument

Overview

About This Chapter This chapter provides an overview of the Shasta High Throughput Oligo Synthesizer hardware and the software components that you will use most often.

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About the Shasta

Overview

The Shasta Synthesizer couples single nucleotides (bases) together in a stepwise fashion to form customized oligonucleotides linked to a solid support at the scale listed in the table below.

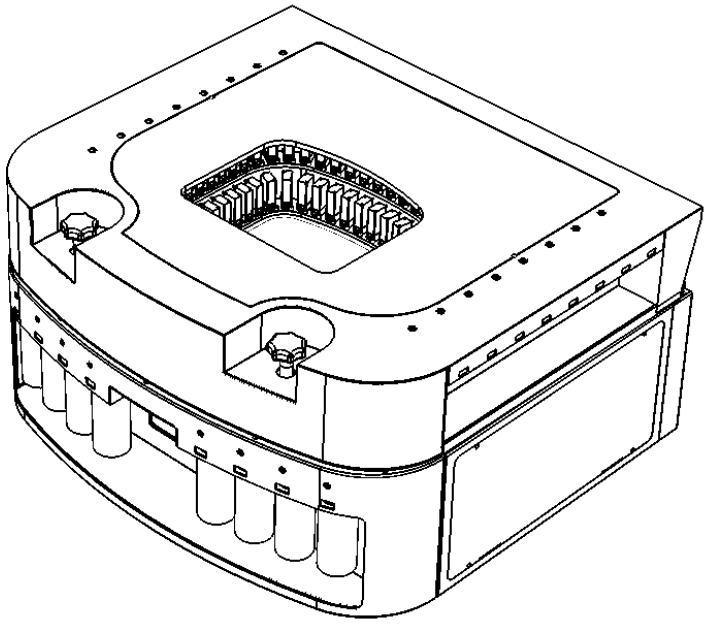


Plate	Scale
384-well plate(s)	2 – 50 nmol
96-well plate(s) / 96 - 192 columns	5nmol - 1µmol
64 syringe bodies and/or columns	40nmol – 20umol

The Shasta performs sequential synthesis; that is, the system can dispense reagents in one row while draining waste in another. Sequential synthesis allows for rapid manufacturing times.

Software

The Shasta software is installed on the system computer. Use the Shasta software to perform:

- Synthesis runs
- Protocol design
- Calibration functions
- Manual Control functions
- Priming functions
- Maintenance and Diagnostic operations

How the Shasta System Works

Chemicals are delivered through dedicated tubing to 64 dispense nozzles positioned over a slide block. The dedicated tubing eliminates the chance of cross-contamination. The slide block moves under the stationary dispense nozzles in the order determined by the Protocol and Sequence files loaded into the Shasta software.

The slide block supports 5 formats:

- 1 x 384 (applicable to plates only)
- 2 x 384 (applicable to plates only)
- 1 x 96 (applicable to both columns and plates)

- 2 x 96 (applicable to both columns and plates)
- 1 x 64 (applicable to columns and syringe bodies)

The pressure of inert gas delivers reagents and amidites to the plates/columns and purges their contents between deliveries. Optionally, the system can also pulse liquids through the resin to better utilize solvents and achieve higher yields.

Multiple Drain Solution

The Shasta is equipped with an array of 16 drain valves that can be plumbed into either column or plate formats.

Each drain apparatus will divide its available synthesis vessels by 16 to give the total complement of columns or wells any 1 drain valve will be responsible for. Though each column/well may be destined to produce a different oligonucleotide sequence, the columns/wells within any drain pathway should be of the same scale, as the columns/wells within this pathway will be processed with the same customer-defined cycle.

Synthesis Protocols

A Synthesis Protocol tells the instrument the steps to perform in order to synthesize the DNA sequences you have entered. An optimal cycle program is provided with the Shasta instrument software and is the only type of oligonucleotide production that will be covered in this manual. However, you can also program cycles to further customize the DNA synthesis process in your laboratory.

The Shasta Synthesizer's "multiple drain solution" offers a high degree of freedom in the creation of Synthesis Protocols, allowing the user to assign a different protocol to every available drain. The options afforded to the user in writing and editing these protocols are discussed further in the Software Walkthrough

About Drain Grouping

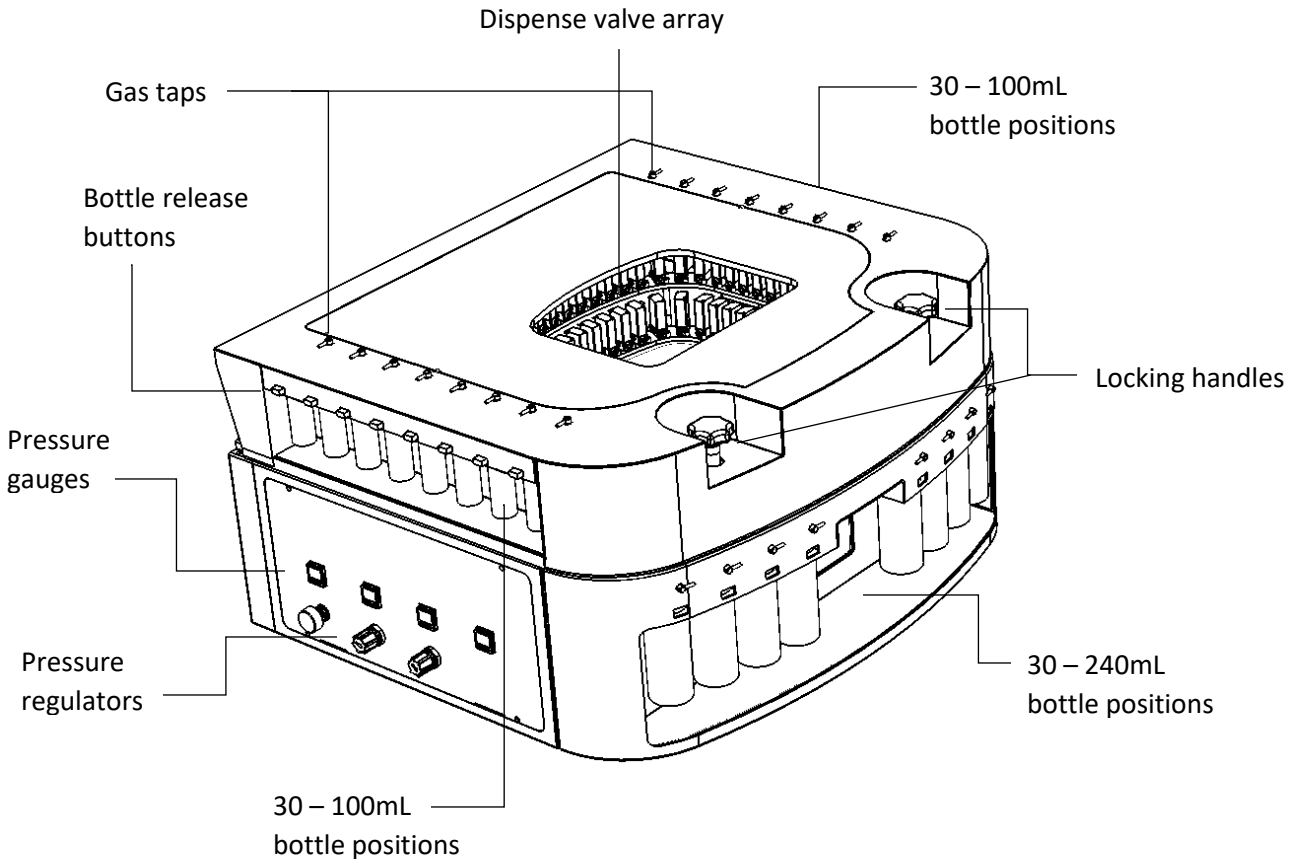
The 16 drains of the instrument can be independent, each with its own protocol; they may also be joined together to run one simultaneous protocol. In most cases, users focusing on high throughput operation will want to utilize one protocol for most of their synthesis. In doing so, the instrument will organize its drains into "groups" to create a more efficient synthesis.

When running one primary protocol, the instrument will organize its 16 drains into 4 groups. Group 1 will consist of drains 1, 5, 9 and 13. Group 2 will consist of drains 2, 6, 10 and 14. Group 3 will consist of drains 3, 7, 11 and 15. Group 4 will consist of drains 4, 8, 12 and 16. Each group will perform the same operations simultaneously. This means initiating all Pulse, Hold, and Drain operations for a given group after their last columns/wells have been dispensed into. These post-dispense operations will begin while the subsequent groups are receiving dispense, awaiting dispense, or finishing a previous section's post-dispense operations.

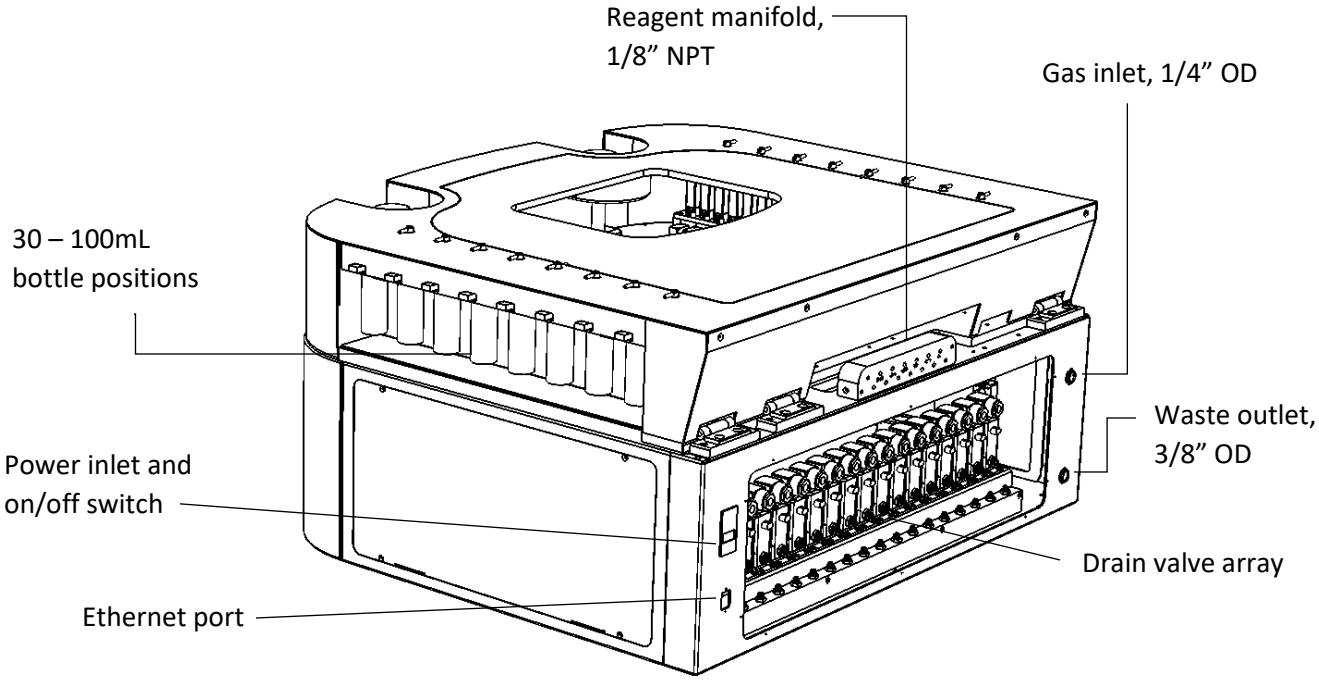
NOTE: Organizing oligonucleotides of similar lengths into a shared group will allow the machine to optimize its movement patterns for a faster synthesis as well as guarantee efficient draining throughout the synthesis.

Shasta Synthesizer Hardware

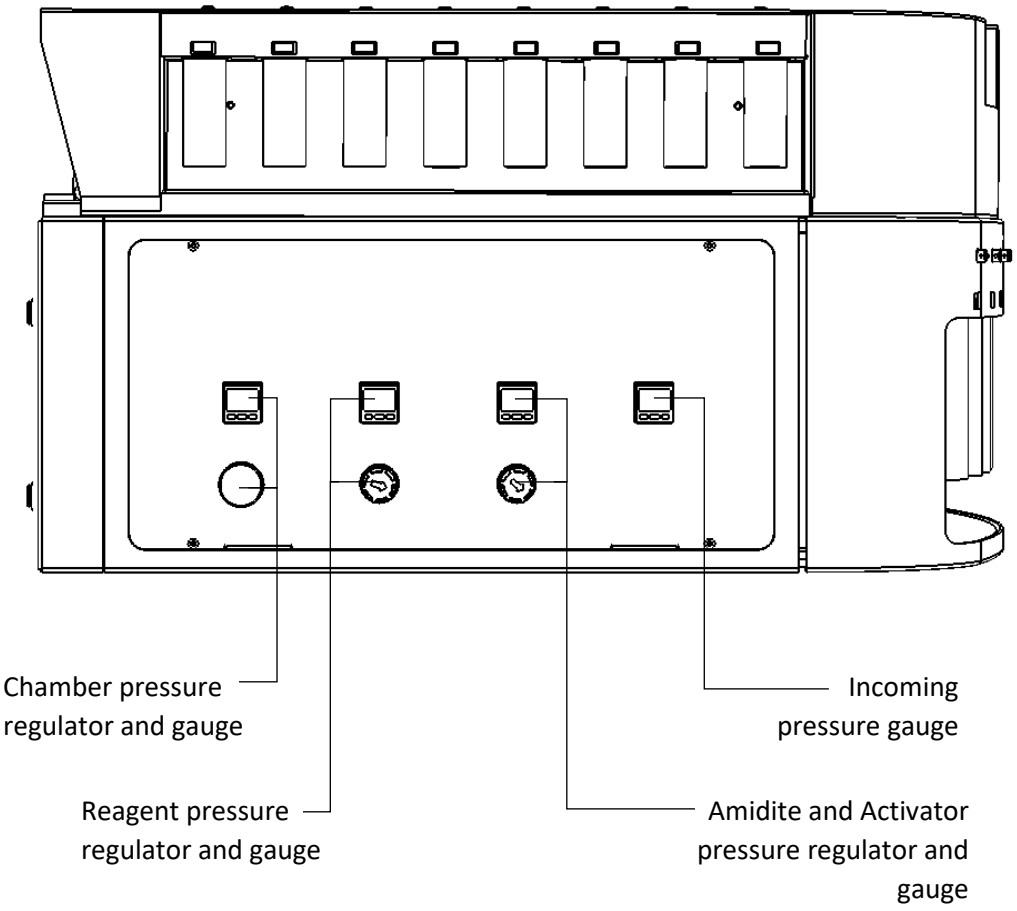
Front and Left Side View This three-quarter view shows you the front and the left side of the Shasta instrument.



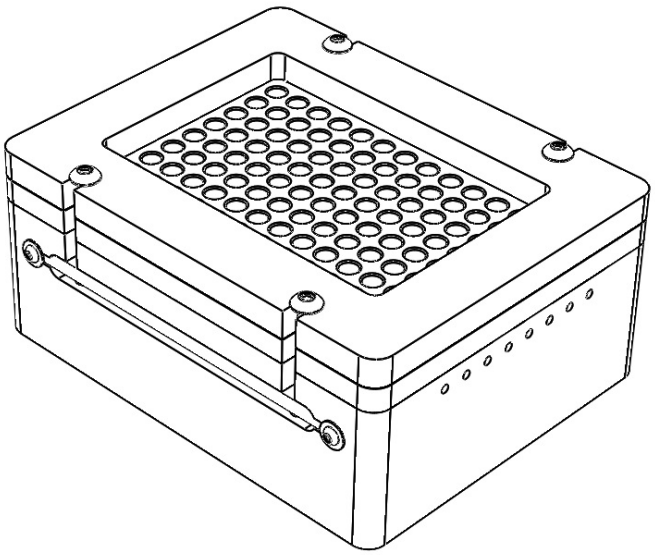
Back and Right Side View This three-quarter view shows you the back and the right side of the Shasta instrument.



Left Side View The components on the left of the Shasta instrument are shown here:



The 96 Column Drain Block

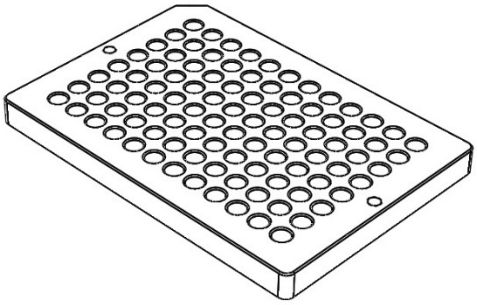


The 96 Column Drain Block is utilized when synthesizing oligonucleotides with standard ABI or Biosearch synthesis columns. The 96 Column Drain Block has been engineered to accept most all available, off-the-shelf columns of this style.

Note: Not all manufacturers produce columns of the same width. If your column does not fit or seal in the 96 Column Drain Block, please contact Sierra BioSystems support.

Inserting Synthesis Columns

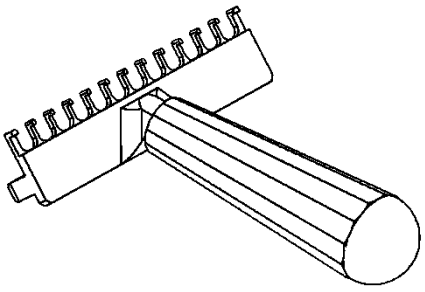
Synthesis columns are pressed into the openings of the column plate to form a "friction fit" seal. Each column must be placed into its respective opening and have force applied against its top to ensure a proper seal.



Removing Synthesis Columns

After a synthesis has been completed, the column plate may be removed from the 96 Column Drain Block for further processing. The columns may be removed simply by turning the column plate upside down and dislodging all the columns with a few hits of a mallet.

To remove sections of columns, use the column removal tool supplied by Sierra BioSystems:



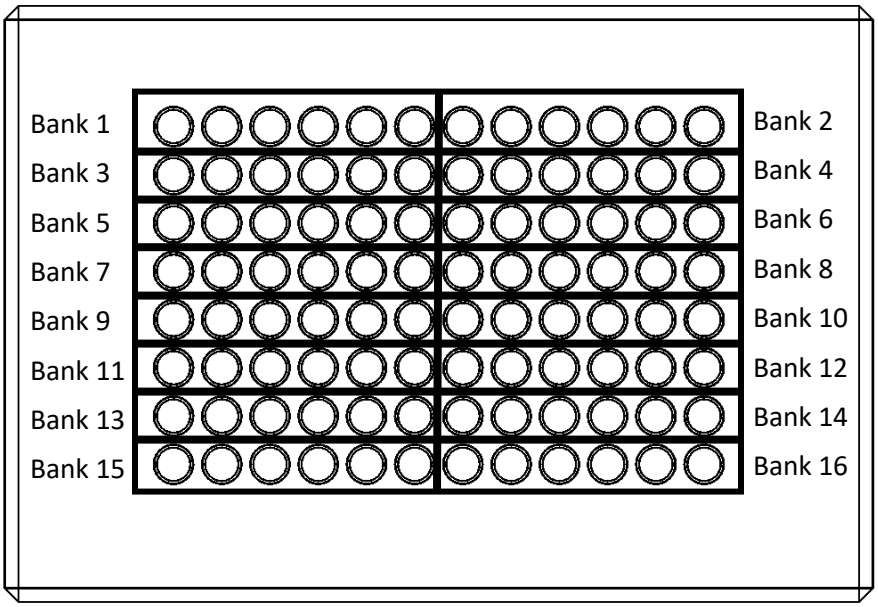
Push the tool firmly against each column in the row so that each column sits into one of its twelve "U" shaped cut outs. Once secure, place a gloved hand over all columns to be removed, and with the other hand, push the handle so

that each column is removed from its opening. Keep a gloved hand over the columns during transfer.

Multiple Drains in 1 x 96

The 96 openings of the 96 Column Drain Block separates into 16 sets of drain banks. Each bank leads to a single drain valve that is responsible for the Hold, Pulse, and Drain operations of 6 columns.

Each bank is assigned a number that differentiates it from the other banks. This number corresponds to its attached drain valve, i.e. "bank" 1 is attached to drain valve 1. Because all flow through the bank will be controlled by its associated drain valve, it is important to note each bank's location and numerical assignment. Observe below:

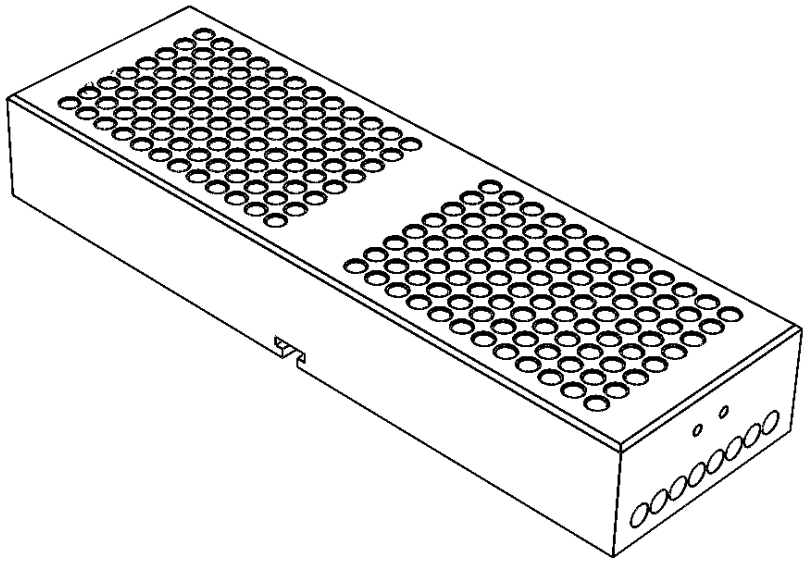


Bank Requirements

IMPORTANT! Each bank synthesizing oligos in a run will need to be closed off to achieve a complete drain. If, for example, 4 of 6 openings in a bank are filled with live synthesis columns, the remaining 2 openings will need to be sealed so that the 4 being used will drain. This can be done by placing closed or used columns in the empty positions. Note that used columns must still contain their frit or filter to perform this function properly.

The 192 Column Drain Block

The 192 Column Drain Block is utilized when synthesizing oligonucleotides with standard synthesis columns. Commonly referred to as ABI or Biosearch columns, the 192 Column Drain Block has been engineered to accept most all available, off-the-shelf columns of this style.



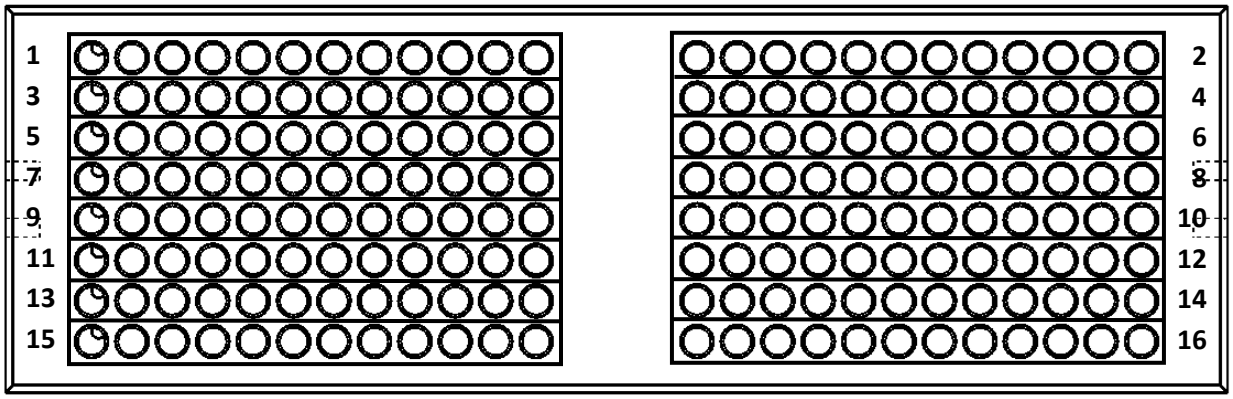
Note: Not all manufacturers produce columns of the same width. If your column does not fit or seal in the 96 Column Drain Block, please contact Sierra BioSystems support.

IMPORTANT! For instructions on properly installing, using, and removing synthesis columns from the 192 Column Drain Block, please see "Installing Synthesis Columns", "Removing Synthesis Columns", and "Bank Requirements" for the 96 Column Drain Block.

Multiple Drains in 2 x 96

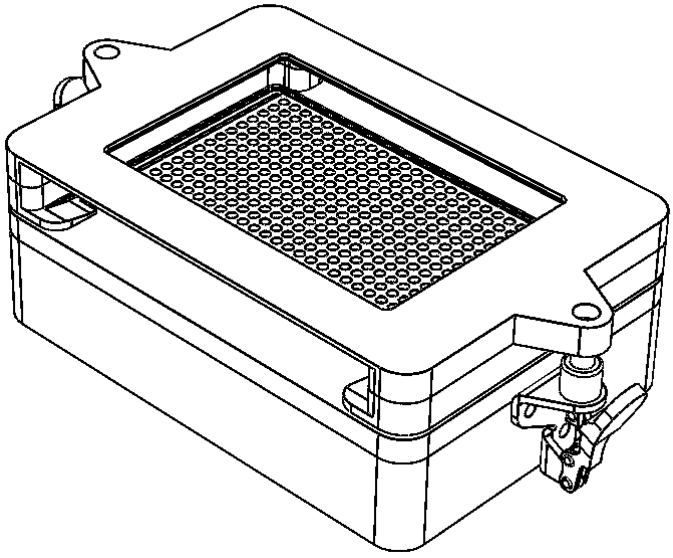
The 192 openings of the 192 Column Drain Block separate into 16 sets of drain banks. Each bank leads to a single drain valve that is responsible for the Hold, Pulse, and Drain operations of 12 columns.

Each bank is assigned a number that differentiates it from the other banks. This number corresponds to its attached drain valve, i.e. "bank" 1 is attached to drain valve 1. Because all flow through the bank will be controlled by its associated drain valve, it is important to note each bank's location and numerical assignment. Observe below:



The 384 Well Plate Drain Block

The 384 Well Plate Drain Block is designed for use with Agilent 384-well filter plates fritted with solid support by PolyDesign.



Like the 96 and 192 drain blocks, the 384 Well Plate Drain Block is designed to separate the synthesis plate into 16 separate drain groups. Each row of the plate is treated as a single drain group. Row “1” or “A” of the synthesis plate is considered drain group 1, row “2” or “B” is considered drain group 2, and so on.

Inserting a plate The plate is inserted into the front slot of the plate carriage and then secured onto the drain block by clamps. The clamps are located on the left- and right-hand side of the drain block. By pushing both clamps down, the plate remains secured throughout the run.

Removing a plate After a synthesis has completed, the plate is removed by lifting both clamps simultaneously and raising the plate carriage. After the clamps are lifted, slide the plate out of the carriage with a gloved hand.

About the Software

Overview

The Shasta Synthesizer software graphical user interface allows you to enter information about the sequences you are synthesizing and to operate the instrument. The GUI allows you to easily:

- Enter custom sequences in several ways:
  - Individually, by typing or cutting and pasting from a text file
  - Importing entire banks of sequences
  - Importing all 16 banks of sequences simultaneously
- Use the optimized protocols that are included in the software, or customize the cycles using the GUI
- Save all sequence and protocol information to repeat the run without re-entry

Conventions Used

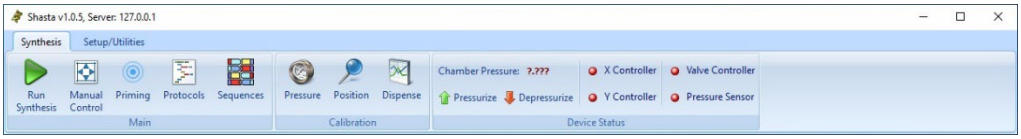
Icon directed submenus are available through the Main Toolbar. Each activity operable in isolation will be accessed through its respective submenu.

Cascading menus and other Microsoft® Windows operating procedures/commands are not used in this software.

Desktop Icon and Main Toolbar

The Shasta Software will be accessible from the Desktop or Windows Explorer software via the Shasta icon shown here:

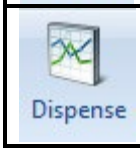
The Main Toolbar will appear as follows:



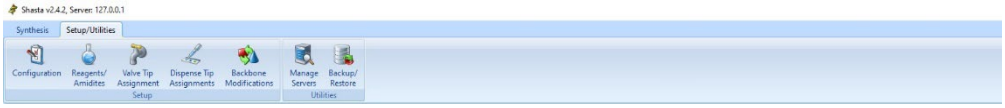
From the Main Toolbar, there are two Menus: Synthesis and Setup/Utilities. Each menu contains icons that direct the user to a synthesizer action.

Synthesis tab icons:

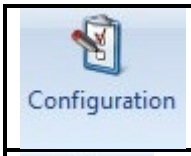
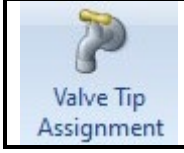
	**Run Synthesis** Allows the user to run syntheses by joining protocol and sequence files together. Other actions such as Pause, Stop, Estimate, and View Plate are available here.
	**Manual Control** Gives the user access to all valves on the instrument as well as control over the positions system. Manual Dispense, Pulse. And Drain commands are available here.

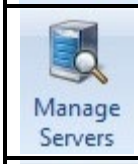
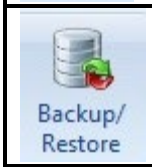
	**Priming** A tool used to prime each delivery tip before the synthesis.
	**Protocols** The Shasta's proprietary protocol writer. Standard loops as well as Meta-Protocol builders are available here.
	**Sequences** The Shasta's proprietary sequence writer. Export and Import functions to and from Microsoft® Excel are available here.
	**Pressure Calibration** Chamber pressure calibration utility
	**Position Calibration** Actuator calibration utility
	**Pressure Calibration** Dispense valve calibration utility

The Setup/Utilities tab will appear as follows:



Setup/Utilities icons:

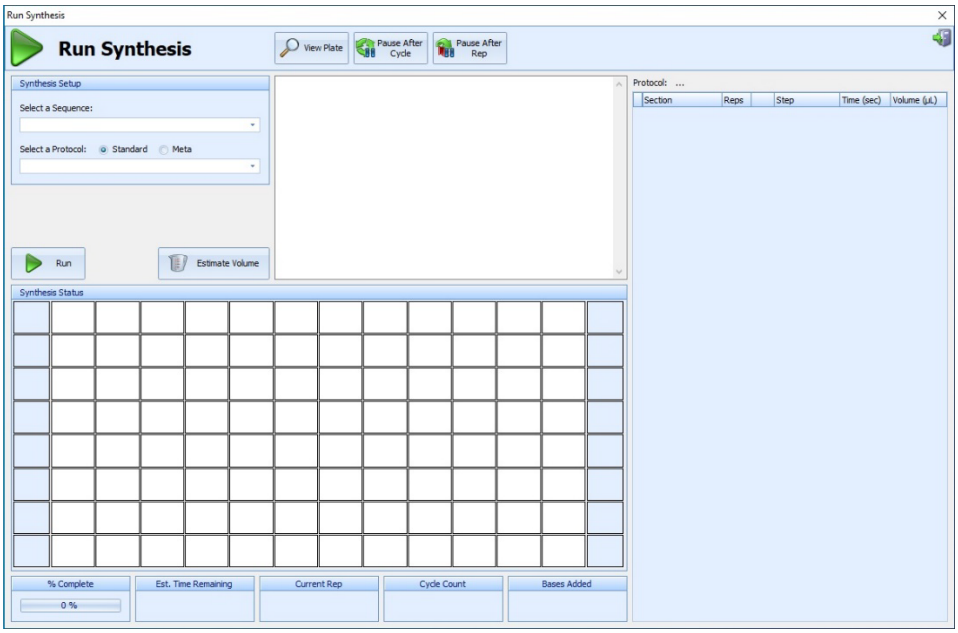
	**Configuration** Window for assigning global variables such as set bands, array patterns, sensor engagement, mid synthesis priming, and other functions.
	**Reagents/Amidites** Stores all information related to plumbed fluids. Identity, density, and other necessary values can be input and retrieved from here.
	**Valve Tip Assignment** Assigns each valve a place on the Valve Controller board.

	**Dispense Tip Assignments** Assigns each valve nozzle a location on the dispense array.
	**Backbone Modifications** Create callouts for backbone modifications in this window, e.g. “Thioate”.
	**Manage Servers** Directs the Shasta client to which server will be delivering database information.
	**Backup/Restore** Allows the user to backup the existing Shasta database or load a new Shasta database.

Software Walkthrough

Below is a cursory overview of each screen that will be regularly employed by the user. A more thorough description of key software elements will be detailed in other parts of this manual.

Run Synthesis



The **Run Synthesis** window is the primary interface from which users will operate the instrument. This window will allow the pairing of Sequence and Protocol files to generate a synthesis run.

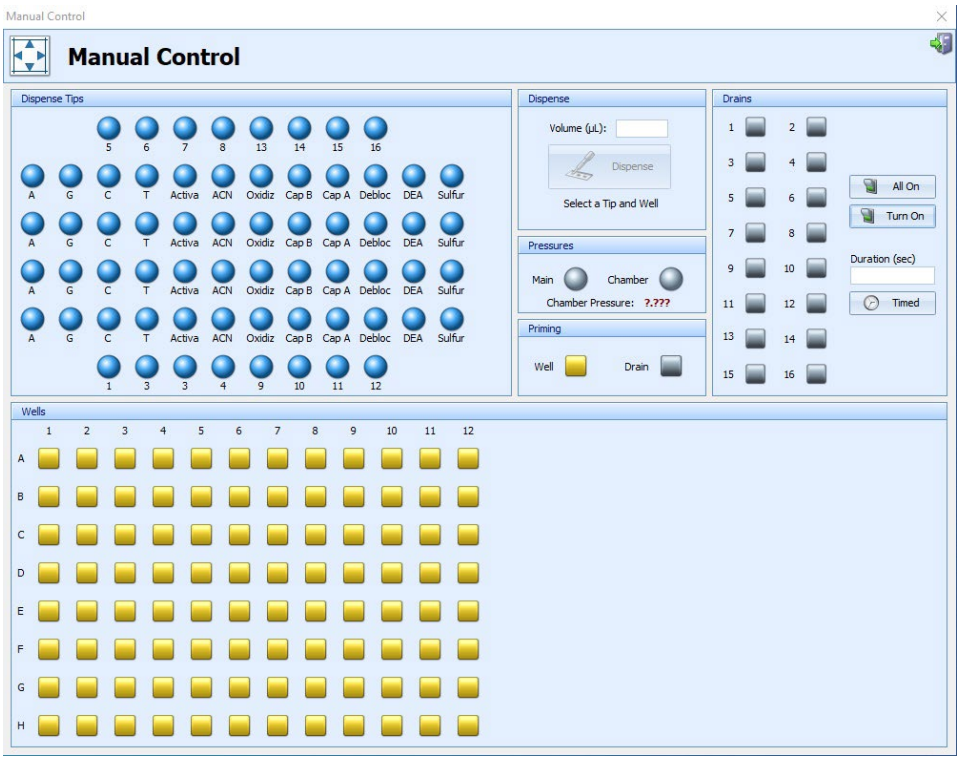
The **Run Synthesis** window offers additional tools and features to supplement the synthesis:

- **View Plate** – An operation that interrupts standard synthesis procedures to allow the user to view all Pulse, Hold, and Drain operations. This feature will take effect at the beginning of the next available Dispense operation, delaying all Pulse, Hold, and Drain operations until all dispenses have finished. **NOTE:** View Plate will require the user to initialize all post-dispense activities. De-select the View Plate feature to resume standard synthesis operations.
- **Pause After Cycle** – Sets a pause point for the end of the current cycle. The synthesis will remain paused until the Resume function is selected by the user.
- **Pause After Rep** – Sets a pause point for the end of the current rep of the current section. The synthesis will remain paused until the Resume function is selected by the user.
- **Select a Sequence –** All sequences generated via the Sequence Writer will be available for selection here.
- **Select a Protocol –** All protocols generated via the Protocol Writer will be available for selection here.

- **Standard** – Standard protocols will run a single protocol for all columns and all bases on the plate.
- **Meta** – Meta protocols are a combination of protocols specified by the user that allow base-specific, column-specific, and sequence-step-specific handling. This will also join Pre-Process, Post-Process, and DMT-off protocols into one cohesive synthesis.
- **Maps –** Map protocols take all the features of Meta protocols and sperate them into drain groups. This allows for 16 separate Meta protocols to be run independently on one plate.

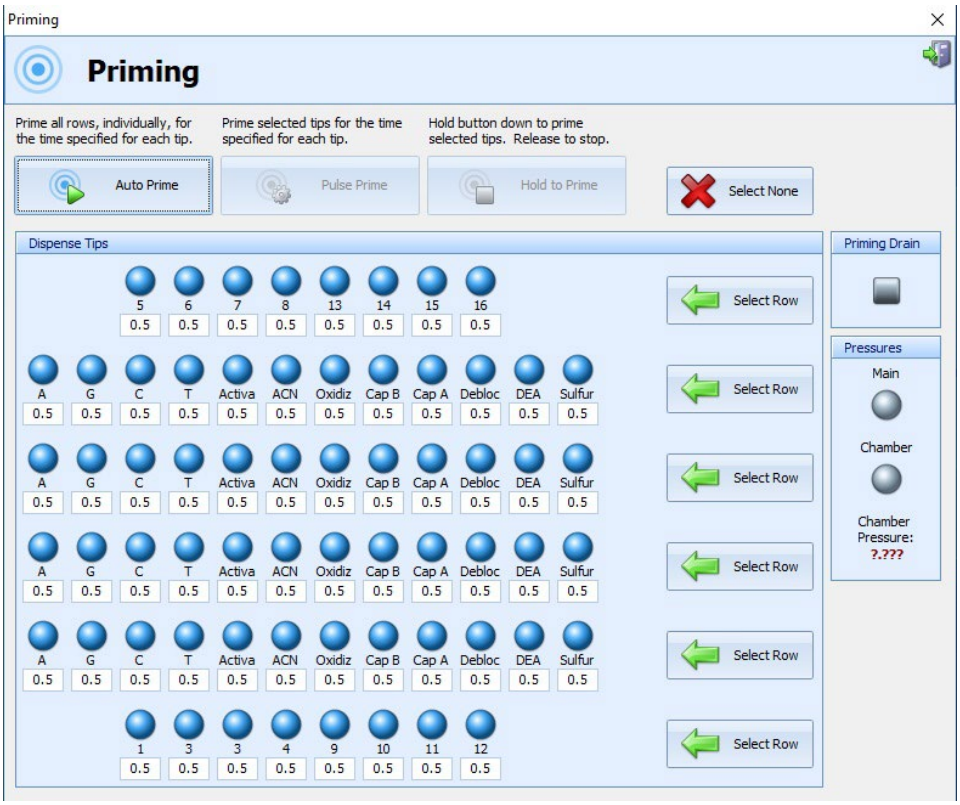
- **Estimate Volume** – Estimates the total reagent and phosphoramidite consumption for the loaded Sequence and Protocol files.
- **Color Display –** This display will show the amidites being handled by the current cycle. The display will also show the column types required by the loaded Sequence file. **IMPORTANT!** Users can terminate a sequence mid-run by *Ctrl + Left Clicking* a well in the Color Display.
- **Protocol Table** – Shows a live feed of the current cycle and the current synthesis step within the file.

Manual Control



The **Manual Control** window offers users access to every valve and actuator on the instrument. From this window, user's can dispense defined volumes of liquid into any well. Users may also perfrom timed drain operations for any combination of drains.

Priming



The **Priming** Window allows users to prime each dispense nozzle of the instrument either in isolation, in combination, or in sequence with other valves.

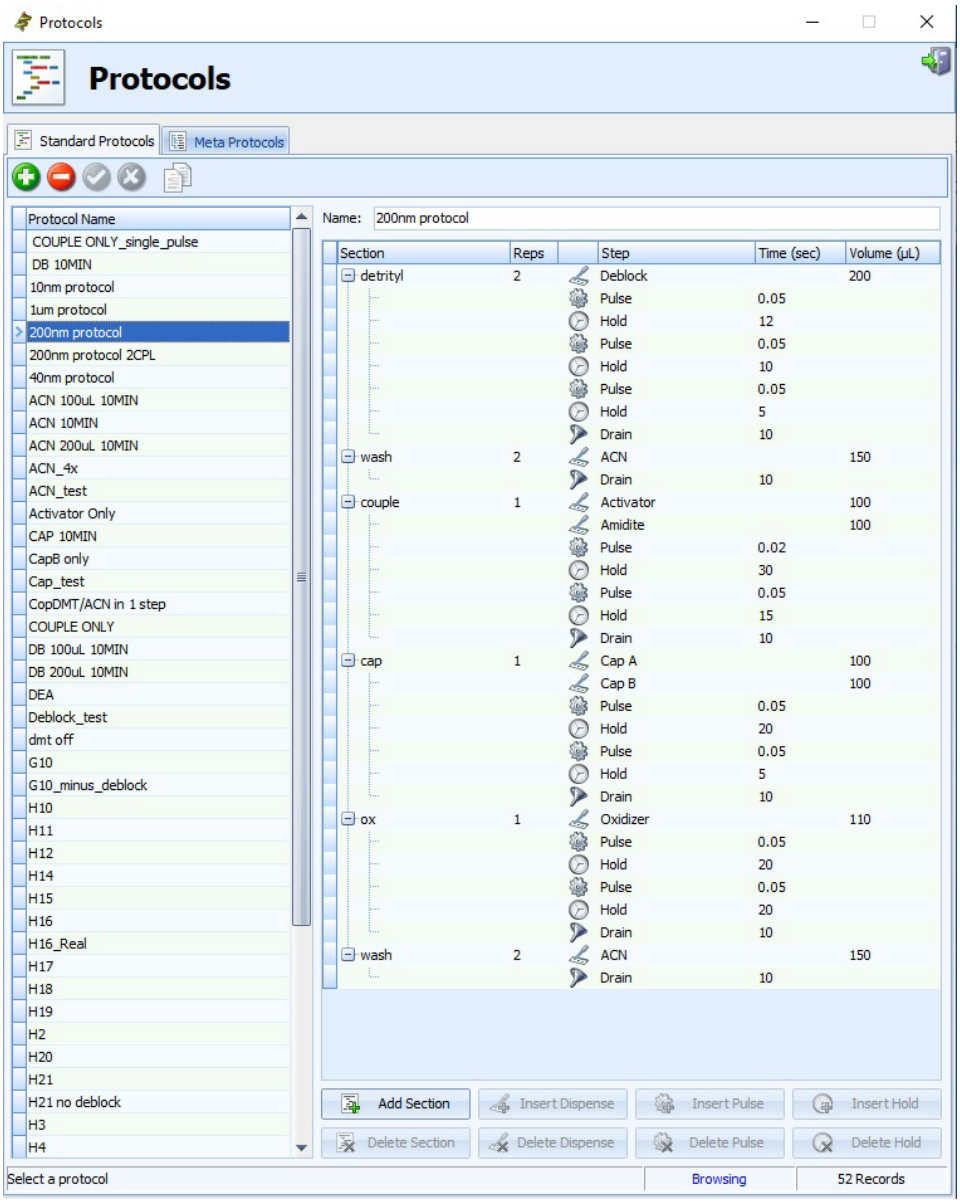
Because the priming channel below can accommodate all dispense nozzles of one row simultaneously, all nozzles selected within a row will remain active until either de-selected or a nozzle in a different row is selected. Entire rows may also be selected.

The **Priming** Window offers a few ways for the user to prime the intrument's nozzles:

- **Auto Prime** – Runs a fully automated sequence in which every nozzle is primed. Each nozzle will be primed for a volume specified under its location in the nozzle array.
- **Pulse Prime** – Will prime the current selection of nozzles for their prescribed volume.
- **Hold to Prime** – Will hold the current selection of valves open for as long the user keeps this button depressed.

IMPORTANT! The priming channel can be overfilled with manual injections. Purge the priming channel periodically by opening the Chamber Pressure valve and the Priming Drain.

Standard Protocols



The **Protocol Writer** is a tool that allows users to write and edit synthesis protocols. Protocols are seperated into Standard, Meta, and Map protocol files.

The **Standard Protocol** writer is the primary protocol editor. From here, individual cycle files can be written and then applied to sequences, columns, bases, drain groups, etc. There are a few operators available to the user when writing a Standard protocol.

- **Add Section / Delete Section** – Allows the user to implement or remove a section. Sections are discrete routines within the cycle that typically consist of one dipense step followed by a series of actions designed to facilitate the use of that dispense. **NOTE:** Section names are labels only and not used by the software in any way. Section names are defined by the user and intended to mark the beginning of its section.

- **Insert Dispense / Delete Dispense –** Adds or removes a dispense step. Once implemented, dispense steps will be defined under the Volume column. As noted, the units for this column are in microliters. **IMPORTANT!** Be mindful of the available space in the plate or columns being used. They can be overfilled. As a rule, 96 column/well formats should not receive more than 300µL of dispense, and 384 well formats should receive no more than 100µL.
- **Insert Pulse / Delete Pulse** – Pulses are brief openings of the drain valves designed to draw liquid into the support of the plate or columns. An ideal synthesis will move liquids across the support as evenly and slowly as possible. A good practice is to implement a Pulse immediately after dispense to allow flow to begin and then implement additional Pulses as the gas underneath the columns equilibrates to chamber pressure. Viscous liquid may require more Pulse steps.
- **Insert Hold / Delete Hold** – Adds or removes a Hold step. Hold steps are waiting periods that have no action other than to allow the reaction on the support to occur.
- **Drain** – Drain steps are timed commands sent to the drain valves. These are like Pulses but typically much longer. The function of the Drain command is to move liquids out of the drain plate as quickly as possible.

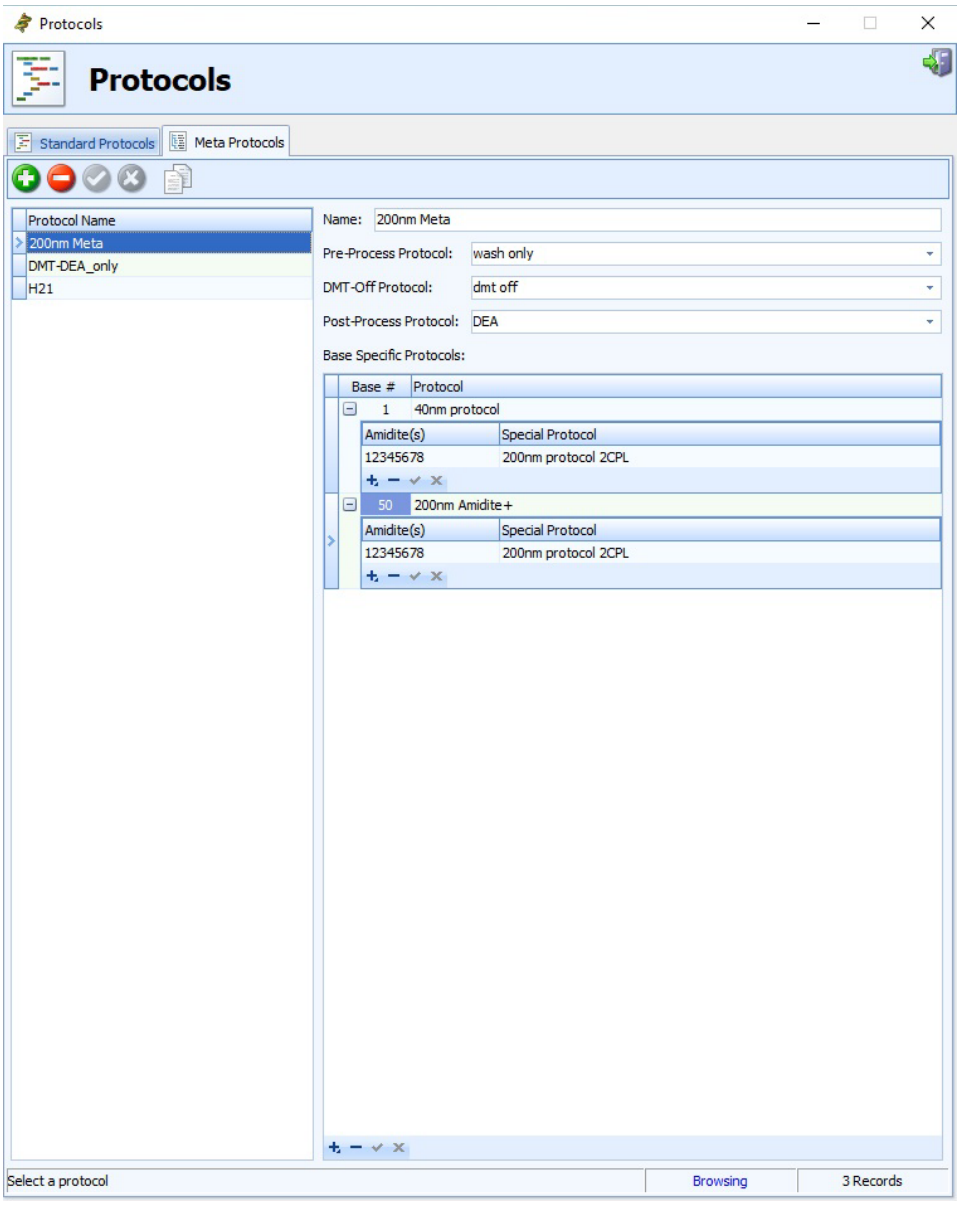
Zero Values are Allowed – If a zero value is input, it is as if the step is skipped entirely. This may be useful if the user wants to:

1) Skip the drain step. If there are liquids in the column that need to remain during the next injection.
2) Skip injections for certain bases within a bank. If only one base requires a unique reagent injection, but it is within a bank of sequences that should not receive this reagent, then typing a "0" for all of the "skipped" bases, and typing the desired volume for the desired base, will keep the injection isolated to the one, desired base.

Multiple Injections – Activator/Amidite and CapA/CapB dispense passes have optimzed movement patterns. Any other combination will inject reagents in no specific order. Reagents will be injected as their column meets the approriate dispense nozzle. **NOTE:** only 2 reagent combinations are allowed. A third will need to be input in a subsequent section.

Single Nozzle Reagent Dispense – Reagents assigned to a single valve (i.e. placed in a spoecialty amidite bottle) will dispense across the whole plate and work like any other reagent would. **NOTE:** Single valve reagents cannot perform more than 1 repition within their section. If additional reptiions of their dispense are required, please create another, subsequent section for that reagent injection.

Meta Protocols



The **Meta Protocol** writer is the secondary protocol editor. From here, Standard Protocols can be linked together to automatically perform case-dependant operations. Base handling, Pre- and Post-synthesis procedures, DMT removal, and bulk synthesis changes throughout the run can be effected here.

- **Pre-Process Protocol** – The Standard Protocol placed in this slot will run before the synthesis begins. No bases will be coupled in this step. It is inteded for a pre-synthesis wash, extra Deblock to remove less labile linker, etc.
- **DMT-Off** – The Standard Protocol placed in this slot will be run immediately after the synthesis has completed. It is intended as a final DMT removal step, but any protocol that does not require phosphoramidite could be performed here. **IMPORTANT!** The

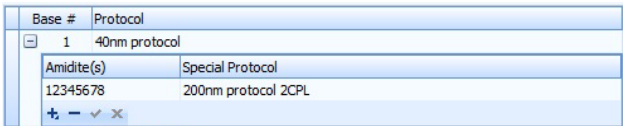
synthesizer will not automatically remove the final DMT group unless specified in both this slot and in the sequence file.

- **Post-Process Protocol** – The Standard Protocol placed in this slot will be run immediately after the DMT-Off step, and it will be the final protocol in the synthesis. This cycle is intended for DEA treaments, additional DCM rinses, etc.
- **Base-Specific Protocols –** The Base-Specific Protocol drop-downs are where the primary synthesis cycles will be defined. Which phosphoramidites will receive particular treatments and *when* they are to receive them will be decided here.
    - **Base #** -- This column defined when certain protocols will be engaged over the course of the synthesis. By default, the first row in the Base-Specific Protocol window will be "1" because we are engaging the decided protocol on Base 1, i.e. the start of the synthesis. **NOTE:** This does not mean that specialty phosphormaidite 1 will use this protocol. It is a time stamp only...
      Additional protocols can be engaged throughout the synthesis by selecting the "+" at the bottom of the window. Assigning this new row's Base # will decided when in the sequence this change is to be made. For example, Base # "20" will begin using the specified protocols at cycle 20 of the synthesis.
    - **Protocol** – To the right of Base # is the Protocol column. This is where the protocol for every base (unless specified otherwise in Special Protocols) will be decided. **IMPORTANT!** This slot cannot be empty. It is the primary protocol to be run during the synthesis.
    - **Amidite(s)** – This is where phosphoramidites that will require special handling can be selected. The column can have many rows, and each row will contain phophoramidites with unique handling requirements. Phosphoramidites that share handling requirements will likely share a protocol, and thus, share a row in this column. **NOTE:** Base names exceeding a single character must be enclosed by "[ ]", e.g. base "FAM" would be inserted as [FAM].
    - **Special Protocol** – The protocol inserted into this space will take effect for each base listed to the left in the Amidite(s) column. **IMPORTANT!** The entire protocol can be changed for each amidite, but because special and standard phophoramidites will often exist in the same drain group, it is important that section and dispense order be the same for special and standard protocols. Injetion volumes, holds, pulses, and drain times can all vary.

Special Base Handling

The Shasta allows the user to modify every step of the protocol for every base. This is done by inserting "sub-amidite groups" underneath the primary protocol.

For example:



The primary protocol in this picture is “40nm protocol”. It will be applied to every base that is not in the sub-amidite group.

The sub-amidite groups consisits of bases labeled 1, 2, 3, 4, 5, 6, 7, and 8. These bases will follow another protocol called “200nm protocol 2CPL”. So, in this example, because A, C, G, etc. are not listed in the sub-amidite group, they will follow the primary protocol, which is “40nm protocol”.

More specialty protocols for bases with different needs (than bases 1-8 in this example) can be added by clicking the “+” button. This will introduce another sub-amidite group that can be populated with more bases and a protocol. This can be done for every sinlge base if so desired.

IMPORTANT! The primary protocol and sub-amidite protocols must have matching section orders. The values within each section can be changed, but the order of injections cannot. For example, this is allowable:

Primary Protocol		
1 Rep	TCA	200uL
	Drain	5 sec
2 Reps	Activator	100uL
	Amidite	75uL
	Hold	60 sec
	Drain	4 sec

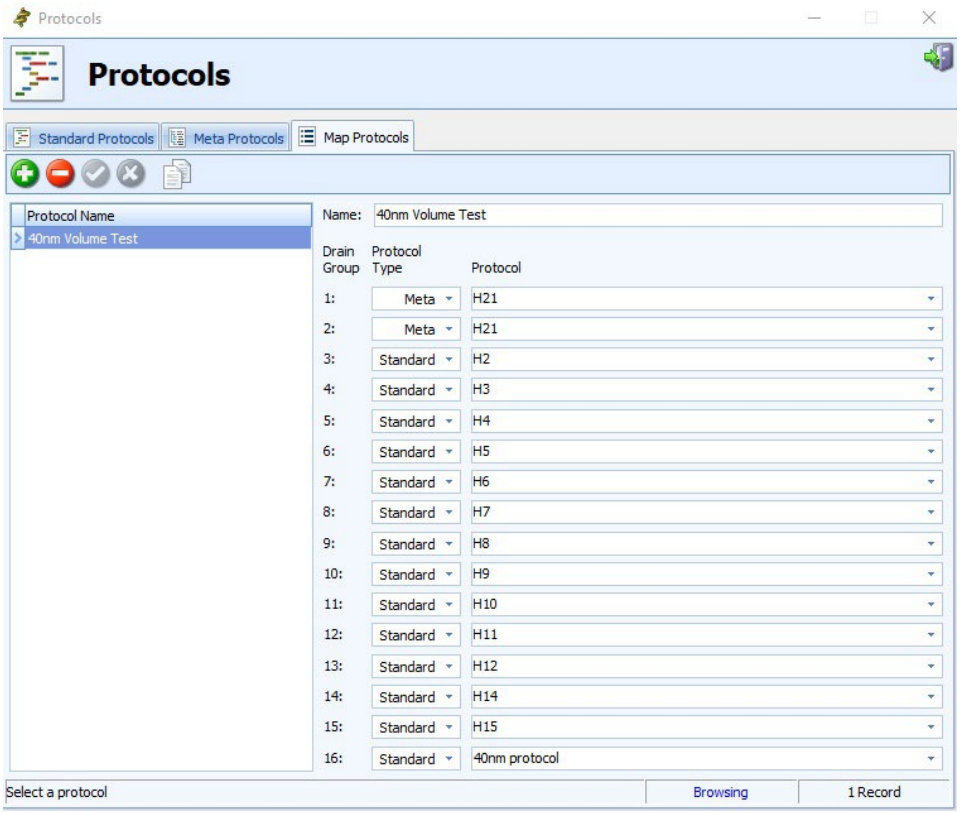
Sub-Amidite Protocol		
1 Reps	TCA	120uL
	Drain	10 sec
4 Reps	Activator	130uL
	Amidite	100uL
	Hold	180 sec
	Drain	4 sec

But this would *not* be allowable:

Primary Protocol		
1 Rep	TCA	200uL
	Drain	5 sec
2 Reps	Activator	100uL
	Amidite	75uL
	Hold	60 sec
	Drain	4 sec

Sub-Amidite Protocol		
4 Reps	Activator	130uL
	Amidite	100uL
	Hold	180 sec
	Drain	4 sec
1 Reps	TCA	120uL
	Drain	10 sec

Map Protocols

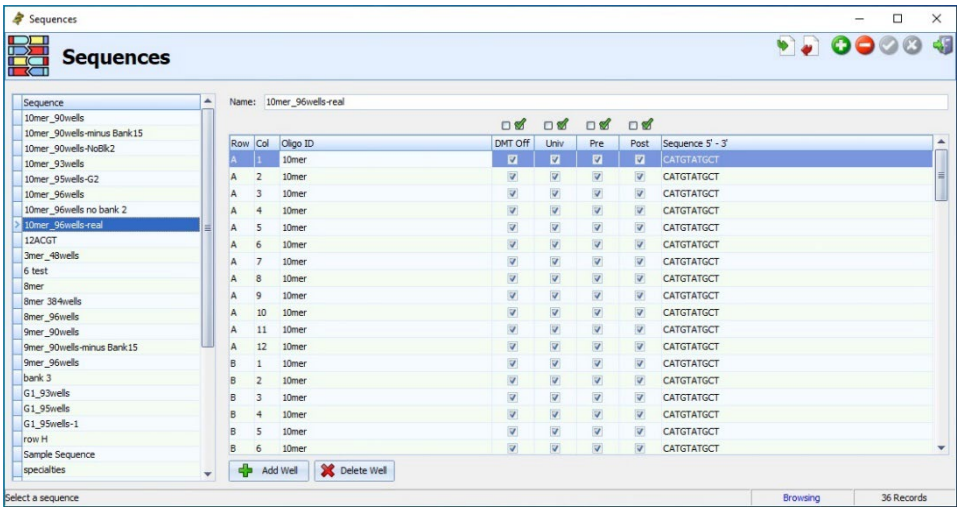


The **Map Protocol** writer is the tertiary protocol editor. From here, Standard Protocols and Meta Protocols can be assigned to drain groups and be performed alongside each other.

NOTE: Not all 16 drain groups need to be filled out in order for the Map Protocol to function. If a sequence file contains sequences in drain groups that do not have a protocol selected, they will not be run.

- **Drain Group** – This column shows which drain group will be affected by the selections made in its row. Refer to the Drain Block explanations to find which groups refer to which sections of the plate.
- **Protocol Type –** Determines which type of protocol will be used here: a Standard or Meta.
- **Protocol –** The drop downs display the available protocols for type selected.

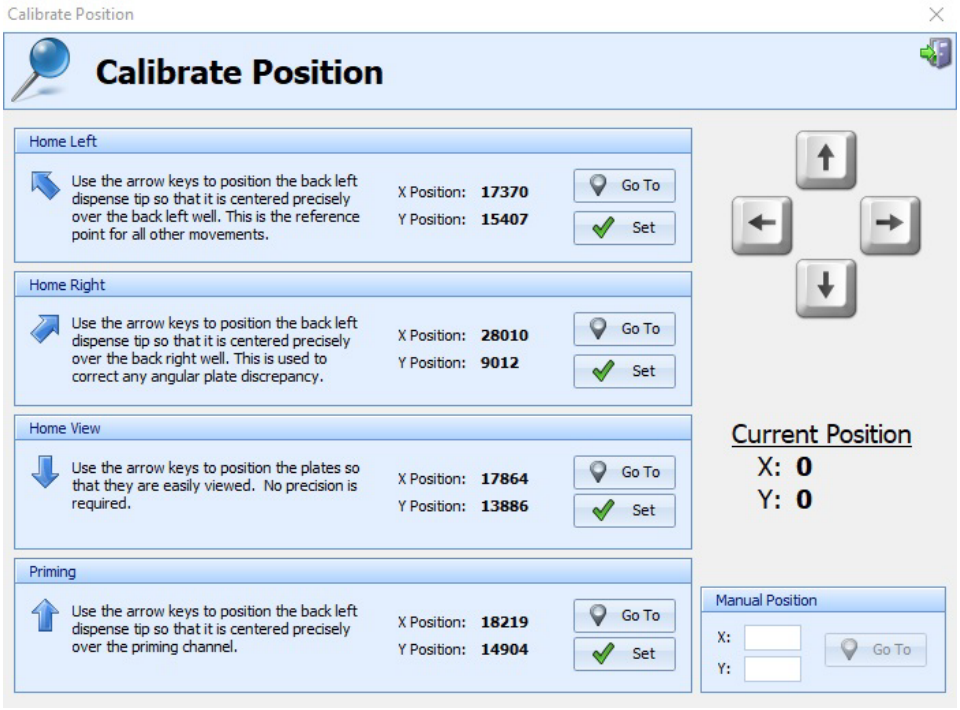
Sequences



The **Sequence Writer** is a tool that allows users to write and edit sequences to be synthesized. Generally, users will create synthesis files in Microsoft® Excel and import them into the Sequence Writer. Sequences can also be exported out of the Sequence Writer to a .xlsx file.

There are toggles for every sequence in the file. These toggles determine whether certain sub-protocols within the Meta protocol will be performed. These include Pre, Post, DMT-off, and Universal Support selections. At the top of the window, selections can be toggled en masse for every sequence in the file.

Calibrate Position



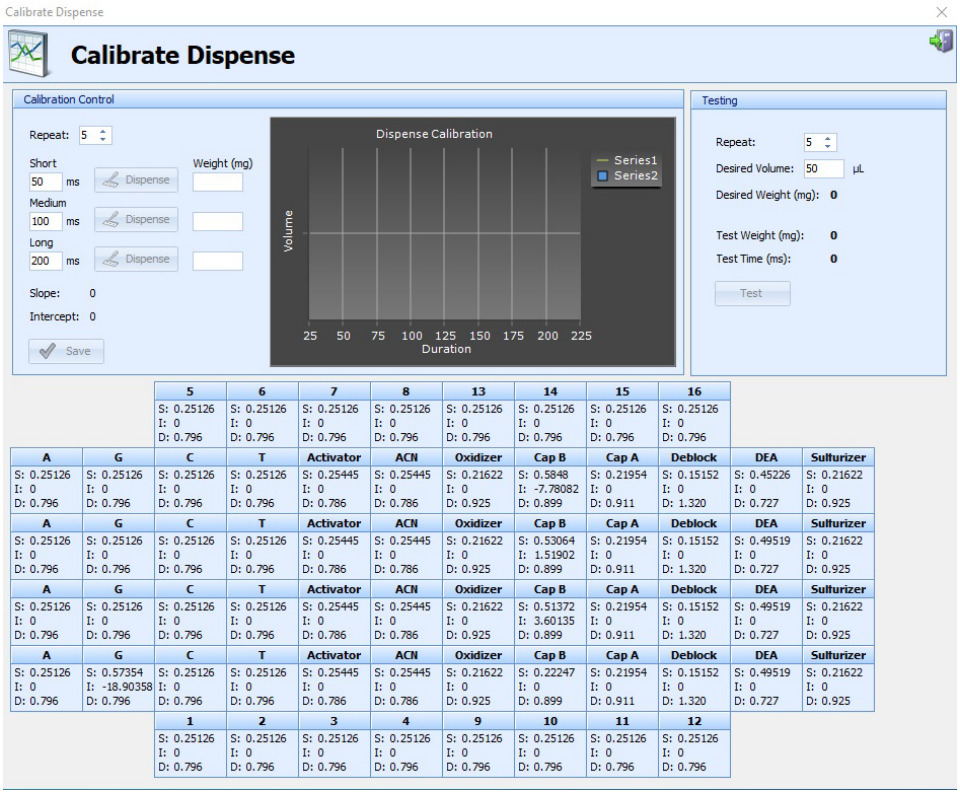
The **Calibrate Position** screen is used to align the actuators within the synthesis chamber to the synthesis, priming, and View Plate paths.

Instructions for each essential coordinate are provided. There are two buttome for each coordinate:

- **Go To** – This will take the drain plate to the saved coordinates for this location.
- **Set** – Will save the current position as the new coordinates for this location.

The arrows on the keyboard can be used for fine tuning the position of the actuators for a given location. Larger jumps should be made by typing coordinates into the Manual Position box in the bottom right and then selecting its Go To button.

Calibrate Dispense



5	6	7	8	13	14	15	16
S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796

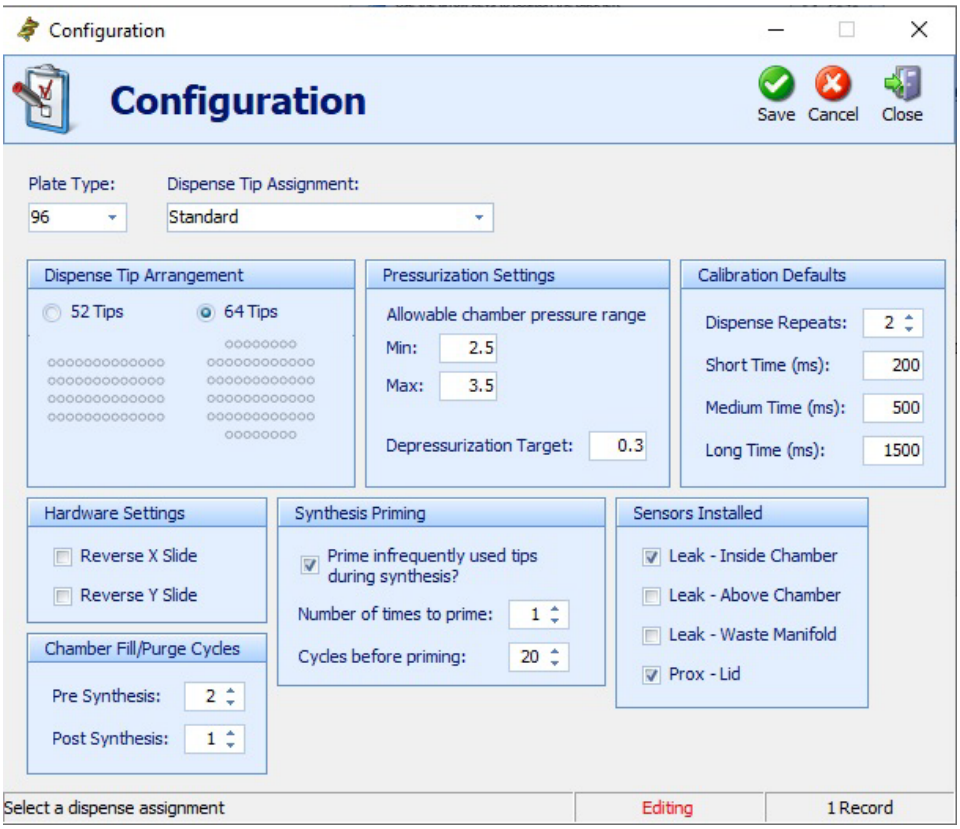
A	G	C	T	Activator	ACN	Oxidizer	Cap B	Cap A	Deblock	DEA	Sulturizer
S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25445 I: 0 D: 0.786	S: 0.25445 I: 0 D: 0.786	S: 0.21622 I: 0 D: 0.925	S: 0.5848 I: -7.78082 D: 0.899	S: 0.21954 I: 0 D: 0.911	S: 0.15152 I: 0 D: 1.320	S: 0.45226 I: 0 D: 0.727	S: 0.21622 I: 0 D: 0.925
A	G	C	T	Activator	ACN	Oxidizer	Cap B	Cap A	Deblock	DEA	Sulturizer
S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25445 I: 0 D: 0.786	S: 0.25445 I: 0 D: 0.786	S: 0.21622 I: 0 D: 0.925	S: 0.53064 I: 1.51902 D: 0.899	S: 0.21954 I: 0 D: 0.911	S: 0.15152 I: 0 D: 1.320	S: 0.49519 I: 0 D: 0.727	S: 0.21622 I: 0 D: 0.925
A	G	C	T	Activator	ACN	Oxidizer	Cap B	Cap A	Deblock	DEA	Sulturizer
S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25445 I: 0 D: 0.786	S: 0.25445 I: 0 D: 0.786	S: 0.21622 I: 0 D: 0.925	S: 0.51372 I: 3.60135 D: 0.899	S: 0.21954 I: 0 D: 0.911	S: 0.15152 I: 0 D: 1.320	S: 0.49519 I: 0 D: 0.727	S: 0.21622 I: 0 D: 0.925
A	G	C	T	Activator	ACN	Oxidizer	Cap B	Cap A	Deblock	DEA	Sulturizer
S: 0.25126 I: 0 D: 0.796	S: 0.57354 I: -18.90358 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25445 I: 0 D: 0.786	S: 0.25445 I: 0 D: 0.786	S: 0.21622 I: 0 D: 0.925	S: 0.22247 I: 0 D: 0.899	S: 0.21954 I: 0 D: 0.911	S: 0.15152 I: 0 D: 1.320	S: 0.49519 I: 0 D: 0.727	S: 0.21622 I: 0 D: 0.925

1	2	3	4	9	10	11	12
S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796	S: 0.25126 I: 0 D: 0.796

The **Calibrate Dispense** window allows users recalibrate valves that are either loaded with a new reagent our are dispensing improperly. Proper calibration converts volume based dispense actions into time driven valve commands that result in volume deliveries accurate to within 5%.

Every nozzle on the array requires calibration. Values for standard synthesis reagents will be supplied with the instrument. As long as this pressure levels of the intrument fall within OEM specification, standard synthesis reagents should not require recalibration, save for a defect in the delivery line.

Configuration



The **Configuration** screen is used for setting global variables. Each variable set is isolated within a sub-window:

Dispense Tip Arrangement -- Selects for 52 or 64 tip configuration. The correct configuration will be whichever pattern mirrors the dispense array on your instrument.

Pressurization Settings -- The *Allowable chamber pressure range* will be the operating range of the chamber pressure during the synthesis. If the pressure falls out of range, the synthesis will pause until corrective action is taken or the synthesis is aborted. The *Depressurization Target* is the pressure that must be reached during the chamber evacuation process.

Calibration Defaults – Values given to new fluids in the calibration screen. They may be altered individually in the calibration screen.

Hardware Settings – Optional slide reversal available on specific Shasta models. These values are set by Sierrra BioSystems before or during installation.

Chamber Fill/Purge Cycles – The chamber can be programmed to fill with Argon and purge X number of times before and after the synthesis. Done before the synthesis, the goal is to reduce oxygen content. Done after the synthesis, the goal is to remove the vapors. A cycle of each is typically sufficient unless the relative humidity of the lab is particularly high.

Synthesis Priming -- This option will trigger the priming of injection tips after going so many cycles without use. Setting the value to 1 would prime a tip on every injection if it was used *every other* cycle. Setting the value to 0 would prime the tip on every cycle. The tip will only be primed once per cycle. The number of priming injections can be set in the *Number of times to prime* box.

Sensors Installed – Depending on your model of Shasta, different sensors will be required. The sensors you have installed will be confirmed here, and these will be selected by Sierra BioSystems before or during installation. **IMPORTANT!** Tampering with the sensor toggles can be potentially dangerous. Please do not alter these settings without instructino from Sierra BioSystems.

Standard and Split Amidite Movement

The Shasta Synthesizer offers a toggling feature for the secondary A, C, G, and T positions. These positions are labeled A/W, C/X, G/Y, and T/Z on the synthesizer's front panel. This feature allows the user to change between two modes of operation for a given run: Standard and Split.

The 1 x 96 configuration will be used in this explanation as an example, but the same will be true for 2 x 96, 1 x 384, and 2 x 384. However, in 384 modes, the number of passes will double for each mode of operation.

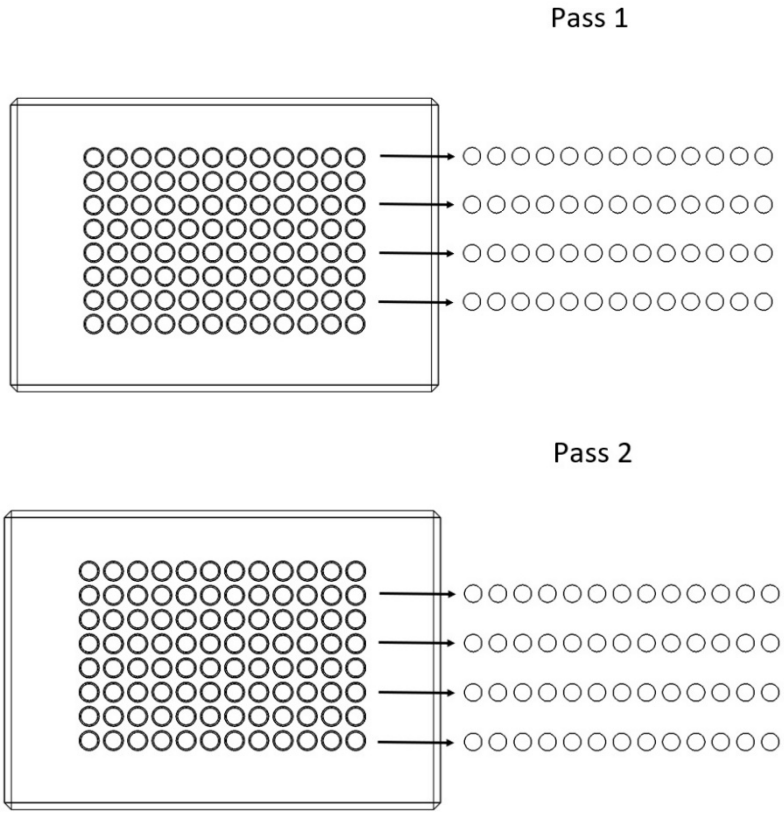
Standard Operation

The use of A, C, G, and T in the in the secondary positions is considered "Standard Operation". In this mode, it is assumed that there will be 2 bottles of A, C, G, and T loaded onto the front of the machine.

The amidite dispense tips are plumbed so that each bottle on the front of the instrument reaches 2 tips (see "Shasta Plumbing" for details). Standard Operation sends the primary bottles to the top 2 dispense tips and the secondary bottles to the bottom 2 dispense tips. The allows for fewer passes during amidite dispense.

If numbered from the top-down, we can see that the tips will pass over the odd numbered rows of columns/wells on the first pass, and the even numbered rows on the second pass.

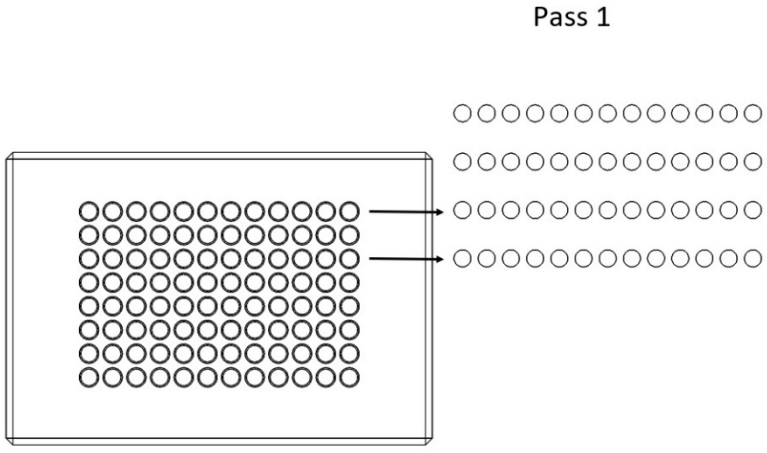
Note: Direction of movement may vary during synthesis.



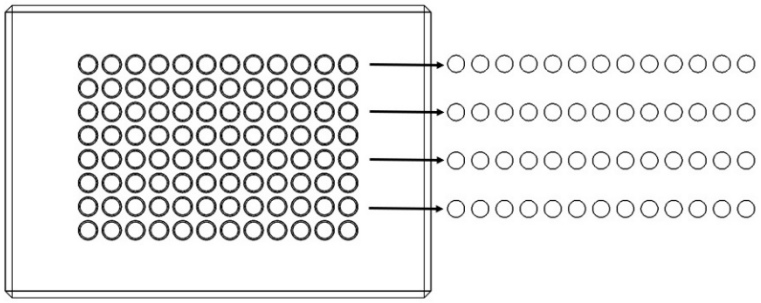
Split Operation

The use of W, X, Y, and Z in the in the secondary positions is considered “Split Operation”. In this mode, it is assumed that there will be 1 set of bottles filled A, C, G, and T loaded onto the primary positions of the machine, and a second set of amidites loaded into the W, X, Y, and Z positions.

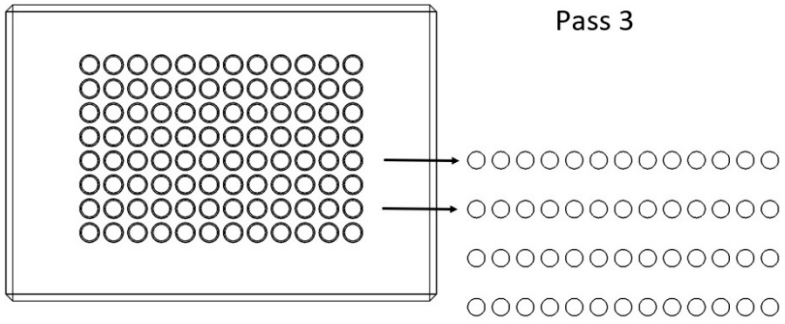
Split Operation sends the primary bottles to the top 2 dispense tips and the secondary (W, X, Y, and Z) bottles to the bottom 2 dispense tips. To ensure every column/well can reach every available amidite, the instrument must make more passes under the dispense tips than in Standard Operation



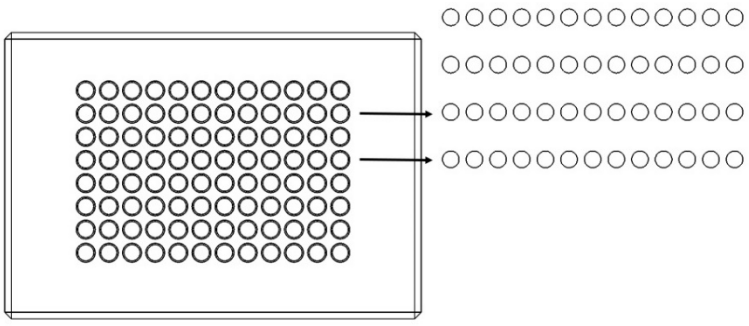
Pass 2



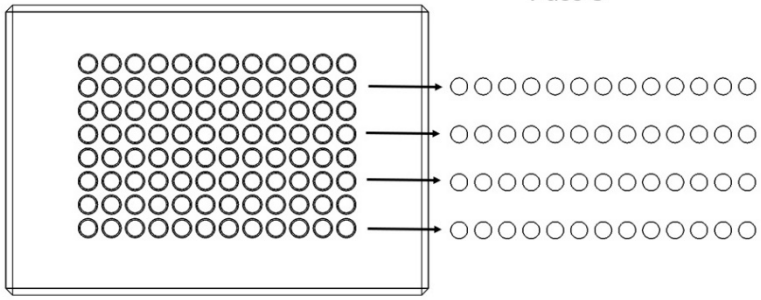
Pass 3

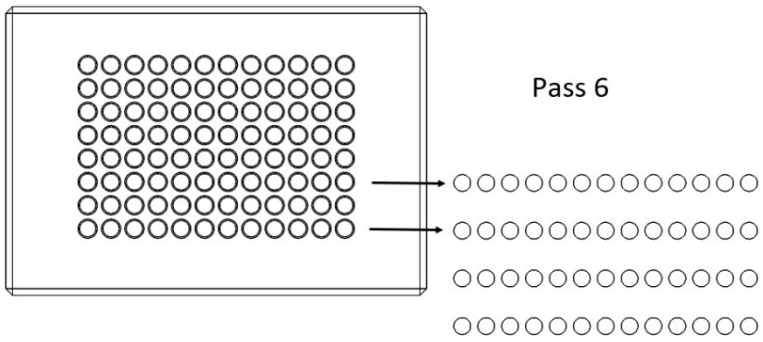


Pass 4



Pass 5



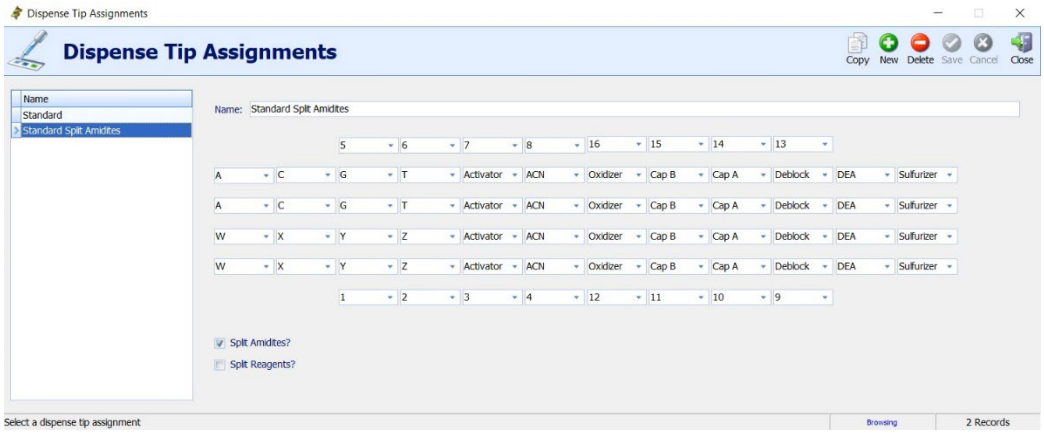


We can see that even in Split Operation, the odd numbered rows of columns/wells receive dispense before the odd ones. This preserves the order of dispense between banks so that the variance and time delay between each row's sections is minimized.

Engaging Split Amidite Movement

Enter "Dispense Tip Assignment" from within the Shasta software. Engage the Split Amidite dispense array from the list on the left of the window.

The window will look as follows:



Note that W, X, Y, and Z have now populated the bottom 2 rows of the amidite dispense array. The "Split Amidites?" box has also been selected. **IMPORTANT!** This box must be selected to ensure the "split amidite" movement pattern is followed.

"Split Reagents" is a similar function that will double the number of available reagent positions.

NOTE: if Split Amidites is engaged during a Map synthesis, the movement pattern will be used for all dispense passes.

Performing a Synthesis

Overview

About This Chapter This chapter provides procedures for performing the various tasks required to synthesize oligos on the Shasta High Throughput Oligo Synthesizer.

Topic	See Page
Set Up the Instrument	38
Setup Tasks: First Run of the Day	38
Setup Tasks: Before Each Run	40
Setup Tasks: After Long-Term Shutdown	43
Preparing a Synthesis	44
Running a Synthesis	46
After the Run	48

Set Up the Instrument

About the Setup Tasks

Perform the setup tasks to prepare the instrument for a run. The setup tasks that you perform depend on how long the instrument has been idle:

If the Instrument has been idle for…	Perform the setup tasks described in…	See Page…
Less than 12 hours	"Before Each Run"	40
12 to 60 hours	"First Run of the Day"	38
More than 60 hours	"After Long-Term Shutdown"	43

Setup Tasks: First Run of the Day

Perform these setup tasks before the first run of each day.

IMPORTANT! After completing these tasks, you must also perform the task described in "Setup Tasks: Before Each Run" on page 41.

Checklist: First Run of the Day

To prepare the instrument for the first run of the day, perform the following setup tasks:

Check		Task
	1	Check the instrument maintenance logs and perform any required maintenance tasks.
	2	Check the chamber door O-ring
	3	Start the system
	4	Verify the Home position
	5	Perform all tasks described in "Setup Tasks: Before Each Run".

Task 1: Check the Maintenance Logs

1. Check your laboratory maintenance logs to ensure that no maintenance tasks are due (See "Maintenance Schedule" on page TBD).
2. Perform any required maintenance tasks.

Task 2: Check the Chamber Door O-ring

1. Inspect the chamber door O-ring for wear or chemical residue:
2. If the O-ring shows signs of wear (for example, cracking), call Sierra BioSystems to replace the O-ring.
3. If chemical residue is present, clean the O-ring with a cotton swab or lint-free tissue.

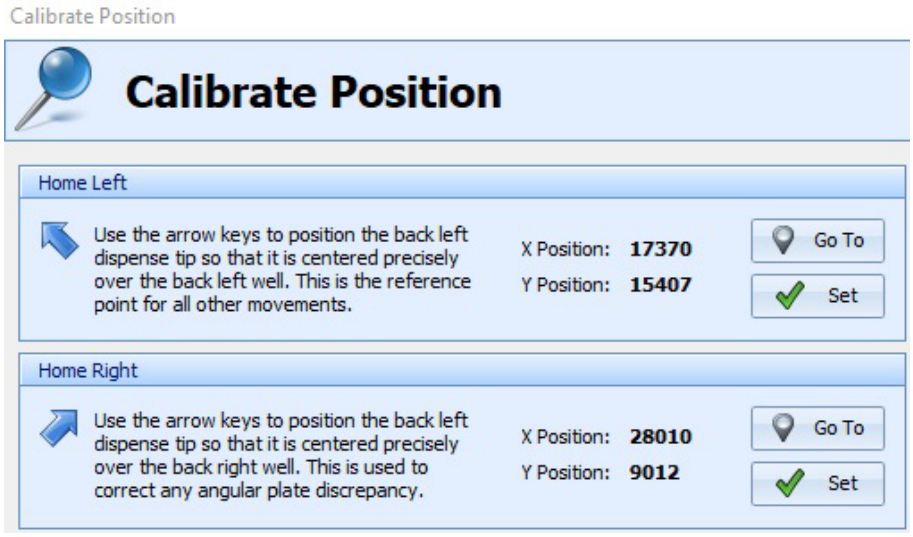
Task 3: Start the System

1. Close and lock the chamber door:
   a. Turn the knobs clockwise until tight.
   b. Lock cam lock with key

2. Power on the instrument, open the Shasta software, then allow the initialization process to complete. The initialize button will be highlighted until initialization is complete.

Task 4: Verify the Home Positions

1. In the Shasta software, select the **Position** button (default view).



2. Click the **Go To** button to move the slide block to the **Home Left** position.
3. Visually inspect the alignment of the dispense nozzles over the slide block:
    - For the 384-well plate, nozzle 1 should be directly over well A1.
    - For the 96-well plate, nozzle 1 should be directly over column/well A1.
4. If nozzle 1 is not aligned with well A1, perform the procedure on page TBD.
5. Repeat 1-4 for **Home Right**

Continue with “Setup Tasks: Before Each Run” on page TBD.

Setup Tasks: Before Each Run

Checklist: Before Each Run

Perform these setup tasks before each run.

Check	Task
	Check the waste container.
	Check the argon tank pressure.
	Check the reagent and amidite bottles.
	Check the pressure gauges for each of the four zones: AMIDITES, REAGENTS, PURGE, SLIDES.
	Check the valves.
	Clean and dispense nozzles.
	Inspect and clean the chamber.
	Prime all dispense lines.

Task 1: Check the Waste Container

Visually inspect the waste container:

1. Check that the waste and vent lines are free of clogs or kinks.
2. Check that the vent line is connected to the laboratory ventilation system.

   IMPORTANT! Waste containers are the low-pressure side of the delivery system and must always be kept vented to the laboratory ventilation system. If a vent line is blocked, back pressure will be generated and will inhibit reagent delivery.

3. Check the fluid level in the waste container. If the waste container is ½ to ¾ full, empty the container per your laboratory's required procedure.

Task 2: Check the Argon Tank Pressure

Visually inspect the two-stage regulator on the argon tank.

1. Check the high pressure. If the valve is below 500 psi, replace the argon tank per your laboratory's required procedure.
2. Check the low pressure. If the pressure is outside the 20 to 80 psi range, adjust the regulator.

Task 3: Check the Reagent and Amidite Bottles

1. Check each bottle to verify that you have a sufficient amount of reagents and amidites for the run. If a bottle will deplete during the run, replace the bottle.
2. Check the installation date on each bottle. If the bottle is out of date, replace the bottle.
3. Be sure that all bottles contain the appropriate filters:

Bottle	**Filter**
Amidite	Amidite Filter, 85 µM
Activator, CAP A, CAP B, Oxidizer, Acetonitrile and Deblock	Filter 1/8" Tubing, Inlet Bottom of Bottle, 10 µM

4. Be sure that the correct reagent or amidite bottle is installed at the correct position, and that each bottle is tightly sealed to its position.

   NOTE: If the instrument was idle for more than 60 hours, the bulk deblock and amidite bottles may have been replaced with acetonitrile to avoid line clogs. Be sure that the bulk deblock and amidite bottles are installed at the correct positions.

5. Verify that there are no unused bottle positions. Install empty bottles or Acetonitrile (ACN) bottles on any unused positions.

Task 4: Check the Pressure Gauges
On the Shasta synthesizer, check the four pressure gauges: AMIDITES, REAGENTS, PURGE, and SLIDES. All gauges should be in the green range:

Gas Pathway	Bottles	Acceptable Range
AMIDITES	• Amidite • Activator	5.0 to 12.0 psi
REAGENTS	• CAP A • CAP B • Deblock (DEB) • Oxidizer (OXI) • Acetonitrile (ACN)	5.0 to 12.0 psi
CHAMBER	Not applicable	~3 psi
INCOMING	Not applicable	40 psi

If one or more of the gauges are not in the specified range, adjust the labeled regulators within the machine. If the pressure has changed since calibration, perform Calibrate a Valve on page X, for either amidites, reagents, or all valves for chamber.

Task 5: Check the Valves
Visually inspect the valves for chemical residue, which may indicate leaks.
If needed, replace leaking valves per the procedure on page X.

Task 6: Clean the Dispense Nozzles
1. Hold a lint-free cloth under the dispense nozzles.
2. Wet each nozzle with acetonitrile to dissolve any residue.
3. Gently dry the nozzles with a lint-free cloth.

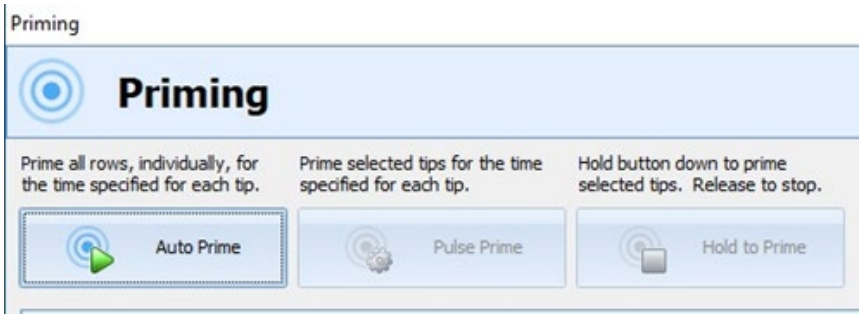
Task 7: Inspect and Clean the Chamber
1. Open the chamber door:
   a. Unlock the cam lock (if present).
   b. Turn the knobs counter-clockwise.

2. Inspect the chamber for foreign material (for example, lint), residue, and/or moisture.
3. If needed, clean the chamber:
   a. Moisten a lint-free cloth with acetonitrile, then wipe the drain plate and X-Y linear stage.
   b. Allow the chamber to air-dry or dry the chamber with a lint-free cloth.

Task 8: Prime All Dispense Lines

Prime all dispense lines to eliminate air bubbles and crystals.

1. In the Shasta software, select the **Priming** button to open the Priming Window.
2. Click **AUTO PRIME**. The instrument begins priming all of the lines into the priming channel.



3. Through the chamber window, visually inspect the dispense nozzles. Fluid should be dispensed in an uninterrupted stream.

Setup Tasks: After Long-Term Shutdown

If the instrument has been idle for more than 60 hours, it should have gone through a Long-Term Shutdown. After a Long-Term Shutdown, all of the instrument bottle positions contain acetonitrile, which means that new reagent and amidite bottles need to be installed on the instrument.

IMPORTANT! After completing these tasks, you must also perform the tasks described in:

- "Setup Tasks: First Run of the Day" on page 38.
- "Setup Tasks: Before Each Run" on page 40.

Checklist: After Long-Term Shutdown

To prepare the instrument after a Long-Term Shutdown, perform the following setup tasks:

Check	Task	Page
	Install reagents on the instrument.	59
	Install amidites on the instrument.	60
	Perform all tasks described in the "Setup Tasks: First Run of the Day" section.	38
	Perform all tasks described in the "Setup Tasks: Before Each Run" section.	40

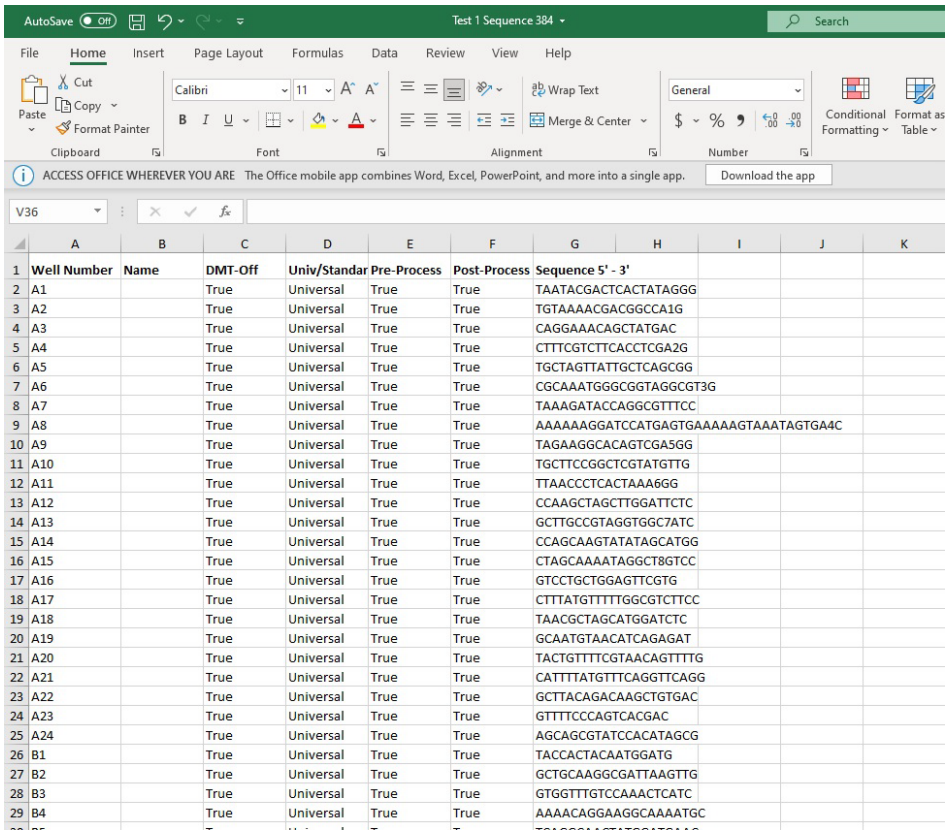
Preparing a Synthesis

A synthesis is specified by two files: a sequence file, that tells what is to be made, and a protocol file, that tells how it is to be made.

About Sequence Files

The sequence file is an Excel document consisting of 1 sheet. The rows of the sheet represent the columns and will be labeled accordingly. Sequence information will include base order, sequence name, and handling procedures that take place before and after the synthesis.

The Excel Spreadsheet



	A	B	C	D	E	F	G
1	Well Number	Name	DMT-Off	Univ/Standar	Pre-Process	Post-Process	Sequence 5' - 3'
2	A1		True	Universal	True	True	TAATACGACTCACTATAGGG
3	A2		True	Universal	True	True	TGTAAAACGACGGCCA1G
4	A3		True	Universal	True	True	CAGGAAACAGCTATGAC
5	A4		True	Universal	True	True	CTTTCGTCTTCACCTCGA2G
6	A5		True	Universal	True	True	TGCTAGTTATTGCTCAGCGG
7	A6		True	Universal	True	True	CGCAAATGGGCGGTAGGCGT3G
8	A7		True	Universal	True	True	TAAAGATACCAGGCGTTTCC
9	A8		True	Universal	True	True	AAAAAAGGATCCATGAGTGAAAAAGTAAATAGTGA4C
10	A9		True	Universal	True	True	TAGAAGGCACAGTCGA5GG
11	A10		True	Universal	True	True	TGCTTCCGGCTCGTATGTTG
12	A11		True	Universal	True	True	TTAACCCTCACTAAA6GG
13	A12		True	Universal	True	True	CCAAGCTAGCTTGGATTCTC
14	A13		True	Universal	True	True	GCTTGCCGTAGGTGGC7ATC
15	A14		True	Universal	True	True	CCAGCAAGTATATAGCATGG
16	A15		True	Universal	True	True	CTAGCAAAATAGGCT8GTCC
17	A16		True	Universal	True	True	GTCCTGCTGGAGTTCGTG
18	A17		True	Universal	True	True	CTTTATGTTTTTGGCGTCTTCC
19	A18		True	Universal	True	True	TAACGCTAGCATGGATCTC
20	A19		True	Universal	True	True	GCAATGTAACATCAGAGAT
21	A20		True	Universal	True	True	TACTGTTTTCGTAACAGTTTTG
22	A21		True	Universal	True	True	CATTTTATGTTTCAGGTTCAGG
23	A22		True	Universal	True	True	GCTTACAGACAAGCTGTGAC
24	A23		True	Universal	True	True	GTTTTCCCAGTCACGAC
25	A24		True	Universal	True	True	AGCAGCGTATCCACATAGCG
26	B1		True	Universal	True	True	TACCACTACAATGGATG
27	B2		True	Universal	True	True	GCTGCAAGGCGATTAAGTTG
28	B3		True	Universal	True	True	GTGGTTTGTCCAAACTCATC
29	B4		True	Universal	True	True	AAAACAGGAAGGCAAAATGC

Each column contains information specific to the sequence:

- **Column A** – The “Well” number. The Well number is the oligos location on the synthesis plate. Neither the oligos nor their labels need to be sequential. The software will sort them alphabetically and numerically once imported.
- **Column B** – The oligo “Name”. These are labels used only by the user. They will not be referenced by the software.
- **Column C** – “DMT-OFF”. Selects for final detrityl step. Values can be: 0, 1, Yes, No, On, Off, True, False, or blank.
- **Column D** – Universal or Standard support selection. In practice, this is a toggle for the first base of the synthesis. Values must be the words: Universal or Standard.
- **Column E** – “Pre-Process”. Selects for an additional cycle that precedes the synthesis. Values can be: 0, 1, Yes, No, On, Off, True, False, or blank.

- **Column D** – "Post-Process". Selects for an additional cycle that follows both the synthesis and the "DMT-OFF" cycle. Values can be: 0, 1, Yes, No, On, Off, True, False, or blank.
- **Column G** – The sequence to be synthesized. Sequences can contain any letter with a corresponding position in the dispense array. All sequences are written 5'– 3'. **NOTE:** Double-digit bases must be enclosed by "[ ]", e.g., base 12 would be inserted as [12].
- Additional Features in the Excel Spreadsheet:
    - **Mixed Backbone Synthesis**: Sequences may contain asterisks "*" that represent a sulfur bond. The sulfurizer will be incorporated into the sequence wherever it is written. For example, in the sequence 5'-ATG*CGA-3', the thioate would be placed between the "C" and "G" nucleotides.
    - **Upper and Lower Case**: Letter case has no effect on the synthesis.

IMPORTANT! To be available from the User Interface, the Excel file must first be imported into the Sequence utility of the Shasta software.

Preparing an Efficient Synthesis

To understand how to use the instrument most efficiently, consider the dispense groups. A dispense group is a set of rows of columns that can be reached by the dispense tips at any one time. When using a 384 well plate, the first dispense group consists of rows 1, 5, 9, and 13. The last group consists of rows 4, 8, 12, and 16. When using a 96-well plate, the first dispense group consists of rows 1, 3, 5, and 7, and the last is 2, 4, 6, and 8.

The number of dispense groups used in a synthesis determines the number of passes required to dispense each reagent to all wells. So, if you want to do a quarter plate, 384-well synthesis, consider using rows 1, 5, 9, and 13 instead of rows 1, 2, 3, and 4. On the other hand, the Shasta 384 uses overlapping: one group can be reacting or draining while another group is dispensing, so using two groups will not take twice as long as using a single group.

About Protocol Files

The protocols used for synthesis will be written within the Protocol Writer and saved in the Shasta software. Protocols can be used to dispense and move fluids, specify reaction times, arrange the reaction sequence, and address the handling requirements of amidites.

Further explanation of the Protocol Writer's use can be found in the Software Walkthrough

Running a Synthesis

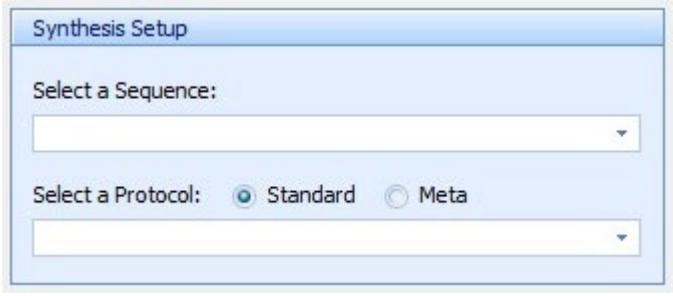
Once the proper sequence files and protocol files have been generated, ensure that all setup tasks have been performed up to and including “Setup Tasks: First Run of the Day”. After setup, proceed to run the synthesis.

Checklist: Running Synthesis

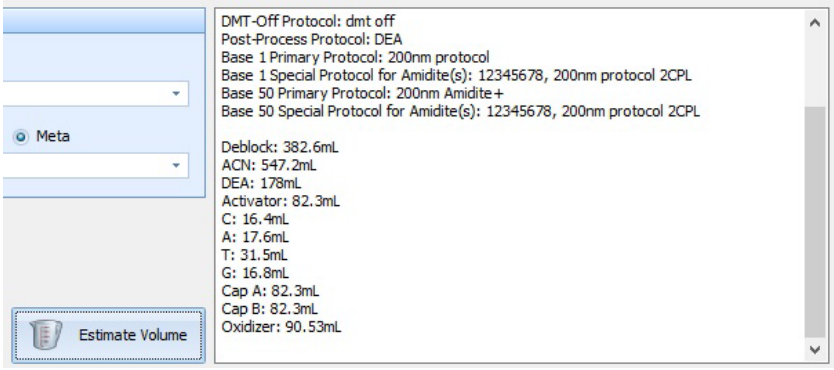
Perform the following tasks to begin a synthesis:

Check	Task
	Enter the Run Synthesis menu and select a file from the Sequence drop-down and the Protocol drop-down. Note whether Standard or Meta is selected.
	Select “Estimate Volume” and ensure enough volume is available for the synthesis.
	Pre-engage “View Plate” if desired.
	Insert columns/plate onto the drain block and follow the color display’s guide for column placement if applicable.
	Apply the Pressure Plate and tighten the four Allen screws.
	Close the lid securely.
	Hit the “Run” button the begin the synthesis.

Task 1: Enter the Run Synthesis menu and select a file from the Sequence drop-down and the Protocol drop-down. Note whether Standard or Meta is selected.



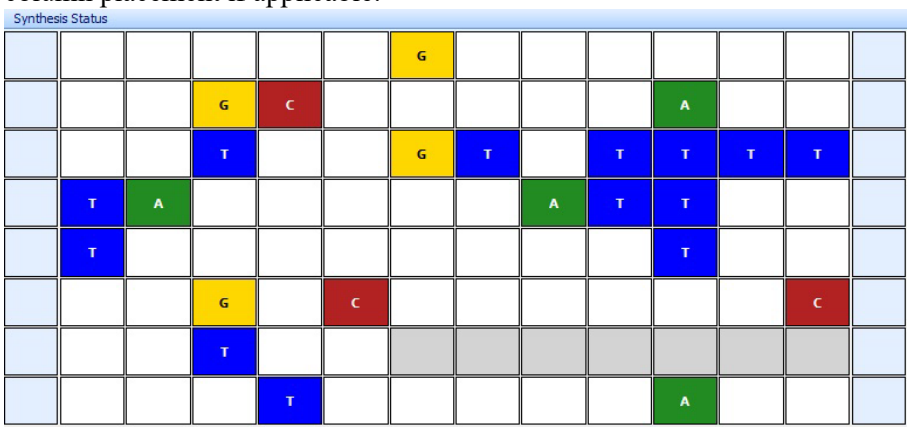
Task 2:
Select “Estimate Volume” and ensure enough volume is available for the synthesis.



Check that the protocols used in estimation match that of the intended synthesis.

Task 3: Pre-engage "View Plate" if desired. **IMPORTANT!** "View Plate" will remain engaged until deselected by the user. After disengaging, wait for the subsequent drain step to run to completion before leaving the instrument.

Task 4:
Insert columns/plate onto the drain block and follow the color display's guide for column placement if applicable.



The color display will act as a column placement guide when using columns.

- Colored spaces will be labeled with the phosphoramidite that should be preloaded onto that column's support.
- White spaces call for universal columns.
- Grey spaces have no sequence to be synthesized.

Task 5: Apply the Pressure Plate and tighten the four Allen screws.

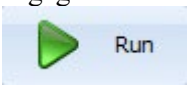
1. Apply the Pressure plate to the drain block.
2. Lift the four Allen screws into position and begin tightening the screws in a "cross" pattern.
3. Ensure that each screw has bottomed out.

Task 6: Close the lid securely.

1. Push the lid down and begin threading one of the two handles into the main plate.
2. Tighten the secondary handle once the first handle has begun to thread into the main plate.
3. Tighten each until they bottom out.

Task 7: Hit the "Run" button to begin the synthesis.

1. Engage the "Run" button.



2. Synthesis has begun. Ensure that chamber pressure has reached the desired threshold.
3. Observe the initial dispense and drain step to ensure proper functioning.
4. Monitor the run from the Row Status indicators, viewing windows, and Protocol Table.

After the Run

Checklist: After the Run

Perform the following tasks to begin a synthesis:

Check	Task
	Verify that synthesis has run to completion.
	Dry the columns/wells if necessary.
	Open the chamber lid.
	Remove columns/plate from drain block.
	Clean the chamber if necessary
	Close the lid.

Task 1: Verify that synthesis has run to completion.

1. Check the software for the "Synthesis Complete" dialogue box and click "Okay".
2. Check the Protocol Table and ensure the desired final protocol ran.

Task 2: Dry the columns/wells if necessary.

1. Observe the columns/wells and check for dampness on the support.
2. If more drying is required, proceed to Manual Control.
3. Within Manual Control, ensure that Chamber gas is applied and then open all drain valves using the "All On" button in the Drain Valves submenu.
4. If moisture was visible in the columns/wells, allow drying to occur for one full minute.
5. Depressurize the chamber.

Task 3: Open the chamber lid.

1. Check chamber pressure and ensure pressure is equal to or less than 0.5psi.
2. Open the lid by unscrewing both handles simultaneously until the lid is free from the main plate.

Task 4: Remove columns/plate from drain block.

1. Undo the four Allen screws securing the columns/plate to the drain block in a "cross" pattern.
2. Push the screws aside and lift the pressure plate from the drain block.
3. Remove the columns/plate from the drain block.
4. Check the drain block for moisture and clean if necessary.

Task 5: Clean the chamber if necessary.

1. Observe the chamber and check for liquid spills during synthesis.
2. Clean any affected areas with a lint-free cloth and ACN if required.

Task 6: Close the lid.

Instrument Maintenance

Overview

About This Chapter This chapter provides the information you will need for preventive maintenance and minor calibrations of the Shasta High Throughput Synthesizer.

Topic	See Page
Long-Term Shut Down	50
Perform a Valve Calibration Verification Test	51
Running the Calibration Verification Test	52
Calibrate a Valve	53
Maintenance Schedule	55
Pressure Testing	56
Cleaning the Device	57

Long-Term Shut Down

About Instrument Shutdown

When the instrument is left idle for up to 60 hours (for instance, over the weekend), perform the following tasks for a Short-Term Shutdown:

1. Clean the dispense nozzles per the procedure on page 41.
2. Clean the chamber per the procedure on pages 41-42.
3. Empty the waste container per your laboratory's required procedure.

Long-Term Shutdown

If you plan to leave the instrument idle for more than 60 hours, you must remove all reagents and run cleanup procedures to avoid line and valve clogs.

1. Clean the dispense nozzles per the procedure on page 41.
2. Clean the chamber per the procedure on pages 41-42.
3. Replace the connected reagent bottles with empty bottles.
4. Remove the remaining bottles, empty them, then fill each bottle with enough acetonitrile to fully prime all the dispense lines.

   NOTE: Sierra BioSystems recommends that you discard reagents and reconstituted amidites rather than store them for reuse.

5. In the Shasta software, select the **Prime** tab, then click **AUTO PRIME** to dispense acetonitrile through all of the nozzles. Adjust number of reps and dispense volumes to thoroughly flush lines with acetonitrile.
6. Empty the waste container per your laboratory's required procedure.

Perform a Valve Calibration Verification Test

Valves control the delivery of reagents and amidites. If valves are not calibrated correctly, oligonucleotide quality and yield may be adversely affected. Valves are calibrated during the manufacturing process to deliver accurate volumes over a specified range, depending on the chemical being delivered. Over time, valves may lose calibration or begin to fail, but may continue to deliver chemicals.

When to perform a valve calibration verification test

- Monthly
- Whenever oligonucleotide yield is lower than expected
- After calibrating a valve

Required Material

- Gloves and safety glasses
- Calibration plate and calibration plate adapter arms
- PCR tubules
- 5/32” Allen wrench
- Reagents and/or amidites

Prepare the System

- Power on the instrument, open the Shasta software, and wait for the initialization process to complete.
- Be sure that there is sufficient reagent and/or amidite installed to test the valves of interest.
- Load and install the calibration plate.
- Place the attachment arms onto the sides of the drain plate and fasten with the drain plate’s rotating screws and a 5/32” Allen wrench.
- Hook the calibration plate onto the attachment arms.
- Insert PCR tubules into the 1 and 3 positions of each labeled space.
- Close and lock the chamber door.
- Prime the lines using the Auto Prime function. Ensure all lines are dispensing in a continuous stream.

Running the Calibration Verification Test

1. In the Shasta software, click the Cal Check button.
2. Remove the calibration plate.
3. After the sequence has run, note the level of solvent in each PCR tubule. Each tube should be 75% full with +/- 5% variance acceptable for a functioning valve.
4. Using a 5/32” Allen wrench, remove the attachment arms from the drain plate.
5. Perform a Calibration procedure on valves not falling within the acceptable range of this procedure.

Calibrate a Valve

When to perform a valve calibration verification test

- When a new valve is installed
- If a Calibration Verification Test indicates that the valve is no longer calibrated to within your laboratory's specifications.

Required Material

- Gloves and safety glasses
- Calibration plate and calibration plate adapter arms
- PCR tubules
- 5/32" Allen wrench
- Reagents and/or amidites
- Balance with 0.1-mg resolution

Prepare the System

1. Power on the instrument, wait for the initialization process to complete, then double-click the icon to start the Shasta software.
2. Open the calibration portion of the software by clicking Calibrate.
3. Be sure that there is sufficient reagent and/or amidite to be installed to calibrate the valve of interest.
4. Install the calibration plate.
    a. Place the attachment arms onto the sides of the drain plate and fasten with the drain plate's rotating screws and a 5/32" Allen wrench.
    b. Hook the calibration plate onto the attachment arms.
    c. Insert PCR tubules into the 1, 2, and 3 positions of each labeled space.
5. Close and lock the chamber door.
6. Prime the lines using the Auto Prime function. Ensure all lines are dispensing in a continuous stream.

Run the Auto Calibration

1. The calibration process happens in 2 segments, Auto Cal Left and Auto Cal Right. Click Auto Cal Left to begin the calibration process for the left half of the dispense valves.
2. When the process is complete, remove the calibration plate.
3. Using a 0.1-mg resolution scale, weigh an empty PCR tubule, tare, and then weigh the PCR tubule filled with solvent.
4. Each reagent/amidite position receives three dispenses: 50ms, 100ms, and 200ms. These dispenses relate to positions 1, 2, and 3 respectively. Weigh each of these positions for the reagent/amidite at hand, record the results in the respectively labeled boxes, then click Done.
5. Perform this process for each valve out of calibration.
6. Hit Save and Close to complete the process.

Maintenance Schedule

Following a maintenance schedule helps:

- Ensure proper instrument operation.
- Reduce service calls.
- Prevent instrument downtime.
- Prevent reagent waste.

Required Maintenance Tasks

Schedule	Maintenance Task	See page
Monthly	Perform a Valve Calibration Verification Test	51
	Inspect and clean the amidites bottle O-rings. Replace the O-rings if inspections show signs of wear.	---
	Inspect the Reagent Cap Assembly Gasket. Replace the gasket if inspections show signs of wear.	---
Biannually	Inspect the chamber door O-ring. Contact Sierra BioSystems if the chamber door O-ring shows signs of wear (for example, cracking).	---
	Perform a Pressure Test.	56
Annually	Replace the amidite bottle O-rings.	---
	Replace the reagent bottle gaskets. **NOTE:** The reagent bottle gaskets are not renewable with each bottle change because the gaskets have a service lifetime of 6 months to 1 year.	---
As needed	Inspect the Reagent Cap Assembly Gasket with every bottle change. Replace the gasket if inspections show signs of wear.	---
	Perform a Valve Calibration Verification Test whenever oligonucleotide yield is lower than expected.	52
	Calibrate a valve if a Valve Calibration Verification Test indicates a problem.	53

Instrument Maintenance Log

Sierra BioSystems recommends keeping an instrument maintenance log to ensure that the required maintenance tasks are performed in a timely manner.

Pressure Testing

Task	Reason
Perform the Pressure Test to verify that the chamber seals properly and the waste valves are functioning correctly.	Pressures gradients between the chemical bottles, the synthesis chamber, and the waste lines affects: • Reagent and amidite delivery • Purging of waste fluid Leaks though gaskets or O-rings may affect chemical delivery and oligonucleotide quality.

When to Perform Pressure Test

Perform the Pressure Test every 6 months.

Perform the Pressure Test

1. Close the chamber door, then turn the knobs clockwise until tight.
2. Power on the instrument, open the Shasta software, then allow the initialization process to complete. The initialize button will be highlighted until initialization is complete.
3. In the Shasta software, select the **Manual Control** button. Select the **Chamber** valve and allow the chamber to reach 3 psi.
4. Deselect the **Chamber** valve and monitor the Chamber Pressure gauge.

If the CHAMBER gauge…	Then the test…	Next Steps
Drops less than 1 psi in 1 minute	Passes	---
Drops more than 1 psi in 1 minute	Fails	a. Check the chamber door O-ring for signs of wear. b. Verify that the knobs on the chamber door are tight (completely turned clockwise). c. Look through the chamber window to verify that none of the dispense nozzles are leaking. d. Contact Sierra BioSystems support.

5. Click **Close** to end the test and retain pressure. Click **Close** and then **Depressurize** to end the test and depressurize the chamber.

Cleaning the Device

If dried solvents collect on or around the chamber or solvent spills occur on the chassis of the machine, follow the instructions below to clean the affected areas.

Required Material

- Gloves and safety glasses
- Acetone or ACN
- Lint-free cloth

Prepare the System

1. Wearing protective safety glasses and gloves, damp a lint-free cloth with Acetone.
2. Clean each dispense tip from top to bottom to remove all accumulated residue.
3. Check underneath the linear actuators for solvent and wipe away as needed.
4. Wipe down the affected area of the chassis the lint-free cloth and let dry.

IMPORTANT! Acetone should never be used to clean the plexi-glass windows of the machine.

Setup Tasks

Overview

About This Chapter This appendix contains detailed instructions for the setup tasks summarized in Chapter 4, Performing DNA Synthesis.

Topic	See Page
Replacing Reagent Bottles	59
Replacing Amidite Bottles	60
Storage Conditions for Reagents and Amidites	61
Storage of External Inert Gas	62
Laboratory Ventilation Requirements	63

Replacing Reagent Bottles

Before each run, check the fluid level in each reagent bottle. If a bottle depletes during the run, replace the bottle.

IMPORTANT If you need to replace reagents, always install a new bottle on the instrument. Do not add new solution to previously used reagent bottles. Some chemicals reduce the integrity of glass bottles; repeated use beyond 6 weeks may cause the bottle to fracture when it is pressurized during instrument operation.

Required Materials

- Gloves and safety glasses
- New reagent bottle
- For acetonitrile/tetrazole bottles only: Medium sized Trap-Pak or molecular sieves

Replace a Reagent Bottle

1. Open a new reagent bottle and record the date.
2. Remove the old bottle:
   a. Slowly turn the bottle counterclockwise until it releases, then remove the bottle from its position.
   b. Using the cap from the new bottle, cap the old bottle to minimize residual vapor release.
   c. Make sure the gasket is in place and does not fall off when the bottle is removed.
3. Place a medium-sized Trap-Pak or molecular sieves into the new bottle.
4. Screw the new bottle snugly into its threaded receptacle on the instrument by turning it clockwise.
   NOTE: The front bottle receptacles have a ratchet cap assembly. A built-in torque-limiting feature reduces the possibility of over-tightening. To avoid leaks in the cap assembly, do not continue turning the bottle when clicking starts.

Replacing Amidite Bottles

Before each run, check the fluid level in each amidite bottle. If a bottle will deplete during the run, replace the bottle.

Required Materials

- Gloves and safety glasses
- New amidite bottle
- Lint-free cloth
- Mini-sized Trap-Pak or molecular sieves

Dissolve Amidites

The amidites are bottled as powders and sealed under argon. In this state, they are stable for 1 year from the date of shipment. Dissolve powdered amidites in acetonitrile (ACN) before installing them on the instrument.

Guidelines for Dissolving Amidites

Amidites are extremely sensitive to acid, oxygen, and water. Follow these guidelines when dissolving amidites:

- Use anhydrous acetonitrile with less than 100 ppm of water.
- After opening acetonitrile, keep it blanketed with argon to avoid contamination with air.
- When transferring acetonitrile to an amidite bottle, use a clean, dry, glass syringe with a needle:
  - The syringe should be dedicated to acetonitrile transfer.
  - Dry the syringe in a 100 to 120 °C oven.
  - Store the syringe in a 100 to 120 °C oven to prevent atmospheric moisture contamination. Before using the syringe, cool the syringe to room temperature in a desiccator.
  - Rinse the syringe with acetonitrile. Do not use water.
  - To avoid cross-contamination between amidite bottles, do not allow the syringe needle to contact the amidites.
  - Shake the bottle until no crystals are visible on the bottom.

Replace an Amidite Bottle

1. Open a new amidite bottle and record the date.
2. Remove the old bottle:
   a. Firmly pull the bottle straight down while pressing the button above its receptacle.
      NOTE: If the bottle sticks, carefully move it from side to side while pulling down.
   b. Using the cap from the new bottle, cap the old bottle to minimize residual vapor release.
3. Use a lint-free cloth to wipe the inlet line.
4. Place a mini-sized Trap-Pak or molecular sieves into the new amidite bottle.
5. Thread the inlet line into the bottle.

Storage Conditions for Reagents and Amidites

Follow the storage recommendations and change bottles when they have reached their expected lifetime. Improperly storing reagents and amidites can impair product quality and can compromise the bottle integrity when the bottle is pressurized under normal instrument operation.

IMPORTANT! Keep all reagents and amidites, on or off the instrument, out of direct sunlight. Sunlight degrades the chemicals and elevates the temperatures within the bottles.

Bottle	**Location**	**Storage Conditions**	**Shelf-Life**	**Lifetime on Instrument**
CAP A	Reagent rack, or as 4-L bottles under the instrument	Room Temperature	1 Year	2 Weeks
CAP B				
ACT (Activator)				
OXI (Oxidizer)				
DEB (Deblock)	4-L bottles, under the instrument			
ACN (Acetonitrile)				
dA	Amidite racks front of the instrument	Room Temperature	1 Year	1 Week
dG				
dC				
T				

Storage of External Inert Gas

Sierra BioSystems recommends the use of industrial grade Argon stored in a size 200 cylinder, CGA-580 fitted, with a maximum loading capacity of 28,000 psi.

WARNING! A regulator must be fitted to the cylinder with a maximum pressure output of 200 psi.

Pressurized Gas and Regulator Requirements

Gas Cylinders

You must supply the required argon gas cylinder and accessories for installation. This instrument requires a pressurized house line, or 1 size 1-A argon gas cylinder that holds approximately 7.2 m3 (257 ft3) of gas when full. Use only pre-purified argon of 99.998 % or greater purity. Damage to the instrument and its products can result from using impure argon, gases other than argon, or an inadequate amount of argon. There should be at least 2 argon cylinders in the laboratory at all times – 1 full and 1 in use.

DANGER! EXPLOSION HAZARD. Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

Pressure Regulator

You must supply a two-gauge regulator with a Compressed Gas Association (CGA) 580-cylinder adapter on the inlet side and a Swagelok® type end-fitting that accepts 6.35-mm (¼-in.) o.d. tubing. The primary gauge (0 to 3000 psi; 0 to 25,000 kPa recommended) measures tank pressure, and the secondary gauge (0 to 200 psi; 0 to 2000 kPa recommended) measures regulated pressure. The secondary gauge must allow regulation between 0 and 50 psi. Compressed Gas Association (CGA) 580-cylinder adapters are supplied with a needle-type shutoff valve on the exit side. The needle valves should have Swagelok-type end-fittings ready for connection to 6.35-mm (0.25-in.) o.d. tubing. The second-stage output of the regulator should be set at 40 psi.

WARNING! BOTTLE EXPLOSION HAZARD. Bottle explosion can cause severe physical injury. To prevent reagent bottles from becoming over-pressurized and possibly exploding, the pressure regulator and the pressure relief valve must be in place and working properly. Failure of these two components will cause bottle over-pressurization and bottle explosion.

Laboratory Ventilation Requirements

Laboratory Ventilation Specifications

The laboratory ventilation system(s) to this instrument must be independent of the room air ventilation system and operating properly whenever the instrument power is on, waste is in the waste container, or reagents are on the instrument. For the K&A Synthesizer, proper ventilation equipment and operation specifications include either of the following:

- A fume hood with an average of 30 linear m/min (100 linear ft/min) face-level velocity of airflow. The minimum velocity at any point in the hood is 24 linear m/min (80 linear ft/min), and the maximum velocity is 38 linear m/min (125 linear ft/min).
- A duct that is dedicated to exhausting chemical vapors with a draw of –5.1 cm H2O (–2.0 in. H2O).

About the Waste System

The waste system is composed of a common fluid and gaseous waste tube that exits the back of the instrument and is attached to a 4-L (1-gal.) polyethylene bottle in a secondary container. The gaseous waste fumes are conducted out of the waste bottle to the laboratory ventilation system for disposal by 9.5-mm o.d. (3/8-in. o.d.) tubing.

WARNING! CHEMICAL HAZARD. Four-liter reagent and waste bottles can crack and leak. Each 4-L bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Connecting the Gaseous Waste Exhaust Line

Follow these guidelines to connect the tubing from the instrument's gaseous waste exhaust to your laboratory ventilation system:

- Use the shortest possible length and the straightest possible run of polypropylene tubing. Tubing length should not exceed 2.4 m (8 ft).
- Make sure that the tubing does not have low points that can trap residue or condensation.
- Fasten the tubing securely. Use polypropylene or Teflon fasteners. Do not use brass; it corrodes. Be careful not to puncture tubing.
- Place the tubing away from sources of potential damage, such as heat, flame, or points of contact (rubbing) with other objects.
- Place the tubing end as far as possible into the duct, canopy, or hood.
- Make sure that oncoming air movement through the duct or canopy does not face the open end of the tubing.

Connecting the Waste Line

IMPORTANT! Connect the fluid waste line from the instrument to the waste bottle so that it drops vertically. Doing so prevents liquid and waste from accumulating and blocking the flow.

Heat Production

The thermal output of the instrument is 853 Btu/h (250 W). Consult your facilities department regarding ventilation requirements for this level of heat output

Fume Hood Operation

Follow these guidelines for fume hood operation and maintenance:

- Operate the fume hood whenever the instrument power is on, waste is in the waste container, or reagents are on the instrument.
- Use a fume hood that is constructed of materials that are compatible with the waste materials/chemicals being generated or exhausted.
- Locate the fume hood away from air currents generated by air-conditioning ducts, fans, windows, doors, and moving equipment and persons.
- Locate the fume hood exhaust vent where gaseous waste cannot be drawn back into the building.
- Affix a sign or label to indicate the position of the fume hood sash that produces an average airflow of 100 linear ft/min face velocity. The minimum flow velocity at any point in the hood is 80 linear ft/min, and the maximum is 125 linear ft/min.
- Ensure that the fume hood meets all local, state/provincial, or national safety requirements. Have a safety professional or mechanical ventilation expert check and record air velocity at least once a year.
- Inspect and maintain the exhaust system, including fans and motors at least once a year.

Duct System Operation

Follow these guidelines for duct system operation and maintenance:

- Operate the duct system whenever the instrument power is on, waste is in the waste container, or reagents are on the instrument.
- Use a duct system constructed of PVDF tubing or other materials compatible with the waste material being generated.
- Do not allow the duct system to come into contact with strong oxidizers, bases, or other chemicals that are incompatible with gaseous waste

Troubleshooting

Overview

About This Chapter This appendix contains detailed instructions for troubleshooting errors on the Shasta Synthesizer.

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Liquid Spills/Leaks	77
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Poor Synthesis Yields

Low yields and excessive impurities are most often the result of suboptimal methods/protocols. Though the Shasta is equipped with stock protocols and reagent suggestions, the available reagents, phosphoramidites, and handling/processing methods are vast, and as such, it is important to understand that method development will be a critical part of synthesizer operation. The table below will attempt to provide an explanation and solution for the most common synthesis errors.

IMPORTANT! The requirements of all phosphoramidites should be ascertained from their vendor(s) and followed closely before proceeding to the below suggestions.

Problem	**Cause**	**Solution**
If high n-1s:	Bad detritylation yields	Double the deblock times, modify pulse times, or increase number of reps to increase the time deblock is in contact with or flowing through the resin.
	Bad coupling *and* bad capping	Double the coupling times or number of reps to greatly increase the time phos/act and capping reagents are in contact with or flowing through the resin.
	Bad Oxidation yields	Adjust Oxidation step to greatly increase the time Oxidizer is in contact with or flowing through the resin.
		Can also try reversing the Ox/Cap steps, or ordering as Cap/Ox/Cap or as Cao/Ox/Ox/Cap.
	Old or wet reagents (particularly phosphoramidites and Activator)	Replace the phosphoramidites with freshly prepared reagents prepared with very dry ACN
		Add 'Drying traps' (Sold by ChemGenes, BioSearch, AIC, etc.) to: • All phosphoramidites • ACN bottle • Activator bottle **NOTE:** Drying traps take about 24 hours to get rid of ~80% of H_2O, so it is important to plan this in advance.

	Slower detritylation and coupling for the first step (reaction rate is typically slower for first deblock and coupling).	Double detritylation time and coupling time for first cycle only.
If high 5'-failure sequences (n-1, n-2, n-3…)	Poor coupling	Double the coupling times or number of reps to greatly increase the time phos/act and capping reagents are in contact with or flowing through the resin.
	Poor Oxidation, but this is rare. If due to poor oxidation yields, the n-1s will likely be accompanied by m+366 and/or m+286 peaks (eluting very late in chromatogram).	Increase Oxidation time.
		Increase concentration of I2 above 20mM (if not using dmf-dG phosphoramidite).
If high 5'-failure sequences (n-1, n-2, n-3…) with phosphate attached *and* significant amounts of 3'-deletions with phosphate (or 3'-deletions with phosphate + 98)	Indicative of excessive depurination	Reduce deblock exposure time (limit to 60s *especially* if using TCA)
		Test different deblocks (different concentration or acid identity, then adjust exposure time).
If high n-1s and very little 5'-failure sequences	Bad Capping yields	Replace capping solutions, increase capping times, and/or test different capping formulations.
If seeing n+1	n+1 oligos are formed because acidic activator removes DMT group, forms dimer, then adds to 5'-OH during coupling step.	Change to a less acidic activator, like DCI or tetrazole.
If seeing n+x (x=2, 3, 4…) impurities (after the FLP peak)	n+x impurities are from exocyclic amine protecting groups coming off the phosphoramidites (in the bottle before synthesis) allowing branching of oligos off of the amine groups.	Replace phosphoramidites.

If high m+275 and/or m+261 when using universal linker (peak will elute after FLP): If high m+302 (peak will elute *long* after FLP):	Note that the molecular weights can vary depending on if Me or Ph is on Unylinker and to what extent the degradation of succinimide ring has proceeded.	longer or otherwise harsher cleavage/deprotection conditions are required.
If seeing m+366 and/or m+286: (Impurities will elute long after FLP)	These impurities are from trityl adding to remaining P(III) if all of oligo was not oxidized to P(V)	Increase Oxidation time. Can also increase concentration of I_2 above 20mM (if not using dmf-dG phosphoramidite).
If seeing large amounts of m+41 impurity:	Bad Capping yields	Experiment with capping times such that m+41 peak is minimized but you don't start seeing increased n-1 impurities from incomplete capping. Trying out alternative capping solutions may also be beneficial.
If seeing excessive m+53 peak (just after FLP in chromatogram):	Incomplete DEA reaction	Longer reaction times with DEA (post-synthesis, pre-cleavage) are required. For the first few additions of DEA (or other hindered amine), it's important to pass the solution through the columns relatively quickly (less than 10s) to quickly rinse the acrylonitrile away from the oligo.

NOTE: It is recommended to analyze and list the following for comparison before analyzing your own oligos by LC-MS

- All 5'-deletions (traditional 'failure sequences', meaning the oligo is truncated from the 5'-end
    - e.g., for sequence 5'-CATGT-3', 5'-failures are 5'-ATGT-3', 5'-TGT-3', and 5'-GT-3' (exclude monomers)
- All 3'-deletions
    - e.g., for 5'-CATGT-3', look for 5'-CATG-3', 5'-CAT-3', and 5'-CA-3'
- All 5'-failures with phosphate attached
    - e.g., for 5'-CATGT-3', look for 5'-ATGT-Ᵽ -3', 5'-TGT-Ᵽ-3', and 5'-GT-Ᵽ-3'

- All 3’-failures with phosphate attached
    - e.g., for 5’-CATGT-3’, look for 5’-Ᵽ- CATG-3’, 5’-Ᵽ- CAT-3’, and 5’-Ᵽ- CA-3’
- **NOTE**: It can seem counterintuitive when the “5’-failures” are actually from the 3’-end of the sequence and the “3’-failures” are from the 5’ end of the sequence. But, for the former, the failure is at the 5’-OH and for the latter, the failure is at the 3’-OH.
- Other common impurities to include in the failure sequences list:

Group	Mass Diff.	Formula
Cyanoethyl	53.0265	C3H3N
CE2	106.053	C6H6N2
m+linkrt-1	275.0195	C9H10N1O7P1
m+linker-2	261.00385	C8H8N1O7P1
10mer-P	79.9663	PO3H
DMT	302.1307	C21H18O2
TransAmination	14.0156236	CH2
DMF	55.0422	C3H5N
BZ	104.0262	C7H4O
Ac	42.0106	C2H2O
m+FAM	537.11896	
n+T	304.046045	
n+G	329.052515	
n+A	313.057611	
n+C	289.046381	
-A+OH	-115.02843	C5H3N5, +O
-G+OH	-131.02333	C5H3N5
-C+OH	-91.01713	C4H3N3
-T+OH	-106.01676	C5H4N2O
-T (in source)	-125.03515	C5H5N2O2
-G (in source)	-150.04172	C5H4N5O1
-A (in source)	-134.04682	C5H4N5
-C (in source)	-110.03552	C4H4N3O

- **NOTE**: all mass calculations require use of exact masses, not average masses.
- Generating mass list is as simple as making an Excel template and pasting in the sequence.

Inconsistent Yields Between Drain Banks

Though a degree of variance is to be expected in stepwise yield (typically +/- .5%), steep deviations in overall yield are typically a sign of mechanical failure or obstruction. This section will describe the likely causes of this issue and the corresponding solutions.

Poor Column or Plate Seal

If the seal between the column and the plate or the plate and the drain block is compromised, solvents will not flow properly through affected the drain bank(s).

First, ensure that no column is loose on the column plate. No column should fall out of the column plate when turning it upside down.

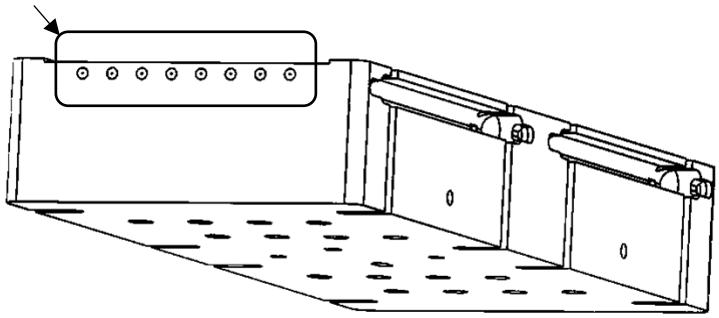
Second, inspect the Kalrez gasket seated on the drain block. The gasket should be free of swelling, tears, or other imperfections. If the gasket is defective, replace it.

Finally, ensure there is no obstruction between the column plate and the gasket when seating the column plate. The plate should seat evenly across the gasket, and the pressure plate should bottom out on the drain block itself.

If using pre-packed synthesis plates, inspect the O-ring seals on the drain block adapter of the poor performing banks. A compromised O-ring will will be evidenced by slow flow throughout the bank. Replace any defective O-rings immediately.

Exhaust Point Blockage

The Shasta Drain Block is designed with exhaust points built into each drain bank.



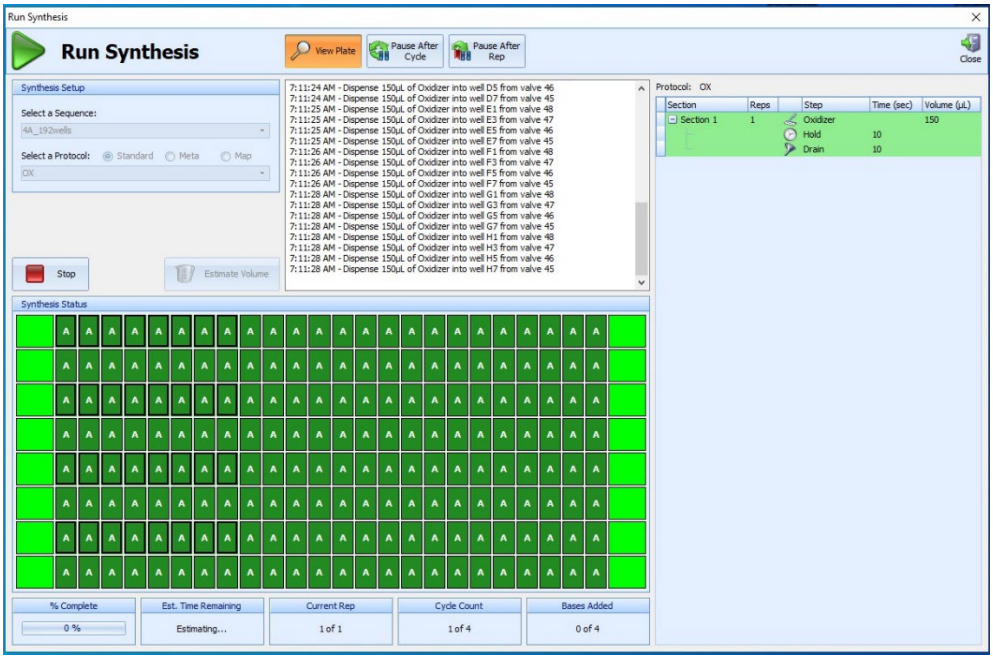
These exhaust points prevent a vacuum from forming beneath the columns.

During the “Pulse” steps, gas is removed quickly, and the solvents attempt to catch up. However, if the solvents completely fill the void left by the removed gas, they will be drained from the plate, and this will end the reaction step too quickly. The small openings allow the space beneath the columns to be filled with gas from the chamber, and therefore, during a “Pulse”, the solvent is briefly moved into support, but it is not drained completely.

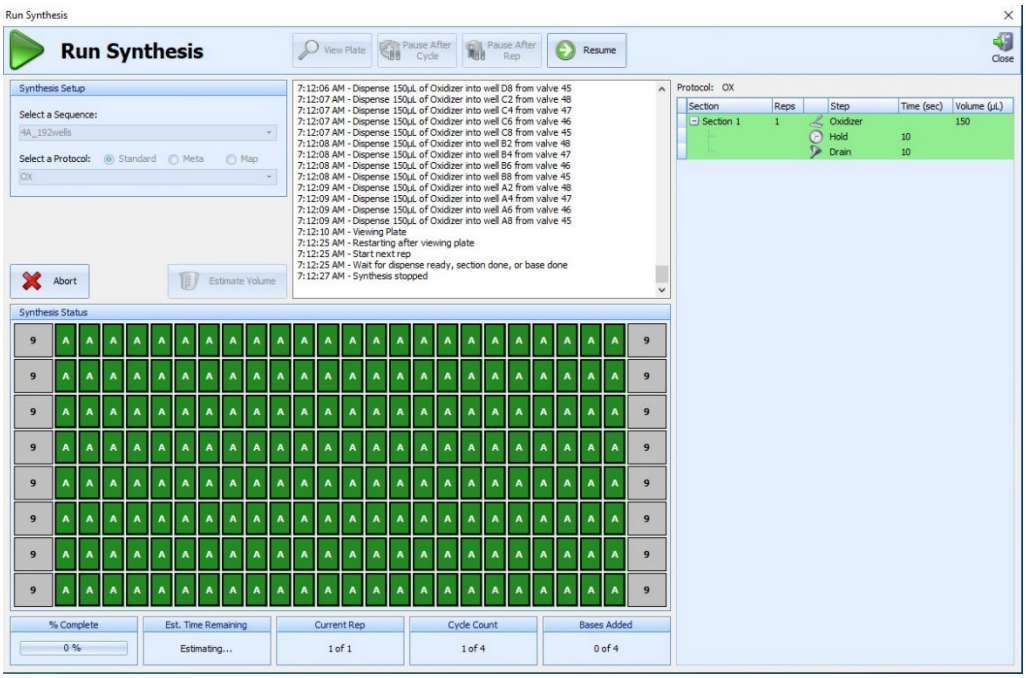
If the holes become clogged. Then the solvents may drain completely before the reaction step is finished. It is easily rectified by inserting some guitar string or wire through the small opening.

How to test for even drain bank flow:

You can test the flow through the columns by making a short protocol of one solvent across the plate. Load a protocol like the one pictured and run it across both plates with “View Plate” enabled.

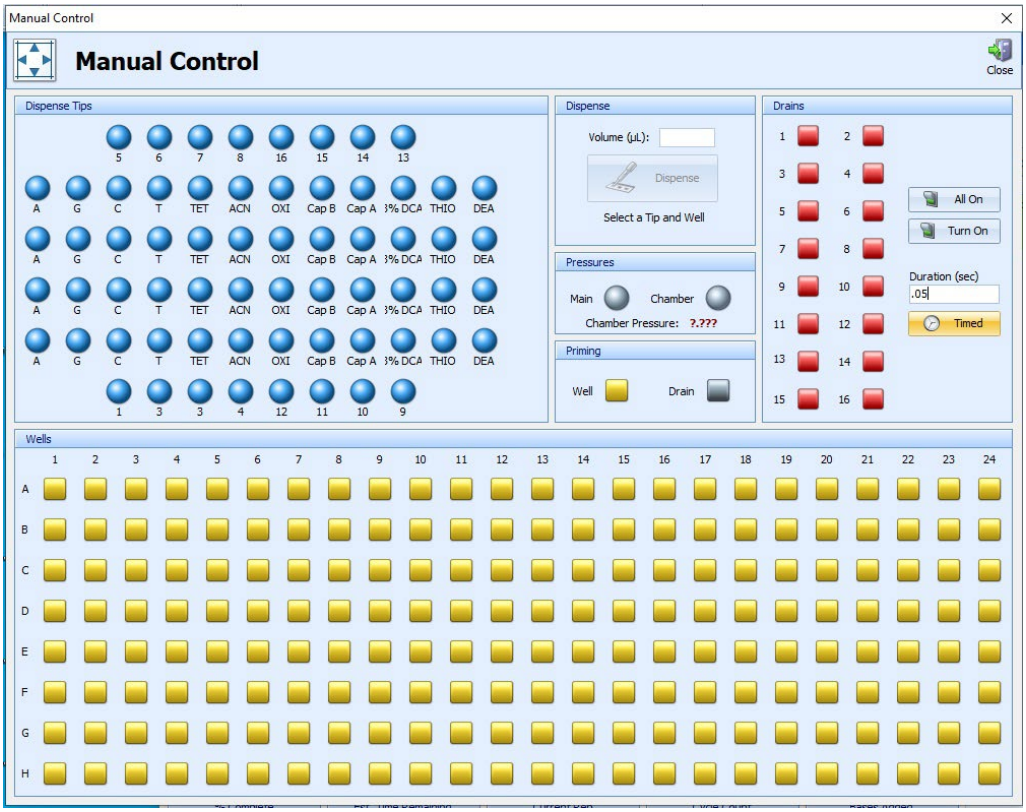


Once the software presents “Proceed with Synthesis?” click “Okay” and then hit “Stop”. The screen will look like this:



Then, go to Manual Control. Both plates will be loaded with solvent. Once in Manual Control, select all Drain Valves on the right side of the screen. Once they are selected,

type in a small value in the “Timed” box (.05 seconds is typically sufficient). While watching the plates, click the “Timed” command. Watch the liquids on both plates.



Make sure you do not lose solvent prematurely from any bank. The fluid movement should look uniform. There may be some variance, but no more than could be attributed to the varied packing of the support.

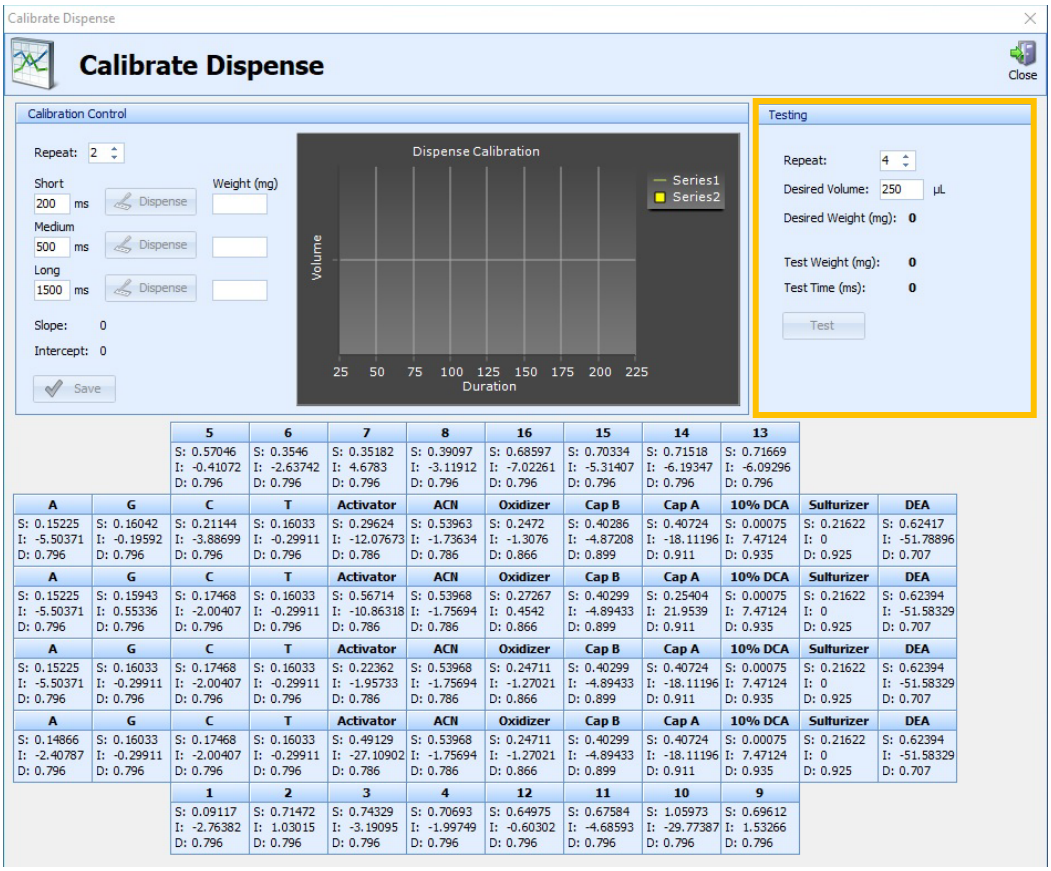
If you lose all fluids from any bank, then that bank’s “opening” should be cleaned with guitar string or wire.

Calibrate Valve(s)

If a whole quadrant of the plate is performing poorly, it is likely a result of one or more valves in need of calibration.

Determine which valve requires calibration by first recognizing the lagging quadrant. In 96 mode, quadrants are in rows of 2 (e.g. rows 5 and 6 are quadrant 3). In 384 mode, quadrants are in rows of 4 (e.g. rows 5-8 are quadrant 2). The quadrant will correspond to the row of nozzles the rows receive dispense from (e.g. quadrant 4 received dispense from dispense row 4).

Once the dispense row has been determined, perform a Dispense Test located on the right-hand side of the Calibration window:



5	6	7	8	16	15	14	13
S: 0.57046 I: -0.41072 D: 0.796	S: 0.3546 I: -2.63742 D: 0.796	S: 0.35182 I: 4.6783 D: 0.796	S: 0.39097 I: -3.11912 D: 0.796	S: 0.68597 I: -7.02261 D: 0.796	S: 0.70334 I: -5.31407 D: 0.796	S: 0.71518 I: -6.19347 D: 0.796	S: 0.71669 I: -6.09296 D: 0.796

A	G	C	T	Activator	ACN	Oxidizer	Cap B	Cap A	10% DCA	Sulfurizer	DEA
S: 0.15225 I: -5.50371 D: 0.796	S: 0.16042 I: -0.19592 D: 0.796	S: 0.21144 I: -3.88699 D: 0.796	S: 0.16033 I: -0.29911 D: 0.796	S: 0.29624 I: -12.07673 D: 0.786	S: 0.53963 I: -1.73634 D: 0.786	S: 0.2472 I: -1.3076 D: 0.866	S: 0.40286 I: -4.87208 D: 0.899	S: 0.40724 I: -18.11196 D: 0.911	S: 0.00075 I: 7.47124 D: 0.935	S: 0.21622 I: 0 D: 0.925	S: 0.62417 I: -51.78896 D: 0.707
A	**G**	**C**	**T**	**Activator**	**ACN**	**Oxidizer**	**Cap B**	**Cap A**	**10% DCA**	**Sulfurizer**	**DEA**
S: 0.15225 I: -5.50371 D: 0.796	S: 0.15943 I: 0.55336 D: 0.796	S: 0.17468 I: -2.00407 D: 0.796	S: 0.16033 I: -0.29911 D: 0.796	S: 0.56714 I: -10.86318 D: 0.786	S: 0.53968 I: -1.75694 D: 0.786	S: 0.27267 I: 0.4542 D: 0.866	S: 0.40299 I: -4.89433 D: 0.899	S: 0.25404 I: 21.9539 D: 0.911	S: 0.00075 I: 7.47124 D: 0.935	S: 0.21622 I: 0 D: 0.925	S: 0.62394 I: -51.58329 D: 0.707
A	**G**	**C**	**T**	**Activator**	**ACN**	**Oxidizer**	**Cap B**	**Cap A**	**10% DCA**	**Sulfurizer**	**DEA**
S: 0.15225 I: -5.50371 D: 0.796	S: 0.16033 I: -0.29911 D: 0.796	S: 0.17468 I: -2.00407 D: 0.796	S: 0.16033 I: -0.29911 D: 0.796	S: 0.22362 I: -1.95733 D: 0.786	S: 0.53968 I: -1.75694 D: 0.786	S: 0.24711 I: -1.27021 D: 0.866	S: 0.40299 I: -4.89433 D: 0.899	S: 0.40724 I: -18.11196 D: 0.911	S: 0.00075 I: 7.47124 D: 0.935	S: 0.21622 I: 0 D: 0.925	S: 0.62394 I: -51.58329 D: 0.707
A	**G**	**C**	**T**	**Activator**	**ACN**	**Oxidizer**	**Cap B**	**Cap A**	**10% DCA**	**Sulfurizer**	**DEA**
S: 0.14866 I: -2.40787 D: 0.796	S: 0.16033 I: -0.29911 D: 0.796	S: 0.17468 I: -2.00407 D: 0.796	S: 0.16033 I: -0.29911 D: 0.796	S: 0.49129 I: -27.10902 D: 0.786	S: 0.53968 I: -1.75694 D: 0.786	S: 0.24711 I: -1.27021 D: 0.866	S: 0.40299 I: -4.89433 D: 0.899	S: 0.40724 I: -18.11196 D: 0.911	S: 0.00075 I: 7.47124 D: 0.935	S: 0.21622 I: 0 D: 0.925	S: 0.62394 I: -51.58329 D: 0.707

1	2	3	4	12	11	10	9
S: 0.09117 I: -2.76382 D: 0.796	S: 0.71472 I: 1.03015 D: 0.796	S: 0.74329 I: -3.19095 D: 0.796	S: 0.70693 I: -1.99749 D: 0.796	S: 0.64975 I: -0.60302 D: 0.796	S: 0.67584 I: -4.68593 D: 0.796	S: 1.05973 I: -29.77387 D: 0.796	S: 0.69612 I: 1.53266 D: 0.796

Using the “Testing” function of the Calibration window and a graduated vial, collect a sample and inspect it for accuracy. If the dispensed value is not the requested value, recalibrate the valve following the method described in “Instrument Maintenance”.

NOTE: before sample collection, be sure to reduce bottle pressure by the operating chamber pressure, e.g. if the chamber fills with 3 psi of Argon during the synthesis, then reduce the bottle pressure by 3 psi before collection/calibration.

Poor Dispense Quality

If the dispense of any tip is angled, sprayed, or deemed too slow to calibrate, please try the following:

Crystallization On Dispense Tips

The dispense tips can become crystallized if they are primed *and* exposed to air for too long. For this reason, it is typically recommended to close the instrument lid when not in operation.

If the tips becomes crystallized, and therefore clogged, a guitar string or wire can be inserted into the tip to dislodge to obstruction.

With the chamber lid open, insert the guitar string or wire through the bottom of the tip. Push the cleaning tool up through the tip and into the connected tubing.

Blockage in the Fluid Line

If no obstruction is present or the dispense remains poor after cleaning, the tubing before and after the valve should be cleaned.

Begin by attempting to clear the tube of fluid.

1) Close the lid
2) Enter the Priming Screen and turn on the Chamber pressure
3) Remove the bottle that the connected tubing draws from, but tleave the bottle in place as a collection for the contents of the tubing
4) In the “Prime” window, select the affected position and click “Hold to Prime” until the contents of the line are fully removed.
5) Leave the bottle disconnected and/or depressurized

Once the tubing is empty, remove the tubing connecting the dispense valve to the dispense tip. Insert a guitar string or wire through the line fully. Repeat this process for the line behind the valve that leads to the bottle. NOTE: the tubing may of significant enough length that inserting the guitar string or wire up from the bottle position is necessary to clear the line fully.

Poor Gas Seal

The affected position may not be reaching the pressurization target. To diagnose this error, perform the following:

1) Turn the individual gas tap to the affected position off.
2) Disconnect gas from the instrument
3) Watch the pressure for the pathway in question by observering its repective gauge on the side of the instrument. Pressure should not drop more that 1 psi in 1 minute.
4) If the pathway passes inspection, open the individual gas valve on the affected position.
5) If the pressure drops, the seal is compromised.

Replace the seal at the bottle if the affected position is deemed to be compromised.

If the seal is replaced and the error persists, the problem stems from either the individual gas valve or the teflon insert at the affected position. Call Sierra BioSystems for further assistance and/or replacement materials.

Chamber Fails to Pressurize

It is necessary that the Shasta chamber reaches its pressurization target. The synthesis will not proceed unless the pressurization target in the Configuration screen is changed. It is not advised to do this however, as consistent Pulse and Drain times come from consistent chamber pressure.

Chamber O-Ring Needs Grease or Replacement

If the Chamber O-Ring is flat or completely free of grease, the chamber will not properly seal.

Begin by applying a small amount of grease to the perimiter of the O-Ring. Suitable grease is supplied with the instrument. If none is available, contact Sierra BioSystems.

If the problem persists, replace the Chamber O-Ring altogether. Replacing the Chamber O-Ring is part of regular maintenance and should be done at least once annually.

When changing the Chamber O-Ring, apply a small amount of grease over the entire surface of the O-Ring before laying into the chamber’s O-Ring groove.

Obstruction is Present on Main Plate

Check the Main Plate below the lid for debris or obsructions. Small items such as allen wrenches, screws, or fittings can create a gap between the lid and the Chamber O-Ring. Double check the space around the O-ring and hinges.

Insufficient Gas Supplied to the Instrument

To fill the chamber in a reasonable timeframe, a sufficient amount of gas must be supplied to the instrument. It is recommended that 40 psi of Argon gas is delivered to the instrument via ¼" OD x 3/16" ID tubing.

If sufficient pressure is supplied but the "Incoming" pressure guage drops when Chamber pressure is engaged, the gas supply no longer contains enough gas to fill the volume of the chamber. Change the connected tank immediately.

Gas Leaks in Reagent and/or Amidite Pathways

Large gas leaks on the instrument are audible and easily diagnosed. Small gas leaks on the other hand are often not a direct contributor to poor synthesizer performance, but they can add significantly to the expense of operating the instrument. Therefore, it is worth checking the instrument for gas leaks periodically.

NOTE: When diagnosing system leaks, it is always recommended to start with a "known good" and then work forward to locate the problem.

Full System Pressure Test

To perform a full system pressure test, supply gas to the instrument and wait for all bottles to equilibrate. This should take no more than a few minutes.

Once the machine has reached its pressure target and has stabilized, remove gas from the instrument:

1. Close the regulator tap leading to the instrument
2. Disconnect the incoming gas tube (gas remaining in the line will escape).

Now, observe the Reagent and Amidite pressure gauges on the side of the instrument. It is ideal for them to remain steady indefinitely, but most instruments will begin to lose some pressure over time.

A reasonable expectation is for an instrument to hold 1 psi over 10 to 20 minutes. Longer is better, but nascent leaks that do not yet cause greater pressure loss are very time consuming to locate.

If the instrument loses more than 1 psi within the 10 minute alotment, start by noting which pathway is leaking: the Reagents or Amidites.

Reagent Path Leak

If there is a leak in the reagent path, it is most likely explained by a loose cap or a seal in need of replacement. Check these first before proceeding.

The next step is to remove portions of the pathway until the leak is no longer present:

1. Block all gas pathways eminating from the reagent pass-thru. Perform the "Full System Pressure Test" again.
    a. If the system passes, reintroduce the gas lines one at a time and perform the "Full System Pressure Test" between the reintroduction of each line. Once the leaking line is found, replace it.
    b. If the system does not pass, proceed to step 2.

2. At the “T” junction that provides gas to both reagent distribution manifolds. Plug both outputs of the “T” junction. Perform the “Full System Pressure Test” again.
    a. If the system passes, reintroduce the gas lines one at a time and perform the “Full System Pressure Test” between the reintroduction of each line. Once the leaking line is found, replace it *and* the push-to-connect fitting it is attached to.
    b. If the system does not pass, proceed to step 3.
3. Open the left panel of the Shasta Synthesizer. Locate the Reagent argon regulator and plug each connection of the regulator.
    a. If the system passes, reintroduce the gas lines one at a time and perform the “Full System Pressure Test” between the reintroduction of each line. Once the leaking line is found, replace it.
    b. If the system does not pass, contact Sierra BioSystems for a replacement regulator.

Amidite Path Leak

Amidite pathway leaks are typically the result of a loose fitting or a poorly seated bottle. Depending on the solvent used to dilute the phosphoramidite, the seal may also have become voided.

To discern the source of the leak, follow the following steps.

1) Allow the instrument to fill with gas, and then turn all of the amidite bottle gas taps to the “off” position (facing downward). Perform the “Full System Pressure Test” again.
    a. If the system passes, begin turning the amidite gas taps to the “on” position (facing horizontally) and observe the amidite pressure gauge while doing so. The guage should remain steady until the leak source is found. Once the leak source is identified, reseat the bottle, inspect the gasket, and tighten the fittings in the pathway. **NOTE:** If a front amidite bottle is leaking, the left side panel will need to be removed to access the fittings on the 2-way splitter connecting both valves to the single fluid line.
    b. If the system does not pass, proceed to step 2.
2) Open the left side panel of the Shasta Synthesizer. Locate the “Y” push-to-connect fitting that distibutes gas to all bottles and the amidite pressure gauge. Plug the output of the “Y” fitting that leads to the bottles (leave the path connected to the amidite gauge). Perform the “Full System Pressure Test” again.
    a. If the system passes, proceed to step 3.
    b. If the system does not pass, contact Sierra BioSystems for a replacement regulator and check valve assembly.
3) Reintroduce the line distributing gas to the amidites. Locate the “T” push-to-connect fitting at the back of the lid. This will be delivering gas to the specialty amidite positions. Plug both positions. Perform the “Full System Pressure Test” again.
    a. If the system passes, proceed to step 5.
    b. If the system does not pass, the leak source is in the gas path leading to the front amidites. Proceed to step 4.

4) Locate the gas manifold for the front amidites and plug every position with a ¼-28 fitting. Perform the "Full System Pressure Test" again.
    a. If the system passes, plug each of the original gas lines in one at a time, and perform the "Full System Pressure Test" again between each reintrofuction. Once the test fails, the leak source has been located. Replace the gas line leading the bottles and/or gas valve.
    b. If the system fails, there is liekly a loose plug. If all plugs are tightened properly, and there is still a leak, contact Sierra BioSystems for a replacement gas manfiold with a new push-to-connect fitting.
5) Locate the plugged "T" fitting at the back of the lid. Reintroduce each side one at a time. Perform a "Full System Pressure Test" after each line is reintroduced. Once the pathway containg the leak source has been indentified, plug each of the original gas lines in one at a time, and perform the "Full System Pressure Test" again between each reintrofuction. Once the test fails, the leak source has been located. Replace the gas line leading the bottles and/or gas valve.

Liquid Spills/Leaks

Liquid spills inside the chamber will be detected by the leak sensor at the base of the chamber. Leaks outside of the chamber are uncommon, but if they are to occur, they will most likely develop behind the delivery or dispense valves. Check the fittings around the leak area and tighten securely (replace if necessary).

Liquids Inside Chamber

If liquids are collecting at the base of the chamber, there are two explanations: 1) the plate is not fully evacuating the liquids between injections, or 2) a dispense valve is leaking.

Poor Draining If there is improper drainage, check that:

1) the column(s) or plate(s) are seated to the drain block properly. Both should be pressed into the plate tight enough so that they do not fall free when turning the plate upside down.
2) the gasket underneath the column plate is seated properly and free of tears or other imperfections.
3) the 4 screws around the plate are securely fastened. They cannot be overtightened. The "keeper" providing the downward force onto the plate must bottom out to garauntee an effective seal.
4) the drain valves are firing. In Manual Control, fire each drain valve manually and listen for an audible "click" of the valve.
5) the drain tubes connecting the bottom of the drain plate to the back of the chamber are properly fastened.

If all of the above have been checked, then proceed to close the lid and inject fluids into the plate (via a Synthesis Run or Manual Control) and obsserve the drain across the plate. If the above criteria have been satisfied, it is likely that there are one or more columns in the plate that cannot move fluids. Find these columns and dispose of them.

If there is any issue proceeding through the above checlist, note the problem area and contact Sierra BioSystems.

Leaking Dispense Valve

A leaking dispense valve is typically caused by loose fittings between the valve and the dispense tip, excessive backpressure behind the valve, or a voided valve seal (though a voided valve seal is typically evidenced by *no dispense at all*).

1. Begin by tightening the fittings at the front of the valve and the dispense tip. Note any looseness in the fittings.
2. If the leak continues, replace the valve completely with one of the spares provided in the spare parts kit.
3. If the leak persists, introduce a section of smaller ID tubing to the back of the valve to restrict the flow just before the valve. Recalibrate after.
4. If the leak persists, lower the pressure for that pathway until there is no leak. Recalibrate after.

Software Communication Errors

Every piece of hardware inside the Shasta Synthesizer communicates over TCP/IP protocol to the connected computer. The software itself acts as a database for all sequences, protocols, calibration values, etc. Any problem connecting to the instrument or running the software will typically stem from a fault in configuring one of these before operation.

IMPORTANT! The Shasta software provides status indicators for the communication of the "X Controller", the "Y Controller", the "Valve Board", and the "Pressure Sensor". The color green indicates that the connection is properly configured, the color red means that there is no connection, or the hardware is configured incorrectly.

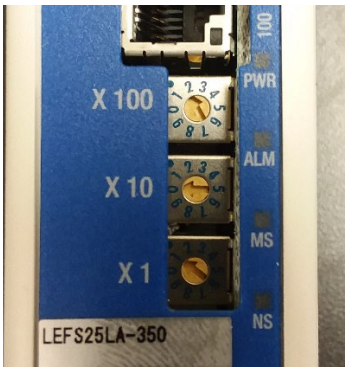
Controller Communication Errors

If either the "X" or "Y" controller is showing a red indicator, then there is no communication between the motion controllers and the Shasta Software. The most likely cause if that the ethernet port on the connected computer is configured incorrectly. To fix this:

1) Open the Network and Sharing Center from the Control Panel on your computer
2) Select "Change adapter settings" located to the left of the window.
3) Select the Ethernet connection and then select "Change settings of this connection"
4) Select "Internet Protocol Version 4 (TCP/Ipv4)" and then select Properties
5) Input the values into the selection pane as shown below:

If this does not resolve the issue, check the IP settings on the controllers inside the Shasta Synthesizer.

1) Set LEFS25LA-350 controller's IP address to 201 via the front dials:



2) Set LEFS25LA-200 controller's IP address to 202 via the front dials.

If this still does not resolve the issue, contat Sierra BioSystems immediately. A replacement actuator may be required if liquids have entered the 12 pin connectors within the chamber.

Pressure Sensor Disconnected

If the Pressure Sensor becomes disconnected, restart the Shasta Synthesizer. It is likely that the Pressure Sensor did not catch the incoming TCP/IP signal upon startup. Power cycling one or two times resolves the issue.

If power cycling does not work, reseting the sensor's IP address may be required:

1) Using an ethernet cable, connect the Shasta and the computer.

2) Run IPSUpdate100.exe (included in installation folder). This is an installer, and it installs a tool from Keyence called "IP Setting Tool". You will get a shortcut for this program on your desktop.
3) Run the IP setting tool. If it doesn't do it automatically, direct it to the network 192.168.1.1.
4) You should now see the NU-EP1 device. Under starting address, it will say BOOTP. Select it and click the "Set IP address" button. ---- If not found, go to Windows Defender Firewall and select “Allow an app or feature…” to allow IP Setting Tool through the firewall. Then restart he IP setting Tool and try again.
5) Set its address to 192.168.1.220.

Valve Board Disconnected

If the Valve Board is disconnected, the internal circuit board controlling the valves has become disconnected from power or its TCP/IP connection.

1) Turn the Shasta Synthesizer off.
2) Check that both the power connection and Ethernet connections are secure.
3) Check the switch that the circuit board is connected to. Make sure that connection is also secure.
4) Turn the Shasta Synthesizer back on.

If there is still a red indicator for the Valve Controller within the Shasta Software, contact Sierra BioSystems.

Cannot Connect to Selected Server

The Shasta software uses what is known as a client/server architecture. The parent program is NexusDB, where NexusDB acts as the server and the Shasta Software is the client. The server stores and manages all the data and the client connect to the server to request and access the data.

The benefit of this architecture is that the server and client do not need to be on the same computer and the server can be used by multiple clients, i.e. one lab could run multiple Shastas on one database. Shastas do not come installed this way however, and establishing this network would have to be done with the assistance of Sierra BioSystems.

When running the Shasta Software, it will need to connect to NexusDB. In standalone mode (standard installation), the IP address that is selected will always need to be 127.0.0.1. This is what is known as the “loopback” address. It is a special IP address that refers to the computer the user is physically using. This is how multiple computers on the same network can all use 127.0.0.1 while keeping individual database information localized to the single, connected computer.

If the software “Cannot Connect to Selected Server”, attempt the following:

1. Try rebooting the computer first. If that doesn’t help, then continue.
2. Open the Services Control Panel
    a. Right-click on the Start Menu button.
    b. Select Run.
    c. Type **services.msc** and click OK.
3. Find the service called NexusDB Server V3.

4. If the Status column is blank, then right-click on the service and choose Start. Skip the remaining steps below and run Shasta.
5. If the Status column says Running, then right-click on the service and choose Stop.
6. Look for a folder called "NexusDB3" inside your C:\ProgramData folder and delete it. Only delete the folder called NexusDB3.
   The C:\ProgramData folder is hidden so you may have to tell Windows to show hidden files and folders. If you open File Explorer in Windows and go to the View tab on the ribbon, there should be a checkbox for Hidden Items that you can check to make the C:\ProgramData folder visible.
7. Go back to the Service control panel and right-click on NexusDB Server and choose Start.
8. Run Shasta. It should ask you if you want to create a new Shasta database. Choose the option to create a new database.
9. You might get an error message. Click OK and run Shasta again.

If the Server does not appear, you may enter it manually:

1. Click the green + icon to add a new record.
2. Enter 127.0.0.1 for the Server Name
3. Enter 127.0.0.1 for the IP Address
4. The Hostname should be left blank
5. Choose LAN / WAN for the Location
6. Click the Connect to Selected Server

If there is a continued issue connecting to NexusDB, then NexusDB must be stopped and started again or reconfigured. Contact Sierra BioSystems directly before attempting to reconfigure NexusDB.

For any questions or concerns, please contact:

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