Takara Bio USA

Shasta™ Single Cell System User Manual

Cat. No. 640282
(071825)

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I. Introduction

The **Shasta Single Cell System** (Cat. No. 640282, referred to as the Shasta system in this manual) is designed to dispense samples into chips, perform imaging on the dispensed sample, and then dispense downstream reagents into the wells identified by the imaging as samples of interest.

Figure 1. The Shasta Single Cell System. For more information about the hardware, see Section IV.

This manual provides instructions for the safe operation and maintenance of the Shasta system. This manual also includes instructions for using the Shasta CELLSTUDIO™ Software.

Review the information in this manual and the documentation supplied with the accessory equipment you are using thoroughly before starting your reactions. If you require technical support, you can contact your authorized Takara Bio service technician or field_support@takarabio.com.

Symbols and Conventions

The following symbols and conventions are used throughout this manual.

Table 1. User manual symbols and conventions

Symbol	Description
	**WARNING:** Indicates a potentially hazardous situation that could result in injury to the user or damage to or destruction of the system.
	**CAUTION:** Indicates a hazard that could result in loss of data or damage to the system.
	Indicates the presence of an electrical shock hazard. Proceed with caution. This symbol may appear next to either a WARNING or CAUTION.
	Indicates the presence of a biological hazard. Proceed with caution. This symbol may appear next to either a WARNING or CAUTION.
	Indicates the presence of a mechanical or pinch hazard. Proceed with caution. This symbol may appear next to either a WARNING or CAUTION.
!	**IMPORTANT:** Provides information on proper system operation.
NOTE:	**NOTE:** Provides helpful ancillary information to support the use of the system.

II. Safety Information

Refer to safety guidelines in the user manuals for all equipment used in this protocol.

Table 2. Shasta Single Cell System safety guidelines.

CAUTION: There are no user-serviceable parts inside the instrument. Service of any internal parts should be performed by a qualified Takara Bio service technician.

Operating conditions:

The instrument is only safe to operate with the covers in place and the doors closed. The covers and doors protect the user from moving parts and electrical shock and must not be removed during operation. If this equipment is not used as specified by the manufacturer, the protection provided by this equipment may be impaired.

Operate the Shasta system only inside an appropriate building. Do not operate the Shasta system outside or in wet environments. Doing so could cause damage to the instrument or harm to the user.

Instrument use:

WARNING: Use of the Shasta system requires users to wear appropriate personal protective equipment (PPE), which should, at minimum, include gloves, eye protection, and a lab coat. However, the choice of PPE used should be dictated by the biosafety level of the biological samples being introduced into the Shasta system. Please consult your institutional biosafety committee for additional information on the necessary precautions for your sample type.

WARNING: Class I Equipment: This equipment must be grounded. The power plug must be connected to a properly wired grounded outlet. An improperly wired outlet could place hazardous voltages on accessible metal parts.

CAUTION: Do not position the equipment so that it is difficult to operate the power switch or remove the power cord.

WARNING: Use only the power cord provided by the manufacturer. Do not replace the power cord with an inadequately rated cord.

Moving the system:

WARNING: If you need to move the system after it has been installed, please contact Takara Bio service technician or field_support@takarabio.com.

III. List of Components

The complete Shasta Single Cell System (Cat. No. 640282) includes the components listed below.

Table 3. Shasta Single Cell System components.

Shasta Single Cell System (Cat. No. 640282)	
Component	**Quantity per kit**
Shasta Single Cell Crate Components (Cat. No. 640284)	**1**
Shasta Single Cell (Cat. No. 640285)	1
Shasta CELLSTUDIO Software	

Shasta Single Cell System (Cat. No. 640282)	
Shasta CellSelect® Software*	
Single-Cell Thermal Cycler (Cat. No. 640002)	1
Barcode scanner	1
Mobile scissor lift	1
Regional Power Cord	3
Universal Power Strip	1
Single-Cell Accessory Kit (Cat. No. 640194)	1
2 x Chip Balance	
1 x 384-Well Plate Balance	
2 x Nanodispenser Centrifuge Chip Spinner	
1 x Nanodispenser Cold Block	
1 x Nanodispenser Chip Blotter	
2 x Nanodispenser Chip Holder	
2 x Single-Cell Extraction Fixture	
1 x Magnetic Tube Stand	
1 x 384-Well Plate Seal Applicator	
Shasta Installation Kit (Cat. No. 640293)	1
1 x 45 ml Nanodispenser Imitation Mastermix	
5 x Nanodispenser Alignment Chip Film	
10 x Blotting Paper	
3 x 384-Well Source Plate Seal	
3 x 384-Well Source Plate	
Single-Cell Loading Kit* (Cat. No. 640206)	**5**
7 x Blotting Paper	
1 x Chip Freezing Film	
6 x RC Film	
Single-Cell Collection Kit* (Cat. No. 640212)	**5**
1 x Single-Cell Collection Fixture	
2 x Collection Tube (2.0 ml)	
1 x Collection Film	
Nanodispenser 384-Well Source Plate and Seal* (Cat. No. 640018)	**1**
1 x 384-Well Source Plate (20/Pack)	
2 x 384-Well Source Plate Seal (10/Pack)	
Single-Cell 350v Chip* (Cat. No. 640019)	**2**
1 x Single-Cell 350v Chip, 5,184 Wells, 2.2 mm (350 nl)	
Single-Cell 250f Chip* (Cat. No. 640193)	**3**
1 x Single-Cell 250f Chip	
Second Diluent 100X* (Cat. No. 640016)	**1**
1 x 100 µl Second Diluent 100X	
SMART-Seq® Pro Reagent Kit (Cat. No. 640259)	**1**
1 x 10 µl Control K-562 RNA (1 µg/µl)	
1 x 25 µl Second Diluent (100X)	
1 x 25 µl BSA (1%)	
1 x 50 µl RNase Inhibitor (40 U/µl)	
1 x 5 µl SMART-Seq Pro CDS (100 µM)	
1 x 20 µl SMART-Seq Pro Oligonucleotide (100 µM)	
1 x 200 µl SMART-Seq Pro RT Buffer	
1 x 40 µl SMART-Seq Pro Lysis Buffer	
1 x 80 µl SMARTScribe™ Reverse Transcriptase (200 U/µl)	
1 x 60 µl SMART-Seq Pro PCR 1 Buffer	
1 x 90 µl Terra™ PCR Direct Polymerase Mix (1.25 U/µl)	
1 x 10 µl $MgCl_2$	
1 x 100 µl SeqAmp™ CB PCR Buffer (2X)	

Shasta Single Cell System (Cat. No. 640282)	
1 x 40 µl 5X Primer Mix	
1 x 350 µl Elution Buffer (10 mM)	
1 x 500 µl Nuclease-Free Water	
1 x 20 ml SMART-Seq Pro Index Resuspension Buffer	
1 x 220 µl SMART-Seq Pro Tagmentation Buffer	

*Component is also sold separately.

IV. Overview: Shasta Single Cell System

A. Main Components

Figure 2. The Shasta Single Cell System (external view).

The Shasta system includes the following main components:

[A] Shasta Single Cell—referred to as the Shasta instrument in this manual.

[B] Touch-screen monitor, wireless mouse, and keyboard

[C] Single-Cell Thermal Cycler

[D] Waste container—the waste container collects the fluid that is discarded through the tip nozzles into the waste well of the tip wash trough. The container sits outside of the Shasta Single Cell.

[E] Handheld barcode reader—it is recommended to use the barcode reader provided with the system for best results.

B. Electrical Inputs and Outputs

On the back side of the Shasta Single Cell there are 5 ports. The top 2 ports are USB 2.0 ports intended for the computer touch-screen input, and the handheld barcode reader. The third port from the top is an HDMI port for the computer monitor. The fourth port from the top is an ethernet port for network connection. At the bottom is the plug for power as well as the power switch.

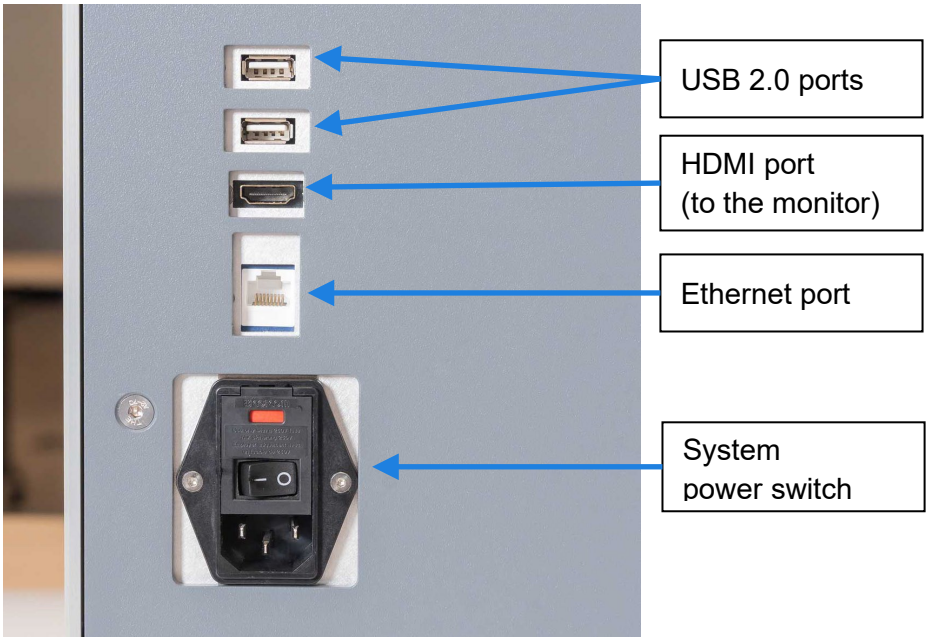


Figure 3. Identification of the electrical input and output on the back of the Shasta Single Cell.

The USB dongle for the mouse and keyboard are plugged into the back of the monitor in the USB type-A port.

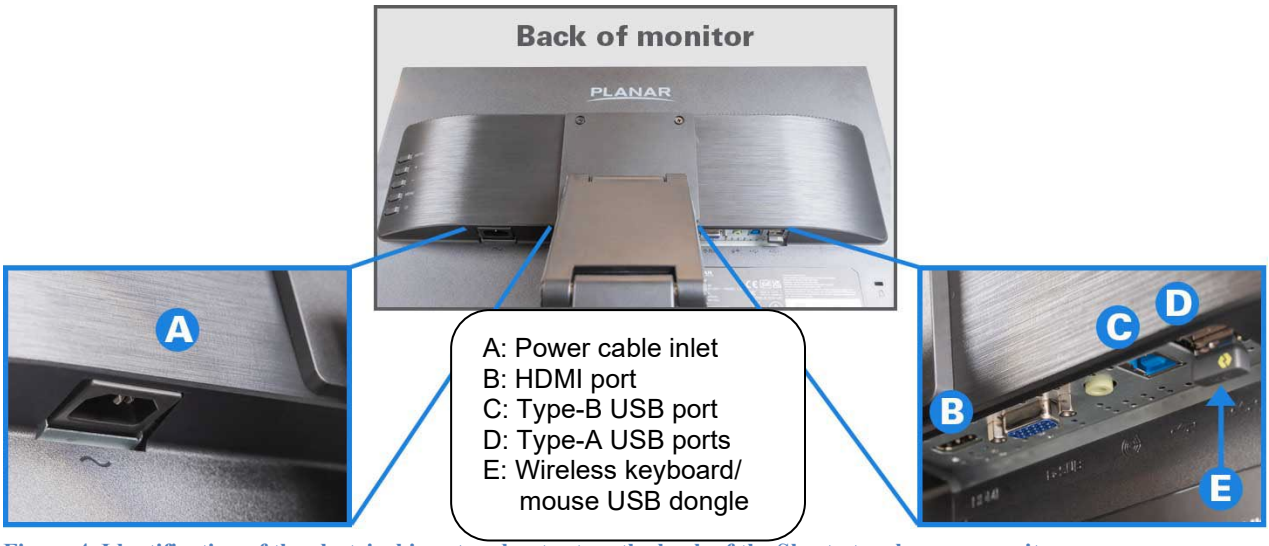


Figure 4. Identification of the electrical input and output on the back of the Shasta touchscreen monitor.

In the front right side (under the product name) is a USB 2.0 port for the user to upload/download data (Figure 5).

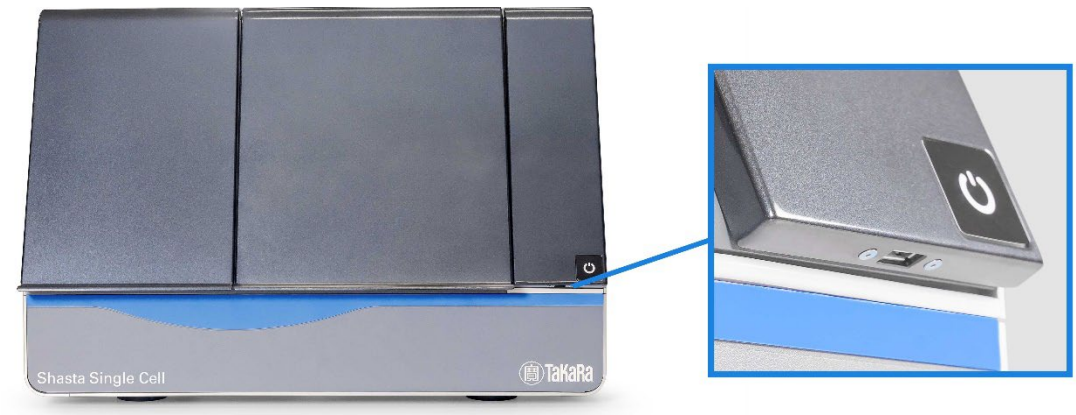


Figure 5. Location of the USB 2.0 port located on the front of the Shasta Single Cell.

C. Internal Components

The following section describes the internal components of the Shasta Single Cell:

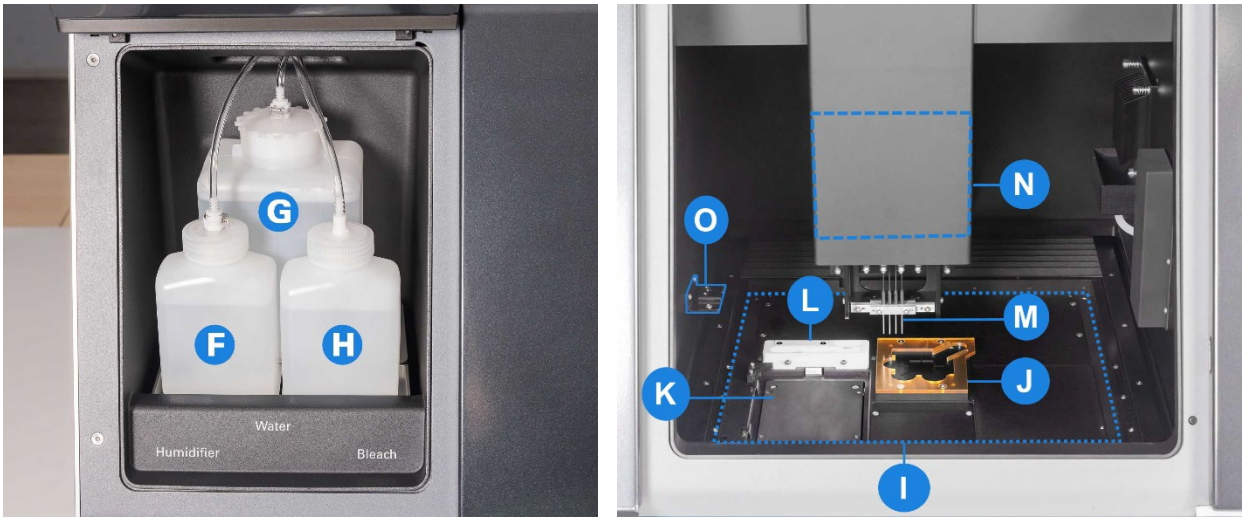


Figure 6. The Shasta instrument (internal view).

Behind the left-hand door:

- Fluidic Source bottles (Figure 6, left)
  [F] Humidifier Source bottle (500 ml capacity)
  [G] Water Source bottle (2 L capacity)
  [H] Wash Source bottle (500 ml capacity)

The right side of the Shasta Single Cell:

- Behind the right-hand door, the environmental chamber (Figure 6, right):
  [I] Dispensing Platform (the XY Stage)
  [J] Chip nest (orange square), with active cooling
  [K] 384-well plate nest (gray square), with active cooling
  [L] Tip wash trough (white)
  [M] Tip nozzles
  [N] Optics module (mounted to the Z axis)—not visible, but indicated by the dotted line
  [O] Temperature/humidity sensor

Figure 7. The Shasta Single Cell (internal view), with a focus on the right-side.

- Right-hand side of the Shasta Single Cell, internal and external features (Figure 7):
  [P] Environmental control hardware (humidifier, dehumidifier, catch tray)
  [Q] Door lock & door sensor
  [R] LED status indicator
  [S] Power touch-button

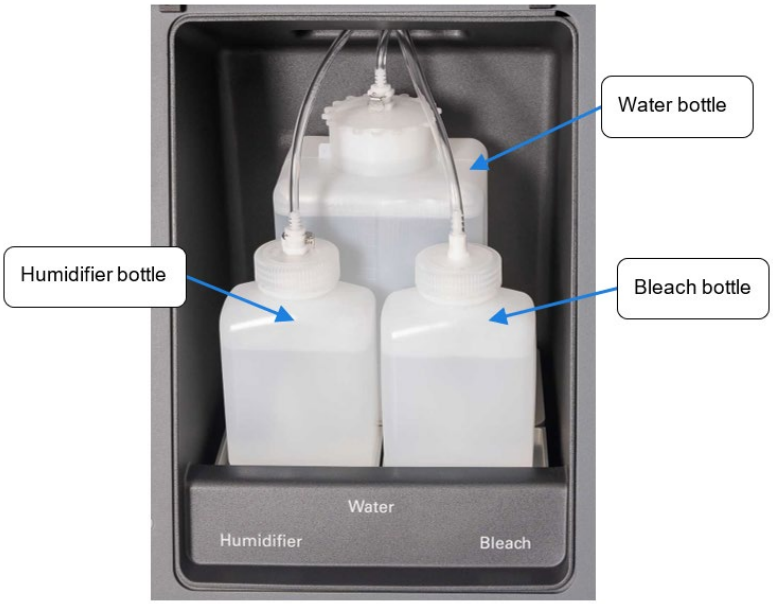


Figure 8. The Shasta Single Cell (internal view), source bottle positions.

Water source bottle (2 L capacity)

The water source bottle should be filled with deionized filtered water (Milli-Q or Elga system or equivalent; 0.2 µm filtration) and this water is used for rinsing the dispenser tips. The water reservoir sits on a scale that monitors the water level so that the system can alert the users prior to starting a chip-dispense operation if the bottle needs to be refilled.

Bleach source bottle (500 ml capacity)

The bleach (wash) source bottle is the source for the wash well in the tip wash trough, and it sits to the front-right of the water source bottle. It should be filled with a 0.2% sodium hypochlorite solution, which is used for cleaning the tip nozzles between dispense rounds to prevent cross-contamination. Like the water source bottle, it sits on a scale so the system can alert users if the bottle needs to be refilled before a dispense.

Humidifier source bottle (500 ml capacity)

The humidifier source bottle is to the left of the wash source bottle and sits on a scale for fluid level monitoring. Like the water source bottle, the humidifier source bottle should be filled with deionized filtered water. The humidifier module itself is located behind the front-right panel (Figure 9, below).

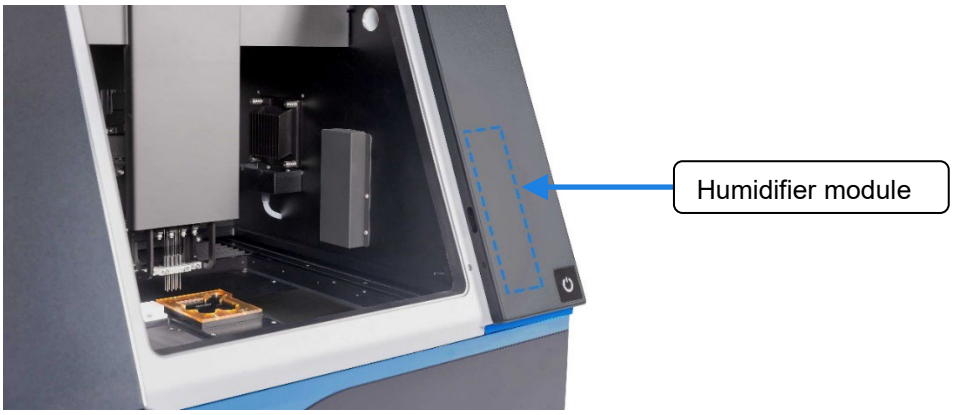


Figure 9. Humidifier module. The dotted-line box indicates the approximate location of the humidifier assembly under the right-side cover.

Dispensing Platform:

The dispensing platform consists of the cooled chip nest and 384-well plate nests, and a tip wash trough. It sits upon an XY Stage, which moves the entire platform underneath the tip nozzles. The tip nozzles are raised and lowered by an independent Z axis.

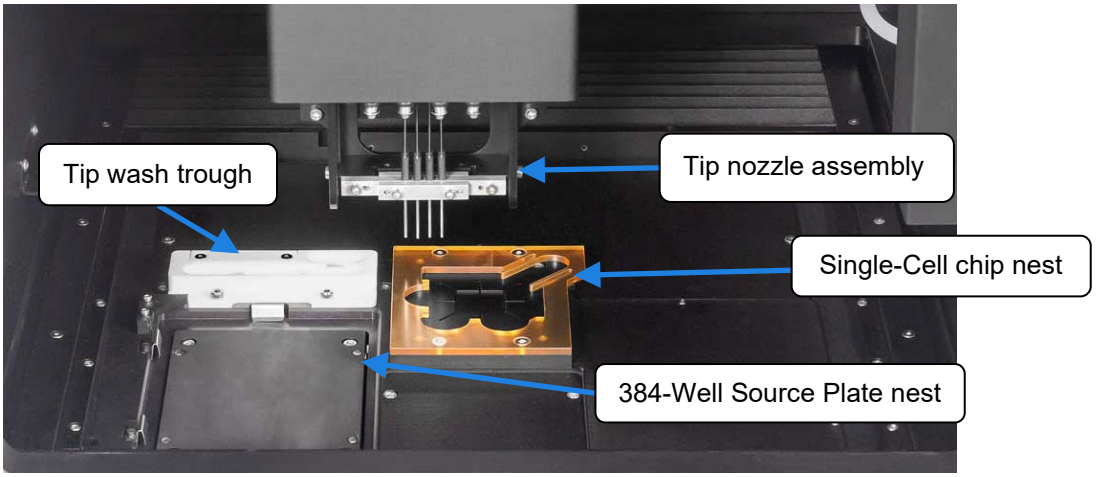


Figure 10. Shasta dispensing platform.

Single-Cell chip nest and 384-well source plate nest

The chip nest (center) and the 384-well plate nest (left) are both actively cooled. The 384-well source plate nest is at a fixed temp (the fluid in the wells will be around 13–15°C), and the chip nest control targets 10°C but will allow for a few degrees deviation in order to maintain dewpoint (no evaporation or condensation of the fluid in the wells).

Tip wash trough

The trough consists of four wells. From left to right they are the "Wash," "Waste," "Secondary rinse," and "Primary rinse" wells. The rinse wells are used for rinsing the inside and outside of the tip with system water and drains to the waste container. The waste well is also connected to the waste container and is where any excess system fluid, reagents, or samples are discarded. The wash well is connected to the wash bottle (which contains 0.2% sodium hypochlorite solution) and used for washing the inside and outside of the tips with the solution.

Figure 11. Tip wash trough well locations.

Tip nozzle assembly and optics

The XY stage houses the chip nest, 384-well plate nest and tip wash trough. The Tip nozzle assembly and optics modules are mounted to the Z axis. The XY stage brings the platform to the desired locations and the Z axis raises and lowers the tips to the desired height for aspiration, dispense, and wash steps. When the door to the stage module is closed, it creates an environmental chamber that allows the system to control the chamber to the optimal humidity levels during sample and reagent dispensing.

Prior to any dispense or scan, the optics module confirms the correct orientation of the chip and identifies the location of the wells. Samples are aspirated from a 384-well plate that sits in the source plate nest and then dispensed into a chip that sits in the chip nest. The tips are brought to the trough to discard excess fluids to waste and to perform tip cleaning. After a sample is dispensed, the optics module scans the chip to generate a map (i.e., filter file) of the wells that contain single cells. The dispensing process is repeated for all downstream reagents, but only dispense into wells flagged in the filter file (if used).

Temperature and humidity sensor

The temperature and humidity is mounted on the inside chamber on the left wall. It is used to monitor the chamber environmental conditions so that the environmental control system can minimize the evaporation or condensation on the chip during dispensing (described below).

Humidifier and dehumidifier modules

The humidifier and dehumidifier modules are the two environmental control units. Based on the temperature and humidity sensor readings, the environmental control system adjusts the humidity in the chamber higher or lower to keep the chip at the condition that minimizes reagent evaporation or condensation during the dispensing process. The control system also attempts to maintain the chip temperature of 10°C but may be a few degrees higher or lower while the humidity conditions stabilize.

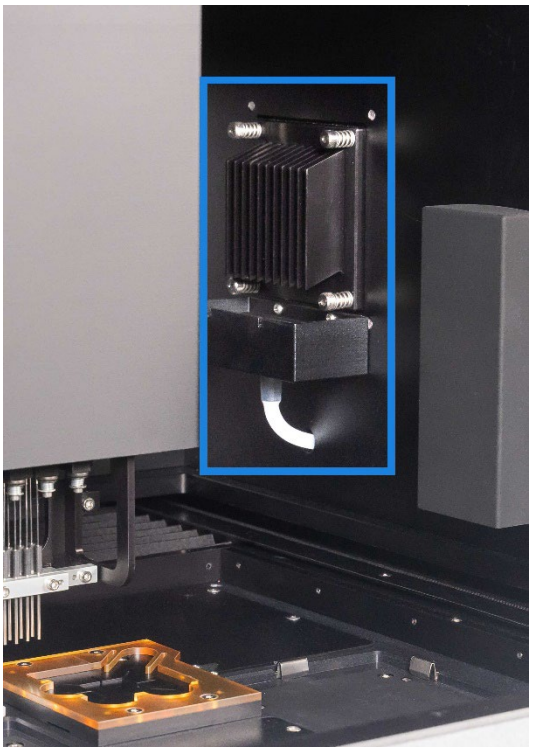


Figure 12. Dehumidifier module.

NOTES: Condensation may be visible dripping from the fins of the dehumidifier module into the trough below. This is expected behavior and means the dehumidifier is functioning as designed.

Door lock and door sensor

The door lock and door sensor are used to ensure safe operation of the Shasta Single Cell. The software will not proceed if it does not sense that the door is closed, and once a dispense operation is started, the door lock prevents users from opening the door and accessing the machine while parts are in motion.

LED Status indicator

The LED Status indicator is in the front-right side of the machine and provides a visual indicator of the status of the Shasta Single Cell. A blue light means that the system is idle. The light turns green during operation. An error status is indicated with a yellow light.

Power touch-button

After the system is powered on from the switch in the back, the system will still be in sleep mode. The power button will be slowly blinking. To wake up the system and start the software, press and hold the power touch-button for 1 sec.

Figure 13. Software power button.

D. Specifications and Lab Requirements

The Shasta Single Cell System requires use of both Shasta Single Cell (Cat. No. 640285) and Single-Cell Thermal Cycler (Cat. No. 640002). Specifications for each product are listed in Table 4 and Table 5.

Table 4. Shasta Single Cell specifications and lab requirements.

Category	Specification
Dispense volume	35–150 nl per nanowell, in 5 nl increments
Software	Shasta CELLSTUDIO Software (preinstalled) Shasta CellSelect Software (preinstalled)
Computer	Enterprise-level Windows 11 PC (included with system)
Power requirements (for different electrical grid types)	**120 VAC/60 Hz mains:** two 15 or 20A outlets **220–240 VAC/50 Hz mains:** two 10A outlets **100 VAC/50–60 Hz mains:** two 15A outlets
Fuses	T 10A H, 250V, 5 x 20mm type
Environmental conditions	Ambient temperature: 18–26°C Temperature variability < 10°C per 10 min Relative humidity, noncondensing: 10–80% RH% variability <10 percentage points per 10 min Altitude: <2,000 m above sea level Dust level: ISO 9 Vibration level: Standard for digital imaging (<12.5um/s VC-C)
Dimensions	32" W x 22" H x 24" D (81 cm x 54 cm x 60 cm)
Minimum required bench space (instrument and monitor)	Includes clearance for Shasta instrument and monitor: 54" W x 26" H x 30" D (138 cm x 66 cm x 76 cm) *Note: Bench space must be capable of supporting 400 pounds (180 kg)*
Floor space	Waste container: 10" W x 10" H x 10" D (25 cm x 25 cm x 25 cm)

Category	Specification
Weight	240 pounds (109 kg)
Minimum space to back wall (for ventilation)	4" (10 cm)

Table 5. Single-Cell Thermal Cycler specifications and lab requirements.

Category	Specification
Power requirements (for different electrical grid types)	**120 VAC/60 Hz mains:** one 15 or 20A outlets **220–240 VAC/50 Hz mains:** one 10A outlets **100 VAC/50–60 Hz mains:** one 15A outlets
Minimum required bench space	11" W x 17" H x 21" D (28 cm x 44 cm x 54 cm)
Weight	20 pounds (9 kg)

E. Setup and Installation

Your Takara Bio Service Engineer will unpack and install your Shasta Single Cell and explain the basic operation of the system. They will use material from the Shasta Installation Kit, shipped with your system, to qualify the system after installation.

NOTE: To avoid contaminating your PCR, do not install the Shasta Single Cell in an area that could contain high-copy DNA or amplicons from previous PCR experiments.

F. Required Reagents and General Lab Equipment

Reagents for Shasta Reservoirs

- Deionized filtered water (Milli-Q or Elga system or equivalent; 0.2 µm filtration)
- 0.2% sodium hypochlorite solution made from reagent-grade sodium hypochlorite in deionized filtered water

Other Reagents and Materials

- 70% isopropanol or equivalent (for example, 70% Reagent Grade Alcohol [VWR, Cat. No. 89370-078]) for priming the dispenser tips.
- Refer to application-specific user manuals for details about required samples and reagents.

General Lab Equipment

- Secondary containers (e.g., 2 L or 500 ml bottles) for refilling reservoirs
- Kimwipes or similar dry wipes for cleaning the stage module
- Refer to application-specific user manuals for any other required equipment

G. Nanowell Technology

Nanowell chip technology distinguishes Takara Bio's platform from other systems. Each Single-Cell chip has a 72 x 72 array of nanowells and can accommodate up to 5,184 reactions in a single run. The chip is engraved with a unique number (Figure 14) that is used to link your chip images and other experimental record files.

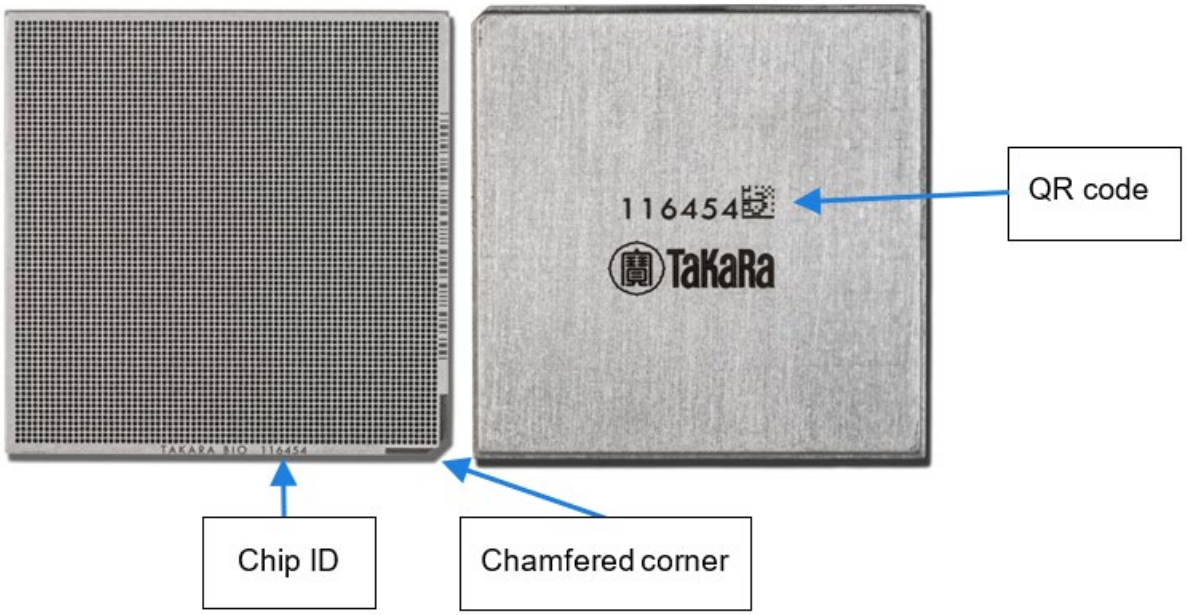


Figure 14. Single-Cell chip features. **(Left)** Top view of aSingle-Cell chip. Note the chamfered (beveled) corner at the bottom right. The "TaKaRa" logo and the chip ID (unique to each chip) are engraved on the chip border, near the chamfered corner. **(Right)** Bottom view of a Single-Cell chip. The chip ID is also engraved on the reverse side of the chip with a corresponding QR code that can be scanned by a barcode reader, allowing for the chip ID to be easily entered into the software.

The Shasta instrument transfers samples, controls, and reagents from a 384-well plate to the chip. After dispensing, the chip can be processed for cell analysis (Figure 15) or additional reagent or index dispenses.

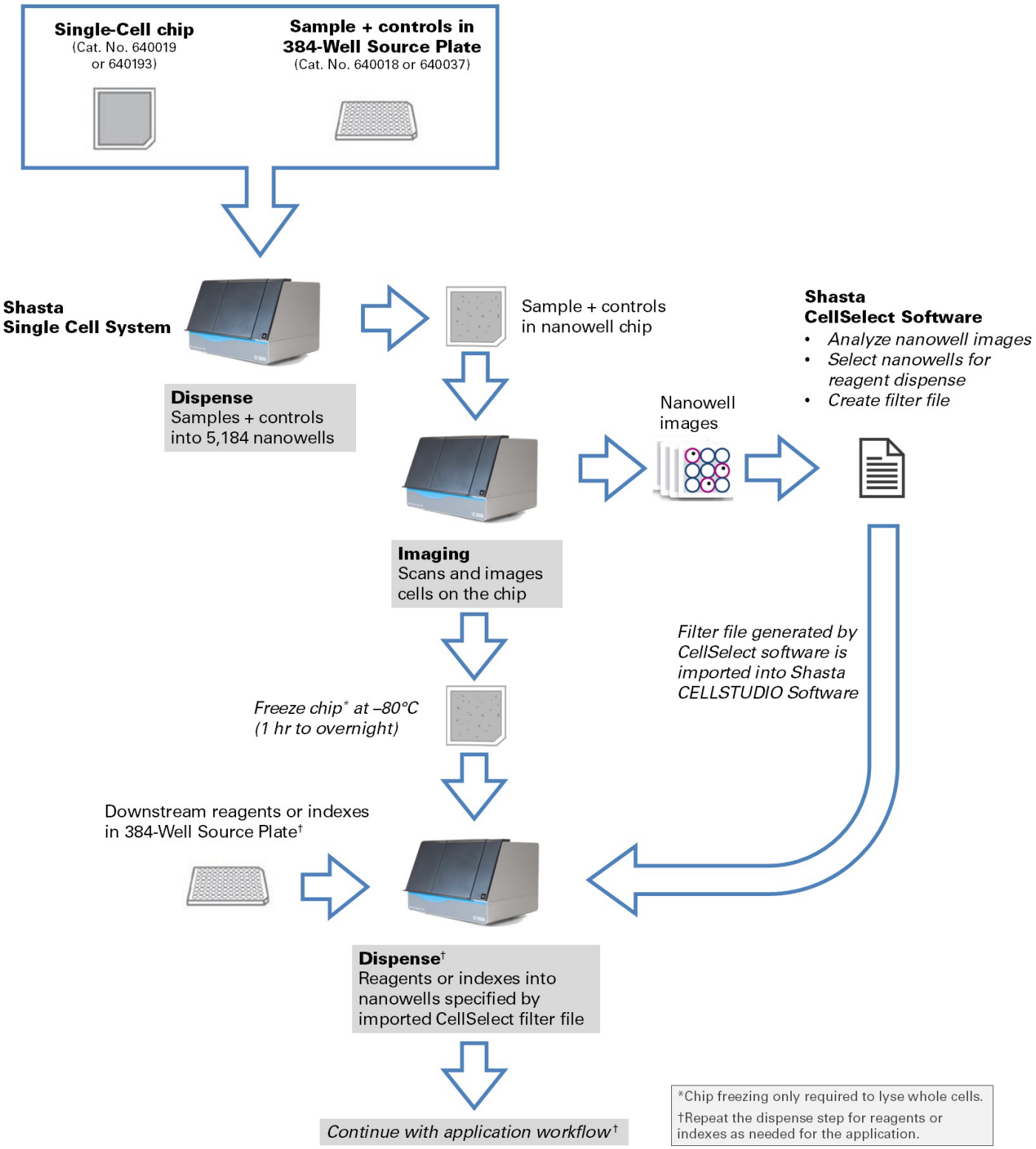


Figure 15. Shasta dispense and cell scan workflow.

V. Overview: Shasta System Software

The Shasta Single Cell System comes preinstalled with two software packages, Shasta CELLSTUDIO Software (referred to as CELLSTUDIO software) and Shasta CellSelect Software (referred to as CellSelect software). Together, the software provide a guided workflow to execute a single-cell experiment.

A. CELLSTUDIO Software

CELLSTUDIO software is the main application software for instrument controls including cells/reagents dispensing and cells imaging. All experiments are organized and saved as independent jobs derived from preplanned applications (apps). The basic operations are described in the following sections. For more advanced functionality, see the Shasta Single Cell Advanced Features User Manual.

1. Starting CELLSTUDIO Software

1. To start CELLSTUDIO, first ensure that the Shasta system is powered ON by toggling the power switch on the back of the instrument (Figure 3).
2. Next, **press and hold the power button for 1 sec** until the LED status indicator light changes from blue to green, indicating the software is initializing. On software startup, the XY and Z axes, dispenser hardware, and optics module will automatically initialize on the system. On the monitor, the initialization splash screen (Figure 16) will display.

Figure 16. Example CELLSTUDIO initialization screen.

3. Upon successful completion of the system initialization, the LED status indicator will change back to blue, and you will be presented with either *Action Center* view (Figure 17), if there are any system actions required, or the *Home* screen (Figure 24) if there are not.

2. Action Center

The *Action Center* displays a list of action items that must be addressed before using the system.

For a comprehensive list of potential action items, please refer to Table 9 in Appendix E, "Troubleshooting Guide".

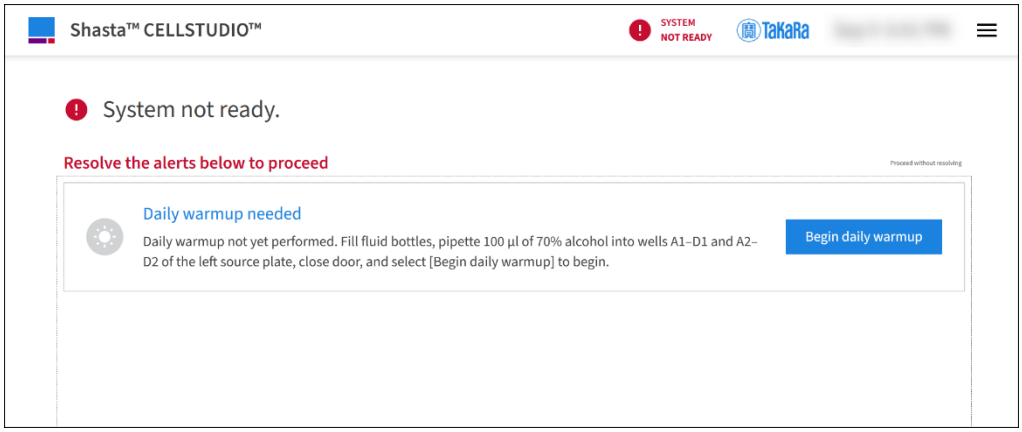


Figure 17. An example *Action Center* view.

Typically, an action item includes:

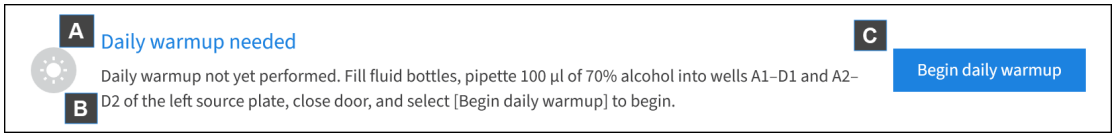


Figure 18. An example action item.

A. **Title**: A brief description or name of the required action.

B. **Instruction**: A detailed explanation of the action needed to resolve the item.

C. **Action button**: A button that, when pressed, performs the necessary action.

3. Title Bar

The title bar on the *Action Center* and *Home* screens display useful information such as system status, current date and time, and more options (Figure 19).

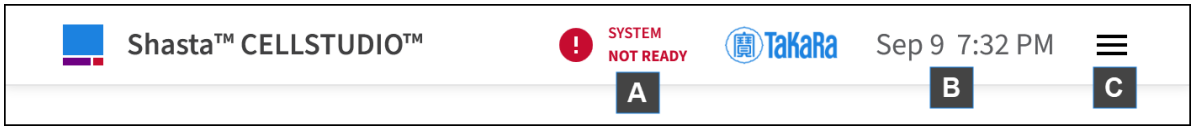


Figure 19. *Home* screen title bar. A. System status indicator. **B.** Current date and time. **C.** The Options menu icon.

On all other pages, the title bar also includes a [Home] icon (see Figure 20). Clicking on the icon will return you to the *Home* screen.

Figure 20. Title bar displayed on screens other than *Action Center* and *Home*. The [Home] icon towards the left side of the title bar is highlighted by a blue square.

4. Experiment Steps

Executing through the steps are accompanied by the easy-to-follow on-screen instructions. In general, the view of the experiment step is generally divided into five regions (Figure 21).

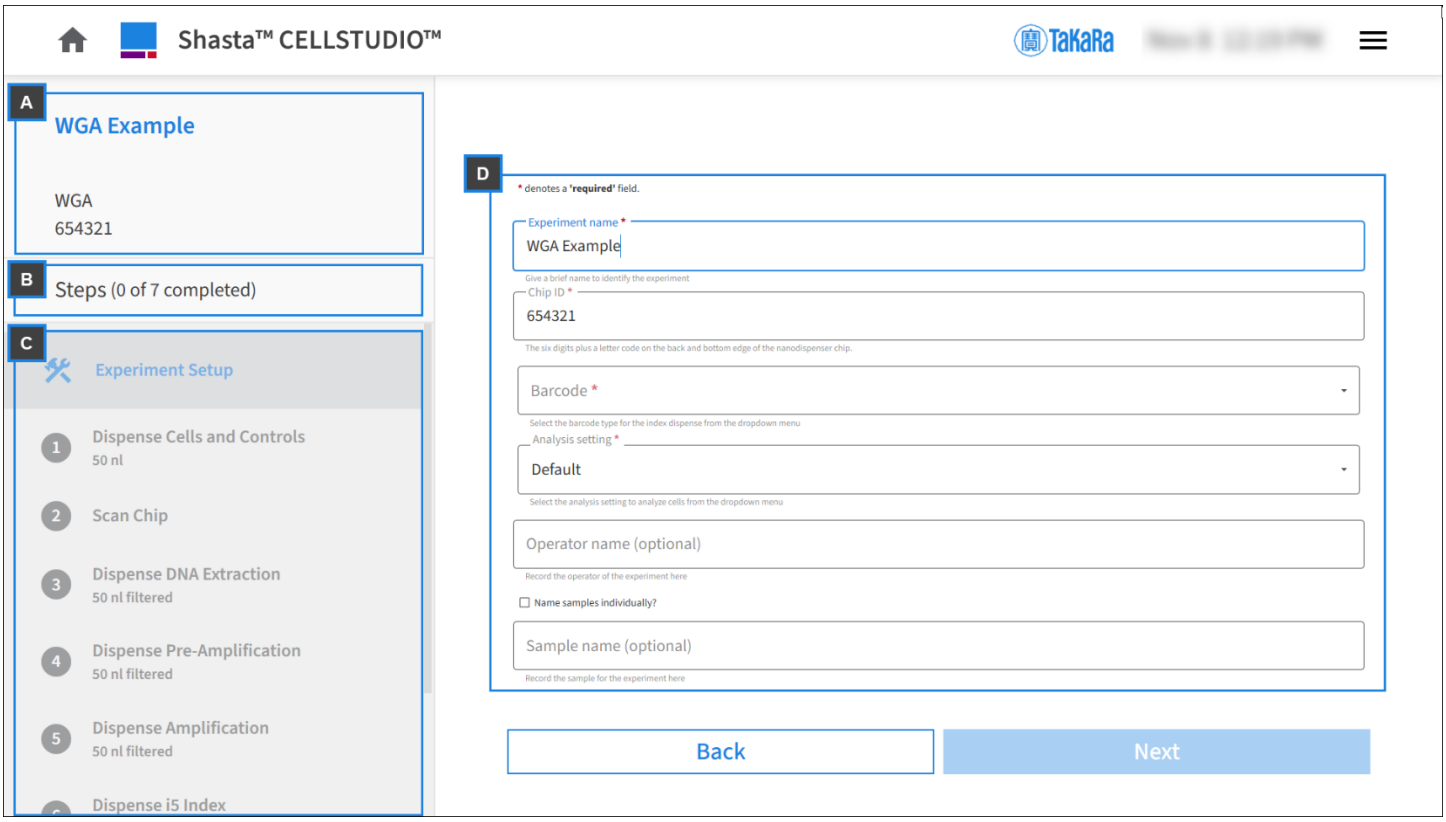


Figure 21. Example Experiment Step View.

[A] Presents an at-a-glance summary of the chosen experiment, detailing essential information for quick reference. In the example of Figure 21, "WGA example" correlates to the Experiment name field (in [D]), "WGA" is the selected application for the experiment, and "654321" is the chip ID.

[B] Shows the current completion status of the experiment, providing a visual indicator of progress.

[C] Enumerates all the procedural steps of the experiment, functioning as an interactive guide for step-by-step navigation. In Figure 21, this area is grayed out because no "Barcode" value has been selected yet; it will resemble areas [A] and [B] once a barcode option has been selected.

[D] Adapts its content to reflect the information pertinent to the active step of the experiment. In addition, it provides step-by-step, on-screen instructions. Figure 21 illustrates what this region will look like during the "Experiment Setup" step, with input field prompts to associate information to the experimental record

5. Dispense Step Summary View

After a dispense step has completed, the icon next to the step name in the left-side menu changes from a number to a check mark (see highlighted step in Figure 22, left, and (4) below the highlighted step). If the completed step is selected by clicking on it, the view that returns (Figure 22, right) displays a short summary of information relating to the metrics of the run.

This information includes:

- The name of the dispense step, from the definition of the application.
- The dispense volume (per well) used for this dispense step.
- Whether the dispense was restricted by a filter file (filtered) or not (unfiltered). For filtered dispenses, the name of the filter file used is also listed with an [Open file] icon to the right of the name. When clicked on, it will open the actual filter file in the program configured on the Windows server to view CSV-format files (e.g., MS-Excel or Notepad).
- The time and date when the dispense started and ended.
- How long the dispense run took from beginning to end, in hh:mm:ss format (hr:min:sec). This value can help you to estimate how long future dispenses in the experiment might take when configured identically (filtered/unfiltered) or in scheduling similar experiments in the future.

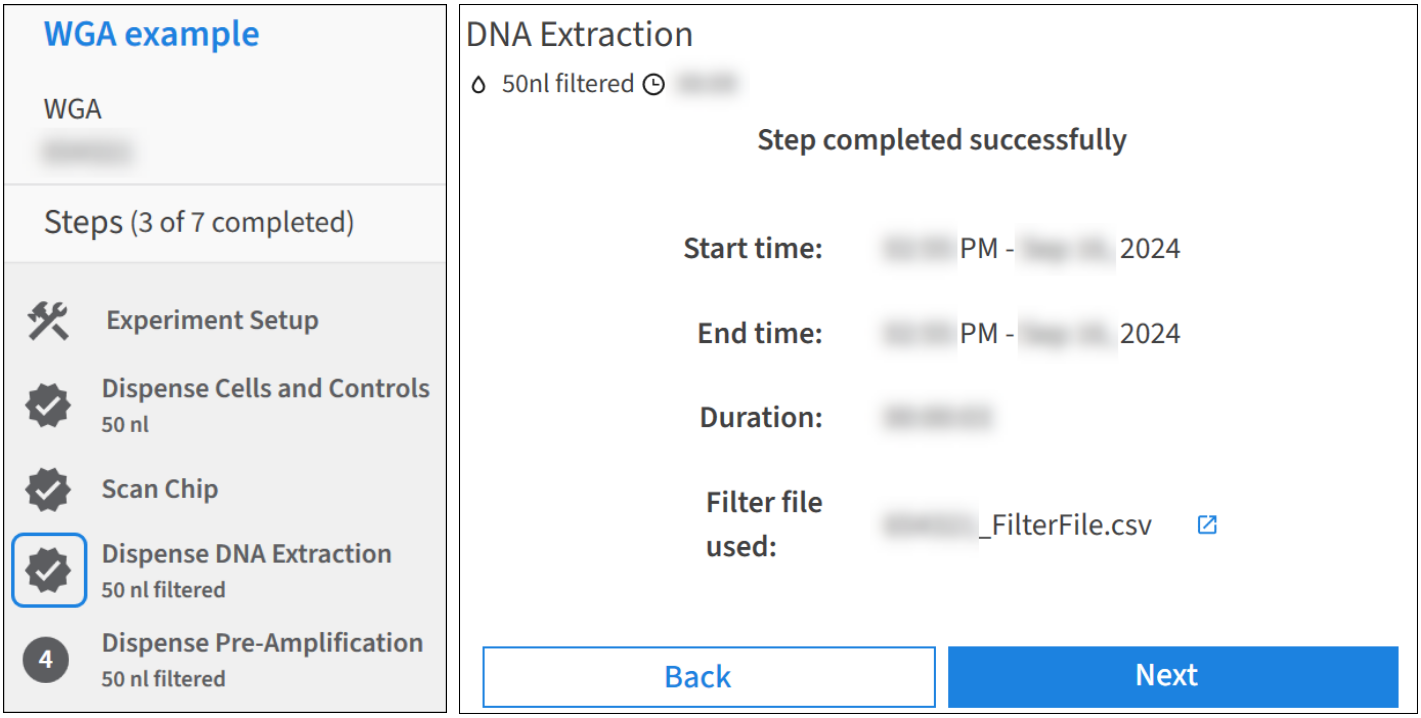


Figure 22. Example summary of a completed dispense step in a workflow. **(Left)** The left-side menu of experiment steps. The icon highlighted by the blue box indicates a completed dispense step. **(Right)** The summary view for the completed Dispense DNA Extraction step.

6. Experiment Summary Page

After the sample dispense step (Section IX) of an application workflow, the **Summary** menu item becomes an active choice at the bottom of the list of steps.

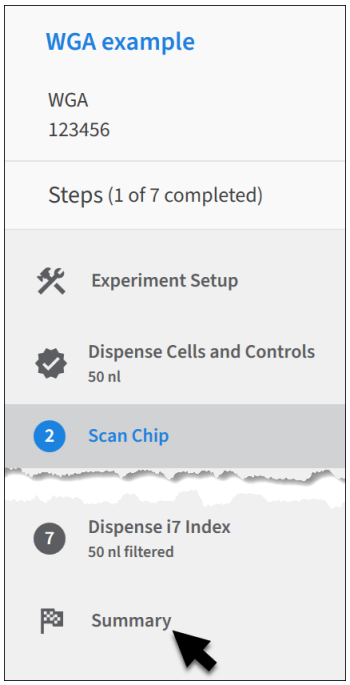


Figure 23. Summary menu option in an application workflow.

The *Summary* page is the user interface for interacting with the results files of the Shasta instrument. A brief description of these files is summarized below.

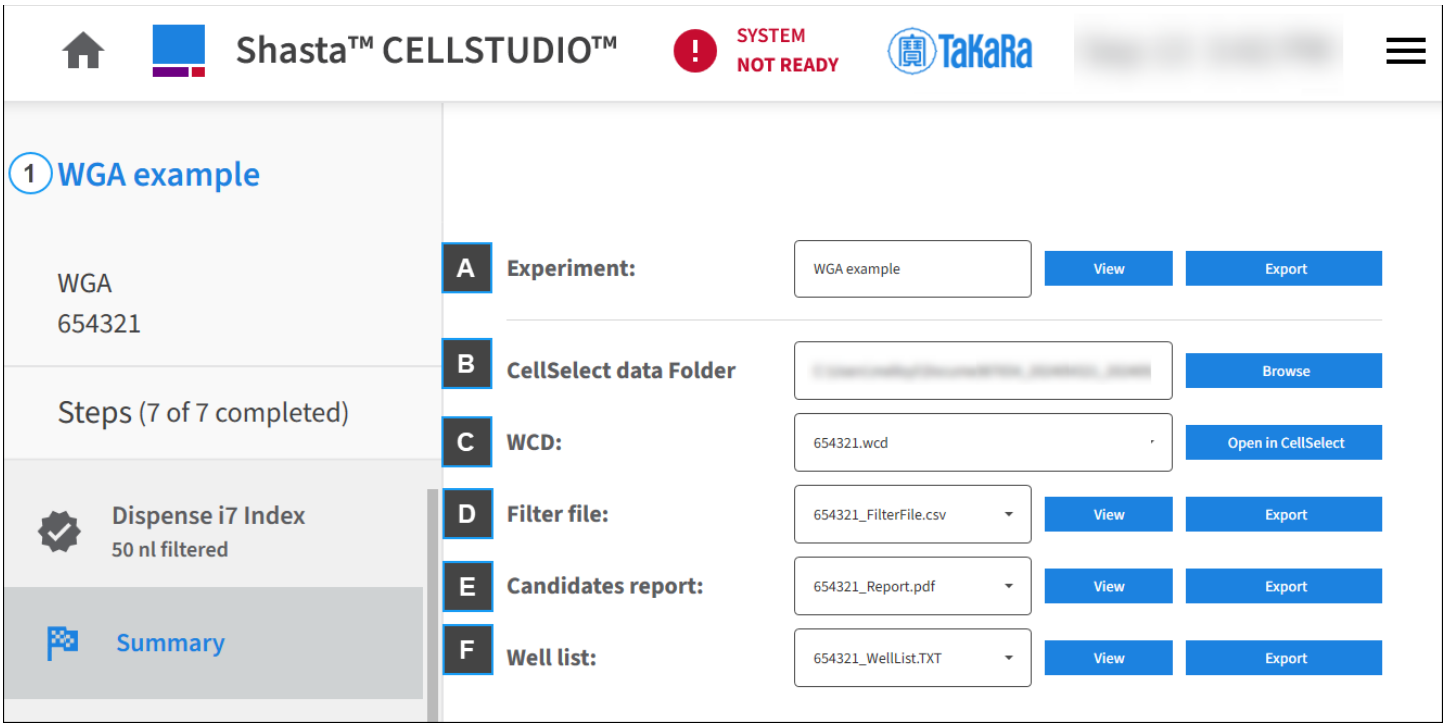


Figure 24. Example *Summary* page. The letter boxes next to each row are added to correspond to the list below and are not present in the actual software interface.

[A] Experiment: The box on this row displays the name of the experiment, corresponding to the element labeled with ①; the box field is not editable. The [View] button for this line brings up a log file in JSON format of all dispenses that have been performed on the chip. The log is updated after every dispense. For each dispense, the date and time, type of dispense, volume, and filter file name (if used) are recorded.

[B] CellSelect data folder: The box on this row (blurred out in the example above) displays the full path on the Shasta computer where the files resulting from the scan chip step and the CellSelect software output is stored and is not editable. [Browse] brings up a window showing a view of the folder contents and can be used to select a different location if the files were moved such as via File Explorer.

[C] WCD: The box on this row is a drop-down menu with one option by default, named as `<chipID>.wcd`. This file correlates to a chip scan results file readable by CellSelect software. If there are multiple WCD files in the data folder location ([B]), they can be selected through the drop-down.

The [Open in CellSelect] button will run CellSelect software and open the WCD file selected in the drop-down menu.

[D] Filter file: The box on this row is another drop-down menu with one option by default, named as `<chipID>_FilterFile.csv`. Created by CellSelect software, the filter file contains a 72 x 72 grid of "candidate wells" instructing the Shasta instrument which of the chip nanowells to dispense downstream reagents and indexes into. [View] will open the file in the default program to view CSV files, allowing you to read the contents of the file. For more information about the filter file or synthesizing one manually, refer to the Shasta CellSelect Software User Manual or Shasta Single Cell Advanced Features User Manual.

[E] Candidates report: The box on this row is a drop-down menu with one option by default, named as `<chipID>_Report.pdf`. Created by CellSelect software, this one-page PDF summarizes the experiment information, a small table of statistics related to the wells filled with samples and positive and negative controls, the number of candidate wells identified of each type, and a map of the candidate wells on the Single-Cell chip. [View] will bring up the report in the default program to view PDF files.

[F] Well list: The box on this row is a drop-down menu with one option by default, named as `<chipID>_WellList.TXT`. This is a comma-separated file containing the nanowell location of every sample and control dispense and its unique barcode identifier. [View] will open the file in the default program to view TXT files. For more information about using the well list, see Appendix D, "Sequencing Data Analysis Guidelines".

For all rows with an [Export] button, clicking it will allow you to save the file off to a different location either on the local server, an external device (such as a thumb drive), or a network drive location (if available).

7. Exiting CELLSTUDIO software

To shut down the software, click on the options button on the upper right side of the user interface and select **Exit Shasta™ CELLSTUDIO™**.

NOTE: Exiting the software generally would be done as part of a more general shutdown of the system. To do that, please refer to the instructions in Section XIV.C, "System Shutdown Procedure (<1 month)", or Section XIV.D, "System Shutdown Procedure (>1 month)".

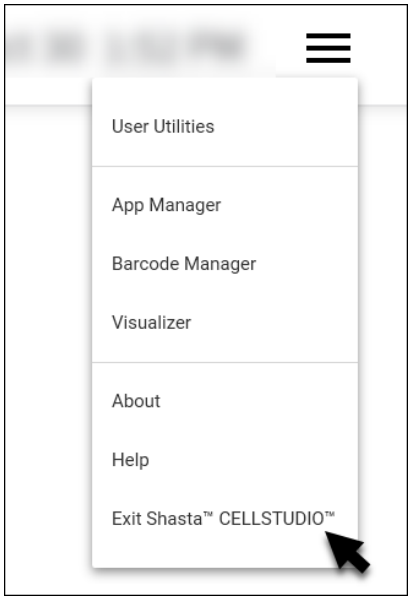


Figure 25. How to exit Shasta CELLSTUDIO Software.

B. CellSelect Software

CellSelect software runs in the background and analyzes images generated with the Shasta Single Cell and provides researchers with the following capabilities:

- Automated or manual image analysis and selection of isolated cells for downstream processing
- Assessment of cell staining to determine viability (live/dead)
- Modify parameters and rerun analyses with the new settings
- Generate the cell candidates "filter file" for selective wells dispensing of reagents by CELLSTUDIO software

More information about CellSelect software can be found in the Shasta CellSelect Software User Manual.

VI. Overview: Application Workflow

The graphic below depicts the overall workflow of a typical application performed on the Shasta Single Cell System. For additional information on any of the steps, refer to the associated section number.

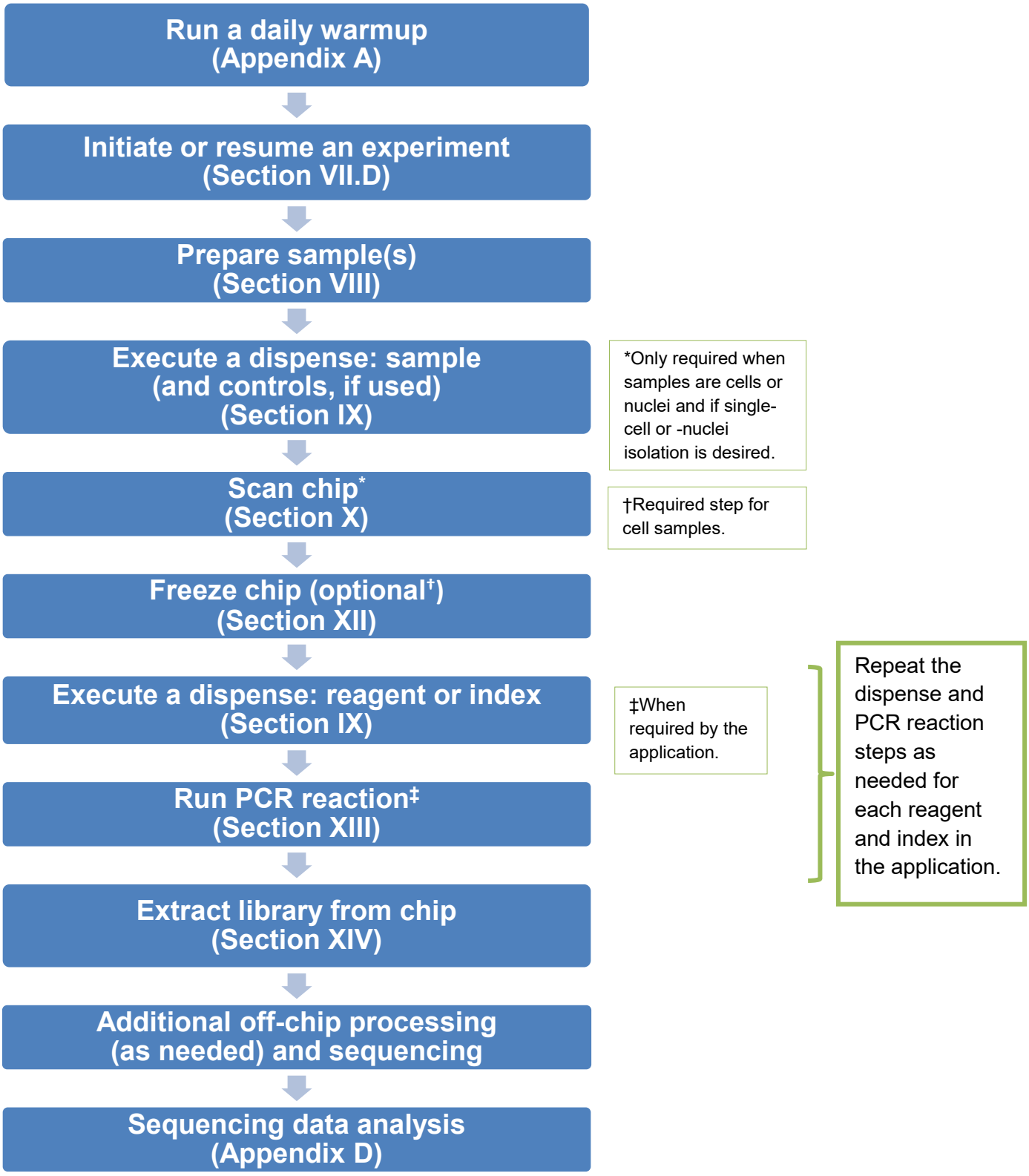


Figure 26. Application workflow overview.

VII. Protocol: Prepare the Shasta System

A. Power on the System (If Needed)

If the instrument is powered down, turn it on by flipping the switch at the back-right of the Shasta unit (Figure 3). The power touch-button (Figure 27) will slowly blink to indicate that the software is ready to be started.

B. Start Up the Software

To start the CELLSTUDIO software, press and hold the power touch-button for 1 sec. Refer to Section V.A.1, "Starting CELLSTUDIO Software" for more information.

Figure 27. Power touch-button location on the front right panel.

C. Preparing the System

When CELLSTUDIO first starts up, it will display the action center (Section V.A.2, "Action Center") for any tasks that should be completed before starting a run. At minimum, it is likely to display as below, prompting you to perform a daily warmup.

IMPORTANT: The daily warmup should be timed to run as close to the first dispense of the day, or it can be repeated before a dispense from the **User utilities** menu. Refer to for how to access the user utilities screen and Section A, "Daily Warmup" for more information about performing it.

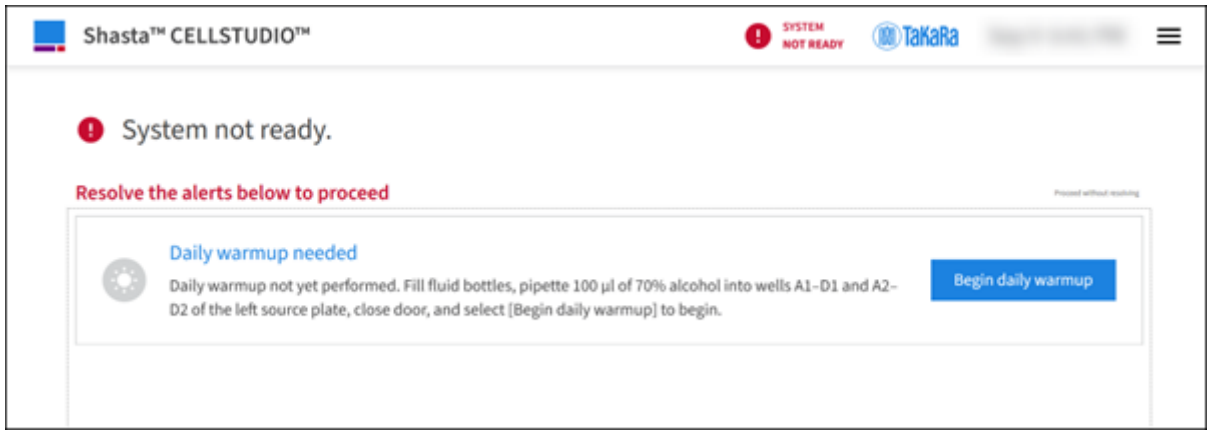


Figure 28. Example of *Action Center* notifications.

The *Action Center* may also be displayed after startup if there is a new, actionable item that comes up (e.g., notification to refill a source bottle). Refer to Section XIV.B, "Daily Maintenance" for detailed instructions on refilling the bottles.

NOTE: Though the action center provides warnings when the fluid-levels in the bottles are low, dispense protocols consume varying amounts of water (water consumption is generally proportional to the length of time of the dispense). It is good practice to periodically check the fluid levels in the bottles and the remaining capacity of the waste bottle visually.

D. Initiate or Resume an Experiment

After preparing the Shasta System and clearing all required action items, the *Home* screen will display. The *Home* screen offers several options to start or resume experiments:

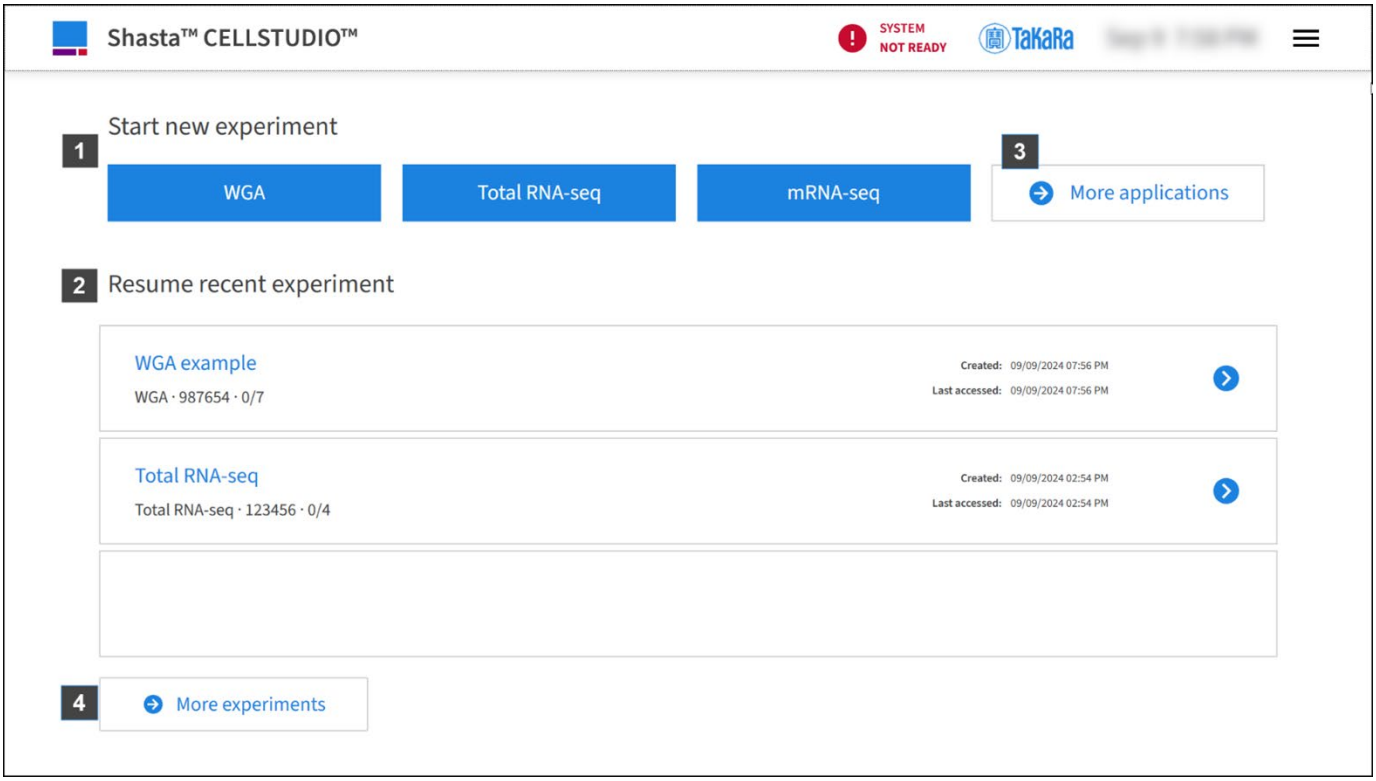


Figure 29. *Home* screen.

[1] **Start new experiment**—select from the recently used applications in this area to begin a new experiment or select [More applications] to browse the entire list.

[2] **Resume recent experiment**—choose a recent paused experiment from the list to continue where you left off.

[3] **More Applications**—access this option to view all application options currently installed on the system. This is an extension of starting a new experiment.

[4] **More Experiments**—access this option to view all experiments started on the system. This is an extension of resuming an experiment.

Select one of the four options, described in more detail, below.

1. Start New Experiment

If starting a new experiment, the first screen to display will be the *Experiment setup* (Figure 30).

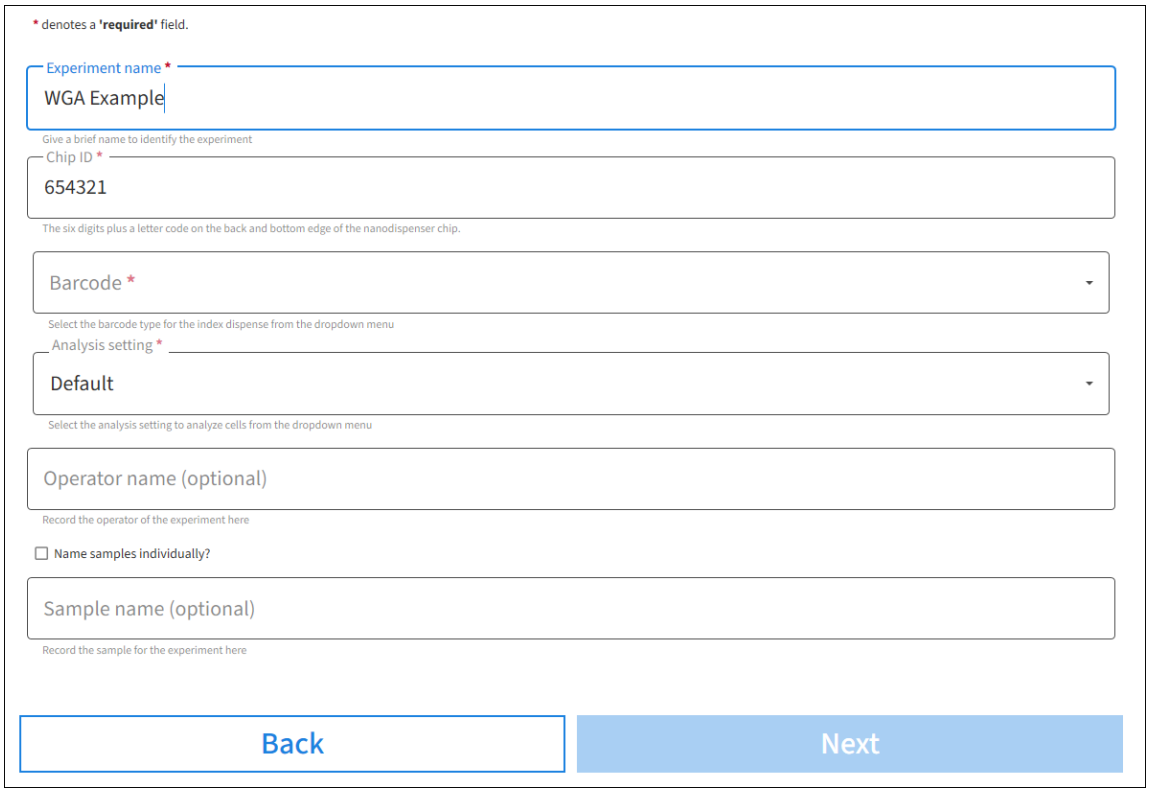


Figure 30. *Experiment setup* view.

1. Enter information about the experiment in the fields in the main part of the display.
   - Experiment name—the unique name of the experiment. This value is also what is displayed in blue font in the top left corner of the screen.
   - Chip ID—the unique ID number printed on the Single-Cell chip. This can be populated automatically by scanning the QR code on the bottom of the chip with the handheld barcode reader or typed in manually from the human-readable code on the edge of the chip (see Figure 14).
   - Barcode—this is the name of the indexes being applied as part of the experiment run. E.g., Long SetA, which refers to the Shasta Long Indexing Primer Set - A (Cat. No. 640283), supplied with the Shasta Total RNA-Seq (Cat. No. 640288) and Shasta Whole-Genome Amplification (Cat. No. 640286) kits.

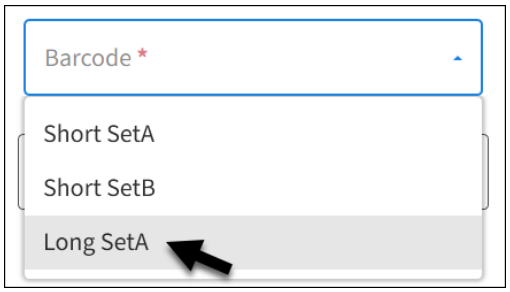


Figure 31. Barcode selection drop-down (default).

- Analysis setting—this option is passed on to CellSelect software to become the parameters by which the cell images are analyzed after scanning. The dropdown menu options are shown in Figure 32.

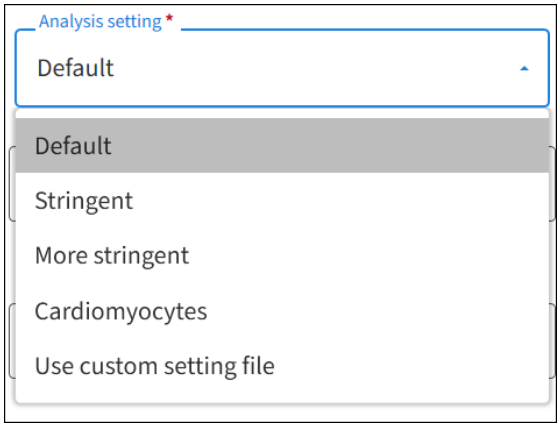


Figure 32. Analysis setting dropdown menu.

A brief description of the settings options are listed below:

  - Default—the equivalent of the default 'V3' setting in CellSelect software, this setting is aimed at a balance between the total number of candidate wells and good accuracy (e.g., a low doublet rate).
  - Stringent—this setting increases detection accuracy (lower doublet rate) but will generally result in a lower total of candidate wells as compared to the default setting.
  - More Stringent—this option further increases detection accuracy (lowest doublet rate) as compared to the 'Stringent' setting.
  - Cardiomyocytes—like 'V3 (Default)', but specifically programmed for detection of cardiomyocytes or other multinucleated cell types.
  - Use custom setting file—after adjusting any *Settings* value in CellSelect (either manually or through the [Tune] feature), there is an option to save the new settings as an XML file. For more information, refer to the Shasta CellSelect Software User Manual, Section II.C, "Saving Settings to an XML File".

    That file can then be applied to a new experiment type by selecting this option, then the saved file name. Using a custom settings file is useful, for example in the instance where you're working with a cell/nuclei type or stains that require some or many adjustments to the CellSelect settings to identify candidates to the threshold criteria to satisfy your experimental needs.

- Operator name (optional)—this field can be used to input the name of the person performing the experiment for record-keeping purposes, or other custom text the user would like to keep associated with the experiment data.
- Sample Name (Optional)—there are two options for this field:
  - One sample name can be assigned to the entire sample source plate.

- Select the "Name samples individually box?" (Figure 33) to assign an individual sample name within CELLSTUDIO software to each of the eight 384-well plate source wells (Section IX.A, "Prepare the 384-Well Source Plate"). This will associate each individual sample name with the nanowells it is dispensed to on the Single-Cell chip, allowing you to track the sample throughout the workflow and in the output data files.

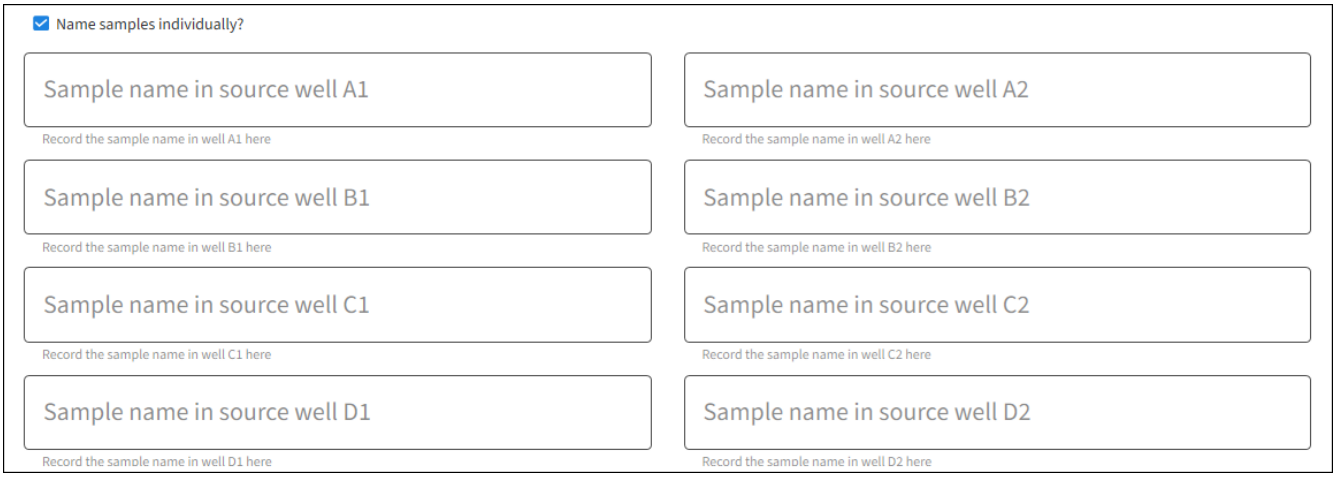


Figure 33. Sample naming option for individual source wells.

2. After the information has been entered, click on the next step, "(1) Dispense Cells and Controls", to proceed.

2. Resume Recent Experiment

If resuming a recent experiment, you will be prompted to enter the chip ID of the Single-Cell chip associated with it.

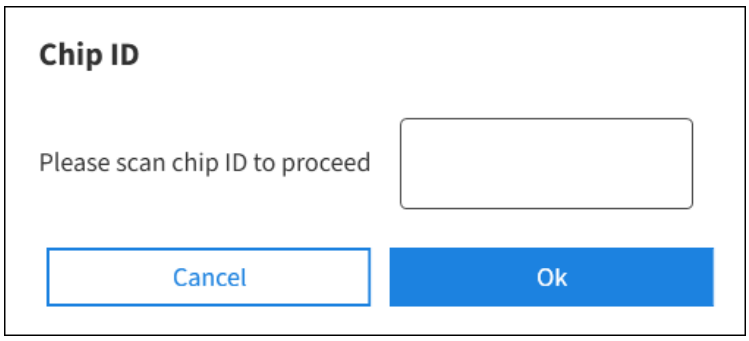


Figure 34. *Chip ID* scan dialog seen when resuming an experiment.

This can be scanned in using the handheld scanner and the QR code on the back of the chip or manually entered via the keyboard.

3. More Applications

The "More applications" option can be used to select a Shasta application configured within the software that is not displayed on the *Home* screen.

Figure 35. *More applications* screen.

There are three areas of interest on this screen.

[A] Applications—the list of all applications (prevalidated and user-defined) in the software.

[B] Search field—suggested if there are a large number of applications defined, making it easier to select the application of interest.

[C] Advanced options—click on the text to bring up the menu.

Figure 36. Advanced options menu on the *More applications* screen.

- The advanced options can be used to sort the applications by a combination of the following parameters.

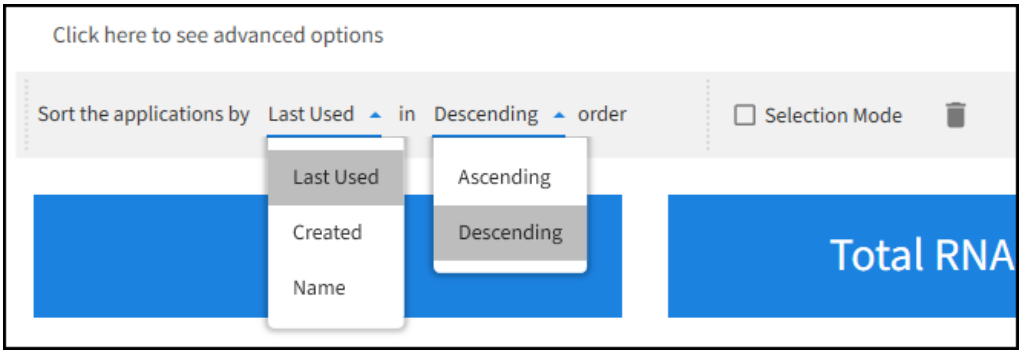


Figure 37. Sort parameters in the *More applications* advanced options.

1. Sort parameter 1
    - Last used—ordered by the date of usage
    - Created—when the application was created. Refer to the Shasta CELLSTUDIO Advanced User Manual for how to create an application.
    - Name—the name of the application

2. Sort parameter 2
    - Ascending—order the selection for Parameter 1 in ascending order (e.g., unused for the longest time to most recently used, oldest existing application to most recently created, A– Z)
    - Descending—order the selection for Parameter 1 in descending order (e.g., most recent to unused for the longest time, most recently created to oldest existing application, Z–A)

- When "Selection Mode" is checked, the trash can to the right of the text (indicated by the black arrow) also darkens. This mode allows a user to select, then delete, an application.

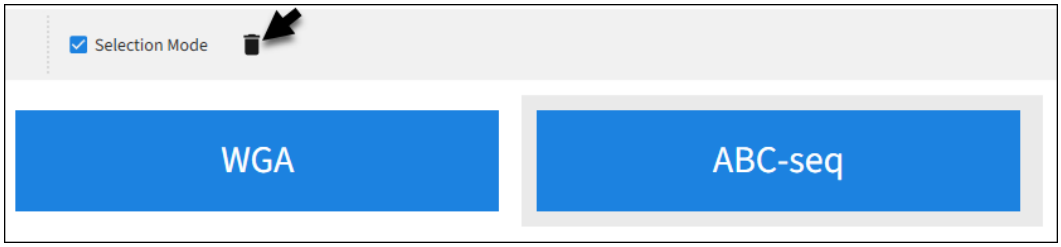


Figure 38."Selection Mode" active in the *More applications* advanced options.

Figure 38 illustrates the "ABC-seq" applications being selected; the button is enclosed in a slightly larger grey rectangle when selected.

When the trash can icon is clicked, a confirmation window will pop up asking you to confirm the deletion. If [Yes] is clicked, the application will be permanently deleted from the system.

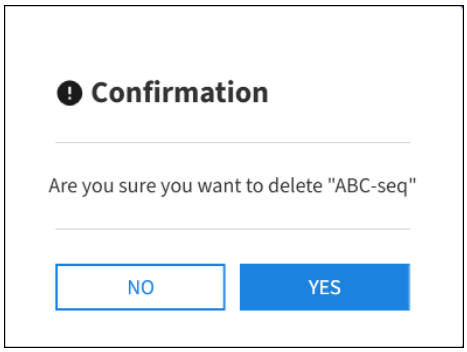


Figure 39. Deletion *Confirmation* dialog when deleting an application in the *More applications* advanced options menu.

NOTE: Only custom-created applications can be deleted through this tool. For more information about creating custom applications, please refer to the Shasta Single Cell Advanced Features User Manual.

Prevalidated applications installed with CELLSTUDIO software cannot be deleted.

4. More Experiments

The "More experiments" option can be used to select an in-progress or completed experiment that is not listed on the *Home* screen.

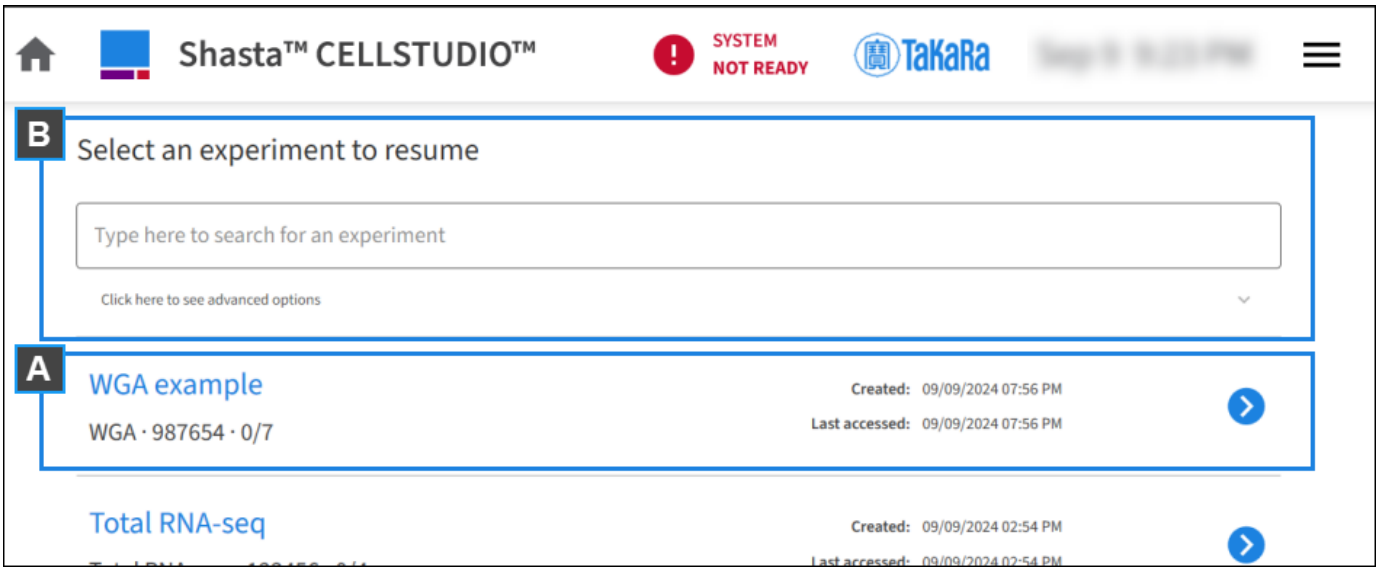


Figure 40. *More experiments* screen. This example shows the most recently accessed experiment (WGA example) and the one just prior to it (Total RNA-seq).

[A] An experiment—each experiment which has been initiated, partially run, or completed and that has not been deleted (see Section [B]) displays on a list on the screen. Figure 40 shows parts of the most recently accessed experiment (WGA example) and the one accessed just prior (Total RNA-seq). The page will continue the list down the screen.

Figure 41. Example experiment entry on the *More experiments* view.

Each experiment in the list (Figure 41) presents the following information:

1. Experiment name—this is the name given to the experiment when created (Figure 30).
2. Experiment configuration—there are three pieces of information listed here, delimited by a dot.
    i. Experiment application—the application option selected when the experiment was created, e.g. 'WGA'.
    ii. Chip ID—this may be a six-digit number or seven-character value (six-digit number + one letter) of the chip associated with the experiment (refer to Figure 14 for where to find the value), e.g., '987654'.

iii. Progress—this displays how many steps out of the total number of steps in the application have been completed before being paused, e.g., '0/7', or zero steps completed out of a total of seven in the application.

3. Experiment dates—this shows the date the experiment was initiated ("Created") and the last time a user ran or resumed the experiment ("Last accessed")

Click anywhere on the experiment entry to resume or to view its details (Section VII.D.2, "Resume Recent Experiment").

[B] The top part of the page contains tools with which to manipulate the experiment entries.

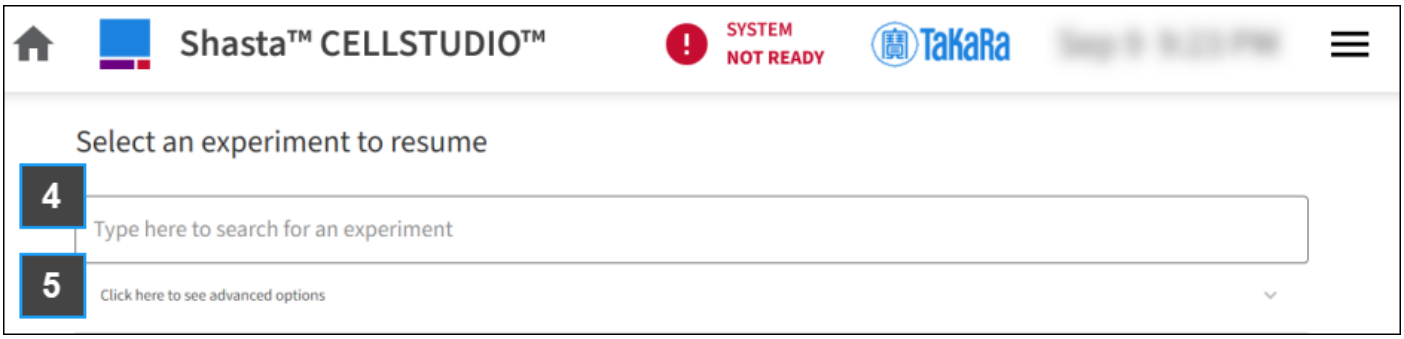


Figure 42. *More experiments* view top of page area.

4. Search field—suggested if there has been a large number of experiments run on the system. The search field makes it easier to locate the experiment of interest.
5. Advanced options—click on the text to bring up the menu, similar to the advanced options under "More Applications" (previous section)

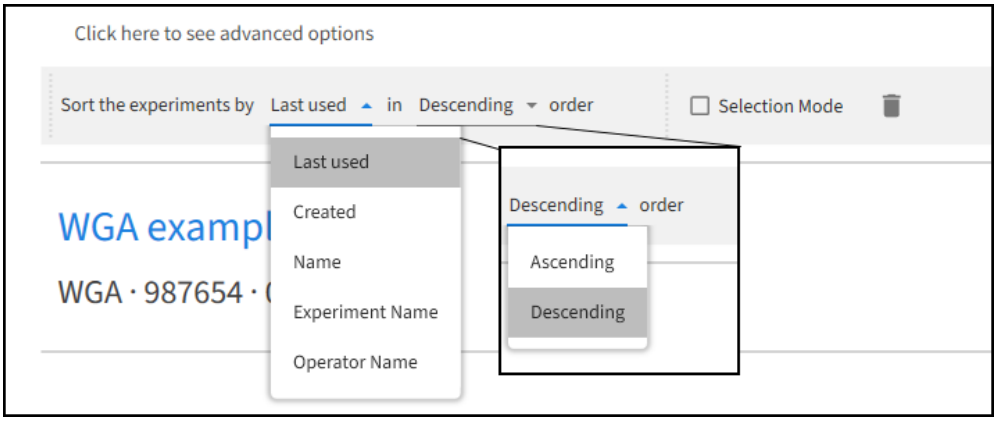


Figure 43. Sort parameters in the *More experiments* advanced options menu.

- Sort parameter 1
  - Last used—ordered by the date of access
  - Created—when the experiment was created.
  - Name—the name of application
  - Experiment name—see [A] above
  - Operator Name—text added to the "Operator name" field when the experiment was setup.

- Sort parameter 2
  - Ascending—order the selection for Parameter 1 in ascending order (e.g., unused for the longest time to most recently used, oldest existing experiment to most recently created, A– Z, etc.)
  - Descending—order the selection for Parameter 1 in descending order (e.g., most recent to not accessed for the longest time, most recently created to oldest existing experiment, Z–A, etc.)
- When "Selection Mode" is checked, the trash can to the right of the text also darkens. This mode allows a user to select, then delete, an application.

Figure 44."Selection Mode" active in the *More experiments* advanced options menu.

Figure 44 illustrates the top experiment being selected; the experiment details are highlighted by a gray rectangle when selected.

When the trash can icon is clicked, a confirmation window will pop up asking you to confirm the deletion. If [Yes] is clicked, all details about the experiment will be permanently deleted from the system.

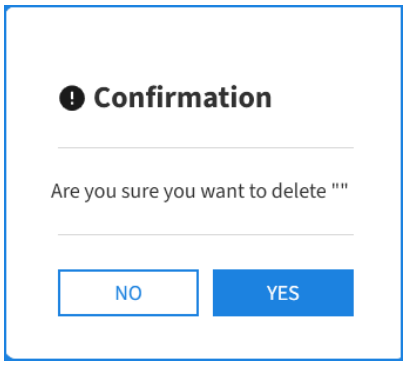


Figure 45. Deletion *Confirmation* dialog when deleting an experiment in the *More experiments* advanced options menu.

VIII. Protocol: Preparing Sample(s)

Prepare your sample or samples. If you're starting with cells, and Hoechst 33342 and propidium iodide are selected for use as dyes, the guidelines in the Single-Cell Minimal Cell Handling and Staining Protocol for Suspension and Adherent Cells Protocol-At-A-Glance can be used for this step.

If using different dyes, a custom staining protocol will need to be tailored to your sample source and experimental needs. Refer to the Shasta Single Cell Advanced Features User Manual, Appendix A, for more information about creating a custom protocol.

IX. Protocol: Executing a Dispense

This section describes the general workflow of performing a dispense within CELLSTUDIO software.

All dispenses have the following steps:

1. Prepare the 384-Well Source Plate.
2. Load the Single-Cell chip into the chip nest of the dispensing platform.
3. Load the 384-Well Source Plate into the plate nest of the dispensing platform.
4. Start the dispense process.
5. Blot, seal, and centrifuge the chip.
6. Discard the 384-Well Source Plate.

In addition to these, many reagent and index dispenses also include a step, just prior to starting the dispense process, to select a filter file (Section D).

See the subsections below for more details about each step.

A. Prepare the 384-Well Source Plate

- If using a prevalidated application, refer to the fill instructions in the associated application-specific user manual available at takarabio.com.
- If creating or using a custom application, source plate fill instructions for samples and reagents will need to be determined during the process of application development. Please refer to the Shasta CELLSTUDIO Advanced User Manual for more information.

! **IMPORTANT:** Avoid introducing dust and debris to solutions that will be dispensed with the Shasta instrument. They can cause the tips to clog.

Observe the following precautions when assembling sample and reagent source plates:

- Consider assembling source plates in a dead airbox to reduce environmental dust
- Wipe down the lab bench every day and wear gloves and a clean lab coat
- Use plates, tips, and tubes from new or carefully covered containers
- Work quickly and cover plates/tubes to minimize exposure to dust in the air

1. Pipette the sample, reagents, or index solutions into the plate wells marked in the CELLSTUDIO display (or according to the user manual, for prevalidated applications).

   Figure 46 shows a 384-well sample source plate map for the cells and controls when performing the Shasta WGA application.

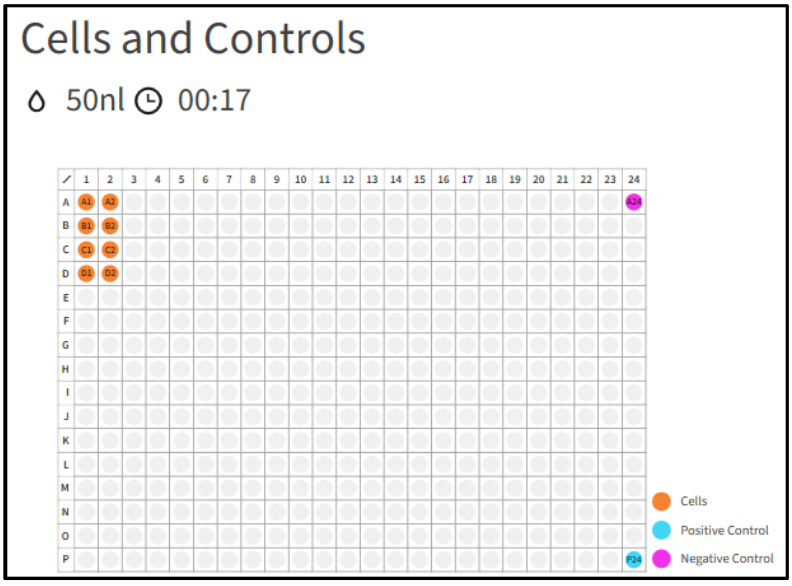


Figure 46. Example source plate map for loading the 384-well plate with samples (cells) and controls. The Shasta whole-genome amplification application (WGA) is shown here.

2. After filling the wells, seal the plate with a 384-Well Source Plate Seal.

> **IMPORTANT:** When applying the 384-Well Source Plate Seal to the plate, sufficient pressure must be applied to activate the silicone adhesive. It is highly recommended that you do multiple rolls of the 384-Well Plate Seal Applicator in both directions to ensure a good seal.
>
> Use source plates immediately.

B. Load the Single-Cell Chip

The workflow step will prompt you to place the Single-Cell chip in the chip nest.

1. If the chip contains dispensed solution that has been frozen, thaw the chip (Section XI.B, "Protocol: Thaw a Frozen Chip").
2. Visually inspect the chip nest and clean it if there is any debris.

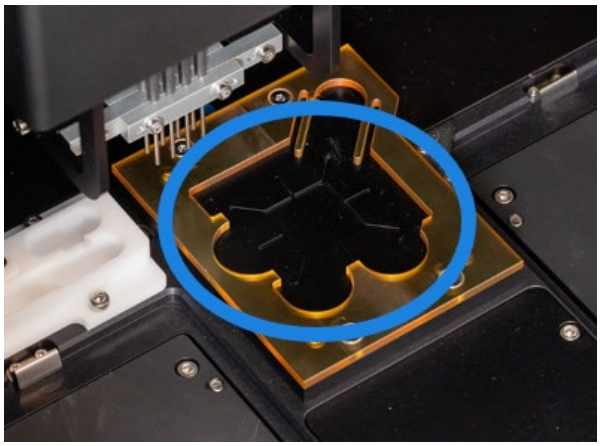


Figure 47. Area of the chip nest to inspect and clean prior to loading a chip.

NOTE: Refer to Section XIV.A, "Clean the Shasta Dispensing Platform" for recommendations on how to clean the chip nest, if needed.

3. In the CELLSTUDIO interface, the area of the experiment step view displaying the active step will change to something similar to Figure 48 with a 384-well plate map on the left and a mini-map of the Shasta dispensing platform on the right.

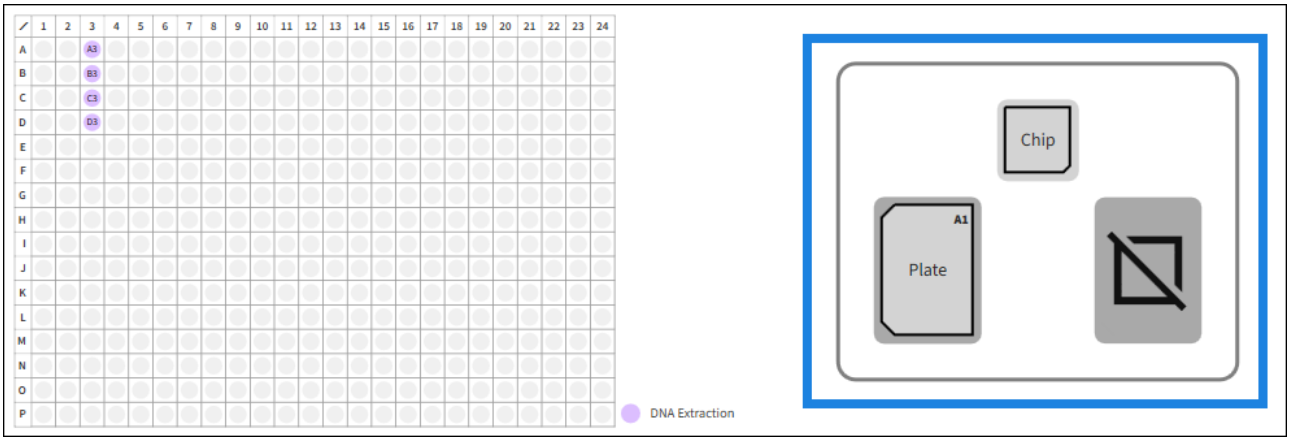


Figure 48. Mini-map of the Shasta dispensing platform displayed in CELLSTUDIO software. The mini-map is highlighted by the blue box (not present in the UI).

4. Insert the chip into the chip nest using the process described below. The film type adhered to the face of the chip will vary depending on what type of step is being performed and should still be in place at this point.

   The parts of the chip nest referenced in the procedure are identified in Figure 49.

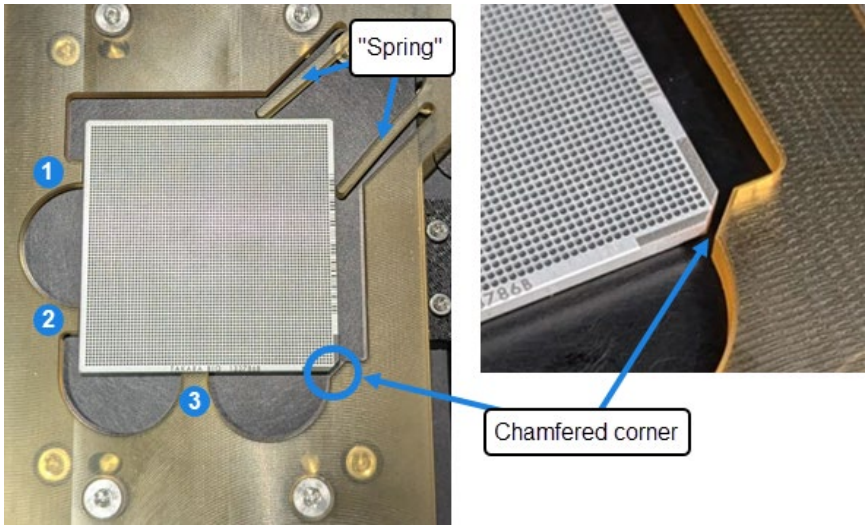


Figure 49. The parts of the chip nest identified. The "spring" is in the top right corner. The chamfered corner of the chip will be oriented towards the bottom right corner. On the bottom and left edge of the nest are three alignment posts (numbered 1–3) protruding into the chip nest well. When inserted correctly, the spring, chamfered corner, and alignment posts ensure the chip is seated correctly in the nest.

   a. Grip the chip in such a way that the film does not become separated from the surface of the chip. The chamfered corner of the chip should be oriented to the lower right corner of the chip nest (Figure 49).

b. Angle the chip so the top right corner of the chip nest (the “spring") is engaged first. To do this, the right index finger should press slightly on the top right corner as shown in Figure 50.

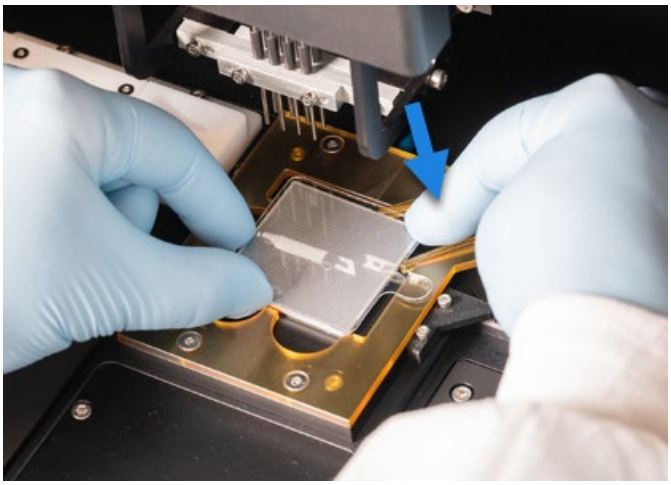


Figure 50. Engaging the spring on the chip nest. Place the right index finger between the two prongs to gently spread them apart so the chip can be fit between them.

c. Guide the chip towards the spring so that it can be engaged (Figure 50, red arrow). The chip should be pushed far enough to the upper right that it will not contact the three alignment posts (Figure 49) when the chip is lowered into the nest.

d. Lower the chip into the nest. The chip can be pressed down on top of the film to ensure it is seated but be careful not to move the film when doing so.

e. Visually check to make sure the bottom face of the chip is flat against the black anodized aluminum surface, and the top face of the chip is flush with the surface of the chip nest (Figure 51).

NOTE: If the chip is not fully seated, the system will not be able to pass the autofocus or vacuum checks successfully in Step 6.

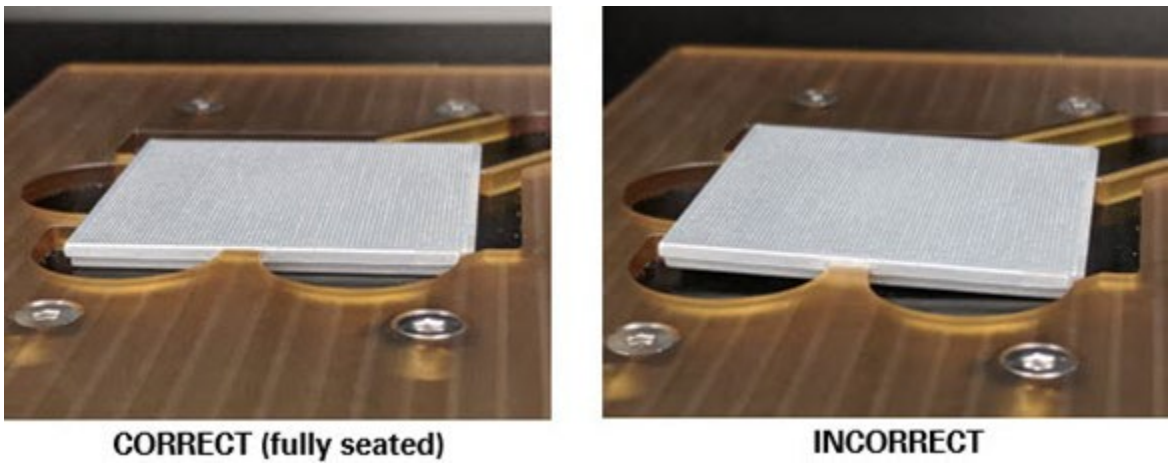


Figure 51. Examples of a fully-seated chip and a chip incorrectly inserted into the chip nest. It is important to check that the chip is fully seated before dispensation to avoid interference with the dispenser tips.

5. Once you confirm the chip placement and click [Next], the system will verify the presence of the chip.

- Upon successful detection, the chip icon's color will change to green, indicating that the presence of the chip has been detected.

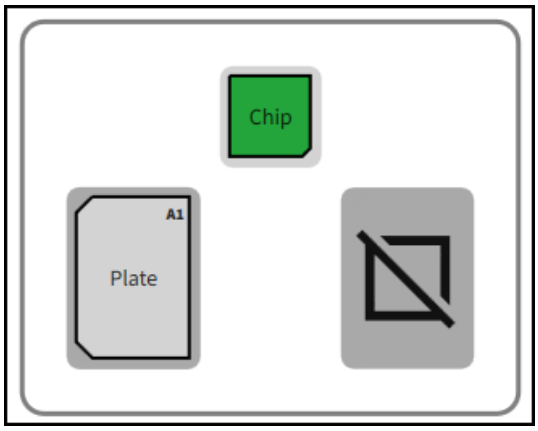


Figure 52. Mini-map displaying successful chip placement.

- If the chip cannot be detected, a warning message display on the screen. Follow the directions on the screen to resolve the issue described in the message and repeat the step.

6. Remove the seal from the chip.
   a. With one finger pressed in the middle of the film (Figure 53), grasp the tab of the film between two fingers of the other hand.
   b. Peel the film from the chip. To avoid pulling the chip out of the chip nest, keep pressing down on the film with one finger until the film is almost fully removed before releasing the pressure.

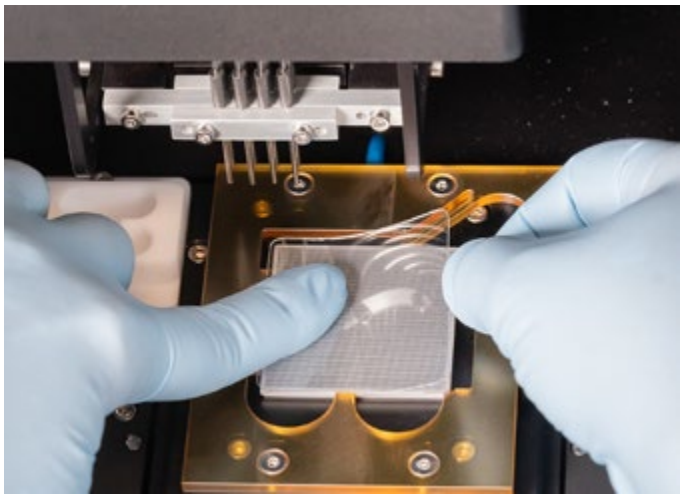


Figure 53. Demonstration of proper chip film removal. One finger (left) should remain pressing down on the film as long as possible while the film is being peeled (right).

7. Click [Done] to proceed.

C. Load the 384-Well Source Plate

The workflow will next prompt you to place the 384-Well Source Plate in the plate nest (Figure 54). A source plate contains either the samples (i.e., a sample source plate) or the reagents (i.e., a reagent source plate) that are to be transferred using the Shasta Single Cell.

IMPORTANT: Only the plates included in the Nanodispenser 384-Well Source Plate and Seal kit (Cat. No. 640018 & 640037) are validated for use on the Shasta system.

1. Place the sample source plate in the plate nest with the A1 position in the top-right corner (Figure 54). Press down on the plate to ensure it is sitting flat against the platform.

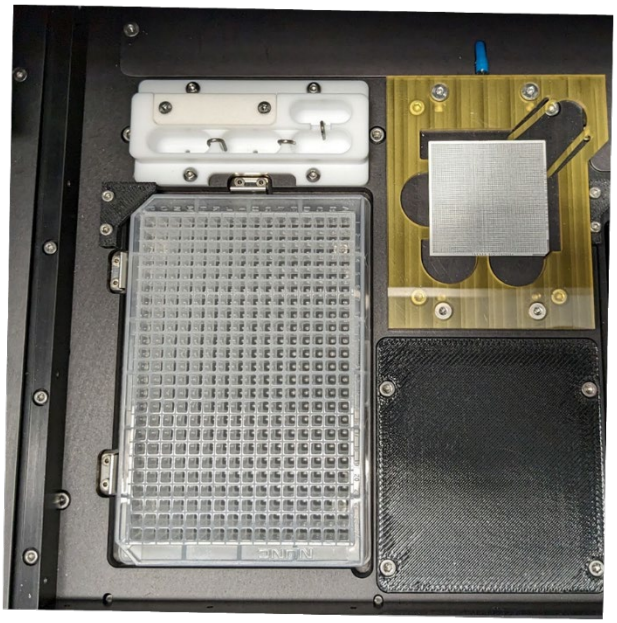


Figure 54. Sample source plate in the plate nest.

2. If a plate seal was used, carefully remove the seal from the plate, taking care not to dislodge the source plate from the nest.
3. Visually confirm the placement of the plate by checking the mini-map in the software (Figure 55). The plate section in the mini-map will turn green if it successfully detects the plate.

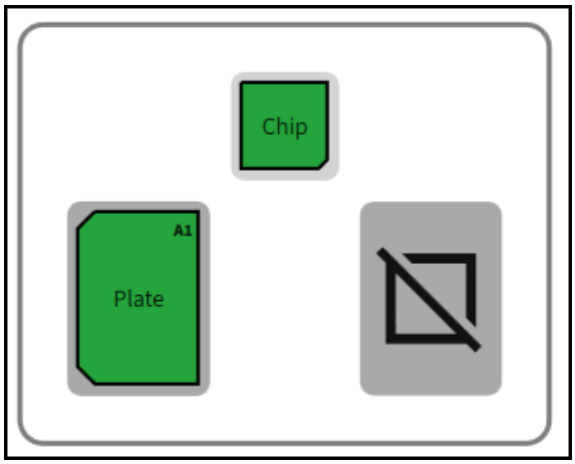


Figure 55. Mini-map displaying successful source plate placement

4. Gently close the front access panels of the Shasta instrument; both doors should remain closed during the dispensing process.

Figure 56. View of the Shasta instrument with the front doors open (left) and closed (right).

5. Once confirmed, click [Done] to proceed to the next step.

D. Select Filter File (Reagents and Indexes Only)

When performing a reagent or index dispense, after positioning the source plate, you will be prompted to select a filter file.

1. Press on the [Browse] button to select a filter file (CSV file) which has been generated by the CellSelect software.

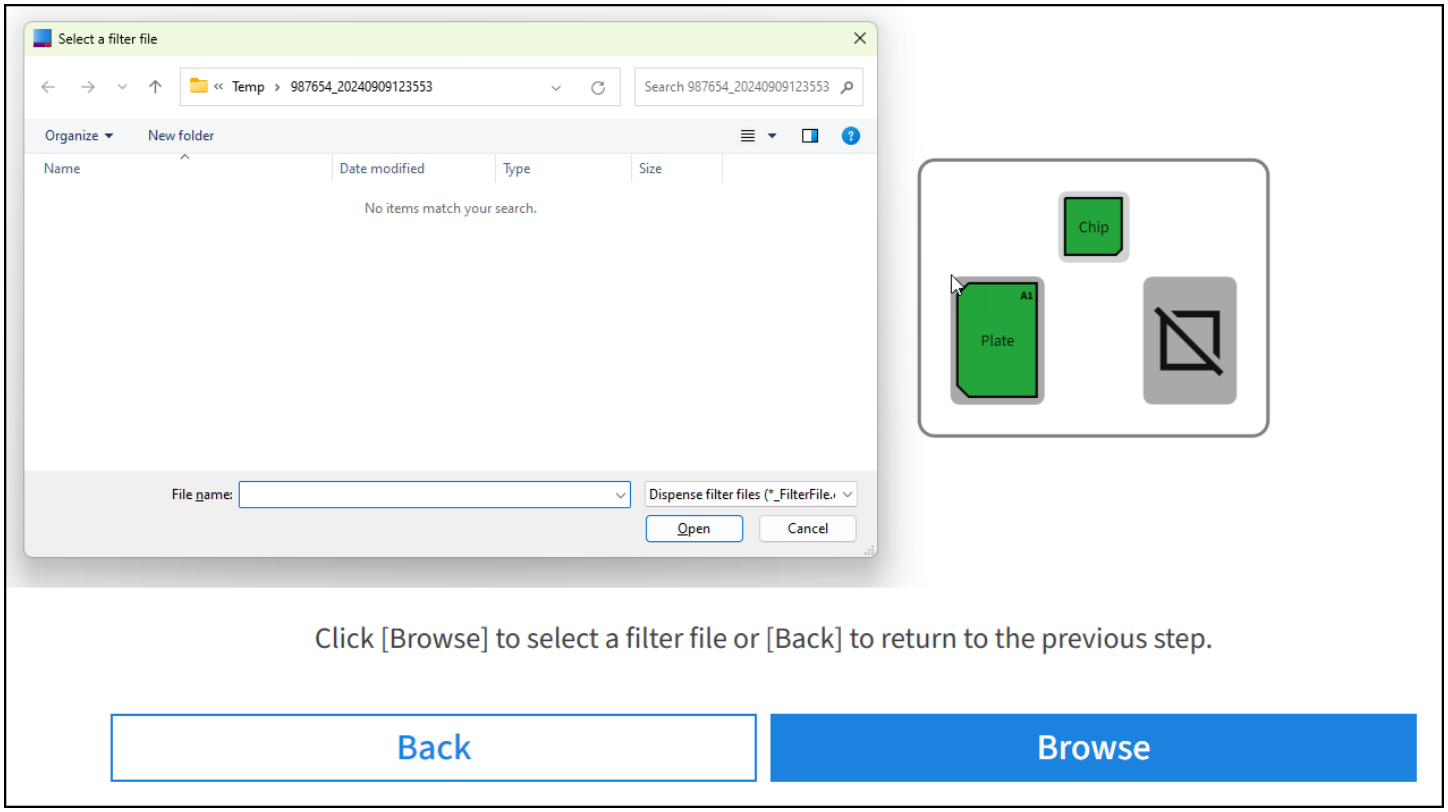


Figure 57. Filter File selection view.

2. After selecting the desired `*_FilterFile.csv` file, click [Open]. The screen will change to display:
   - The name of the filter file.
   - The number of wells that will be dispensed to (affected by the filter file).
   - An option box will display under the mini-map, [Click here to select a different filter file]. Click on the box if you want to change which filter file to use for the dispense.
   - The largest action button on the bottom will change to the name of the dispense step.

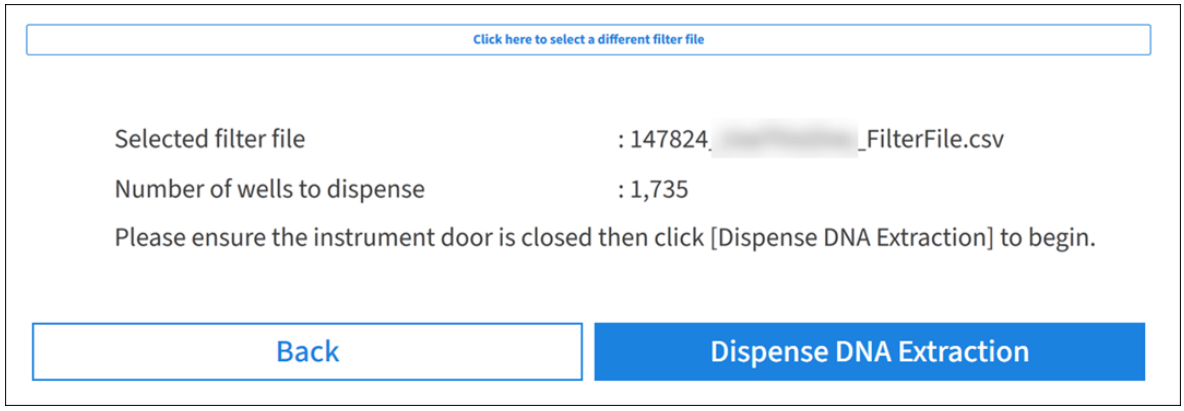


Figure 58. Dispense step display after selection of a filter file.

E. Start Dispense

After completing the preceding steps, the system is ready to dispense.

As a reference, the screen will display the minimum volume required to carry out the dispense. The minimum volume will vary by protocol; for the recommended amount, refer to the user manual for the specific application you are running.

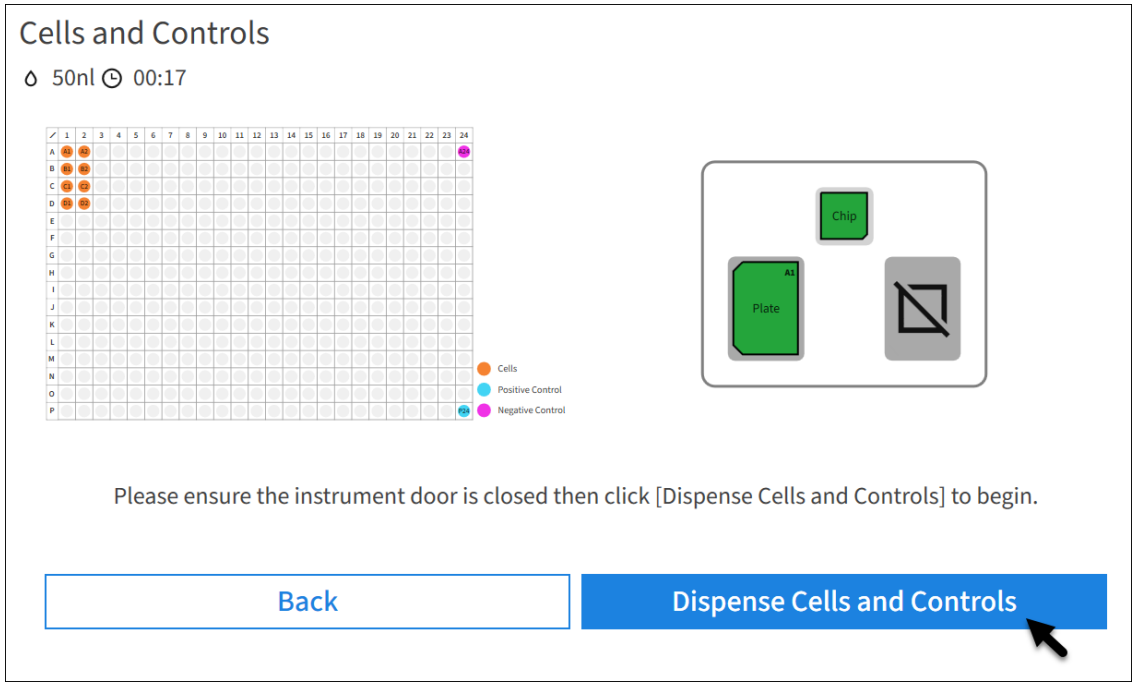


Figure 59. CELLSTUDIO workflow step, ready to dispense. This example depicts the cells and control dispense for the Shasta Whole-Genome Amplification workflow.

1. To initiate the dispense process, click on the [Dispense <STEP NAME>] button. The door of the instrument will lock and remain so until the dispense process is complete.

   The software will display an in-progress view (Figure 60).

Figure 60. Dispense in-progress view.

NOTE: The "Estimated" time displayed indicates the total time expected for the dispense step to progress to completion and may vary, such as in the case of reagent dispenses restricted by a filter file. The actual "Elapsed" time may slightly exceed the estimated time; this is normal behavior.

2. (Optional) If the application has been configured for a "Pause Before Aspiration" during the dispense step, a message similar to the one in Figure 61 will display. In most instances, the directions listed on the screen should be followed but check the user manual for specific instructions relating to prevalidated applications.

   The number of pauses experienced and the total number of pauses configured for the application is shown after the list of instructions; in the screenshot, this is "1 of 4 pauses", or the first of four pauses to expect.

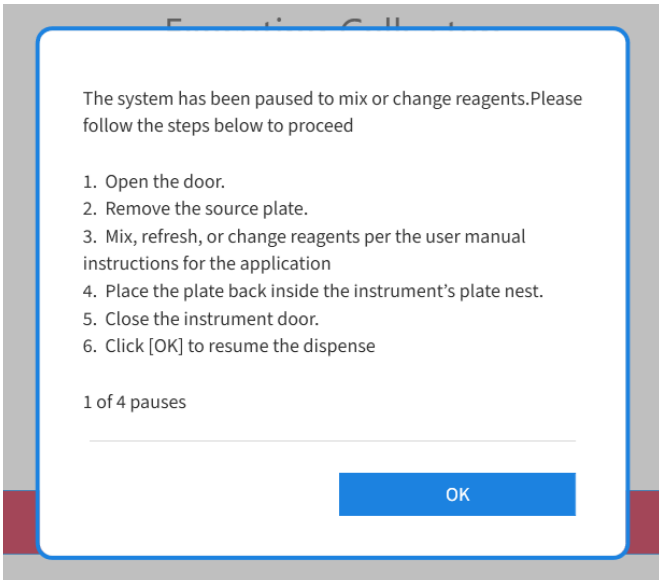


Figure 61. Example dialog window when the dispense is paused, prompting to remix the source plate. This image was generated from the Shasta Total RNA-Seq workflow.

3. Upon completion of the dispense, including all pause steps, if applicable, the software will display a screen indicating the dispense is finished.
4. When ready to continue, click the [Next] button.

NOTE: The finished view may also include optional instructions for offline processes that need to be completed before you can proceed to the next step in the experiment. These are intended to supplement, but not replace, a user manual. Please refer to the user manual for the most up-to-date details of prevalidated workflows.

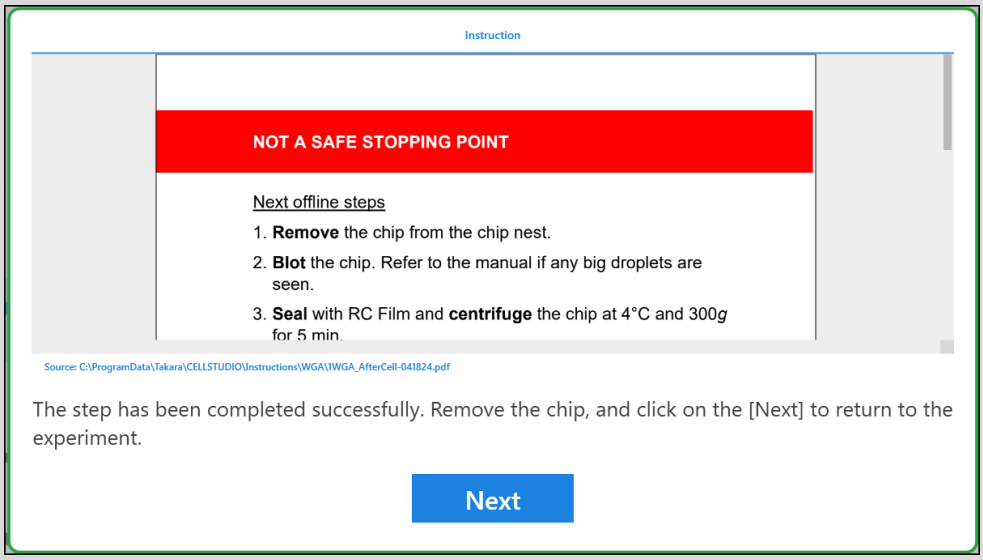


Figure 62. Optional instructions for offline processes included in a prevalidated workflow. The instructions above are an excerpt from the Shasta WGA workflow, after cell and control dispense.

F. Blot, Seal, and Centrifuge the Chip

After a dispense is complete, promptly blot, seal, and centrifuge the chip, as described below.

1. Carefully remove the chip from the chip nest and place the chip, wells facing up, on a clean lab wipe.
2. Gently place a piece of blotting paper directly on top of the chip. Make sure that the blotting paper covers the entire face of the chip.
3. Pick up the Nanodispenser Chip Blotter (Figure 63, below) by the top handle and place the flat face of the blotter against the blotting paper on the chip. The blotter should extend beyond the edges of the chip.

Figure 63. Nanodispenser Chip Blotter. The blotter is provided with the Shasta Single Cell System.

4. Let the blotter rest on top of the blotting paper for exactly 2 sec without pressing down; the weight of the blotter is sufficient for adequate blotting.
5. Remove the blotter, then gently remove the blotting paper. Check to see if there are any big droplets blotted, as shown in Figure 64. If larger droplets are seen, refer to Table 11 in Appendix E, "Troubleshooting Guide".

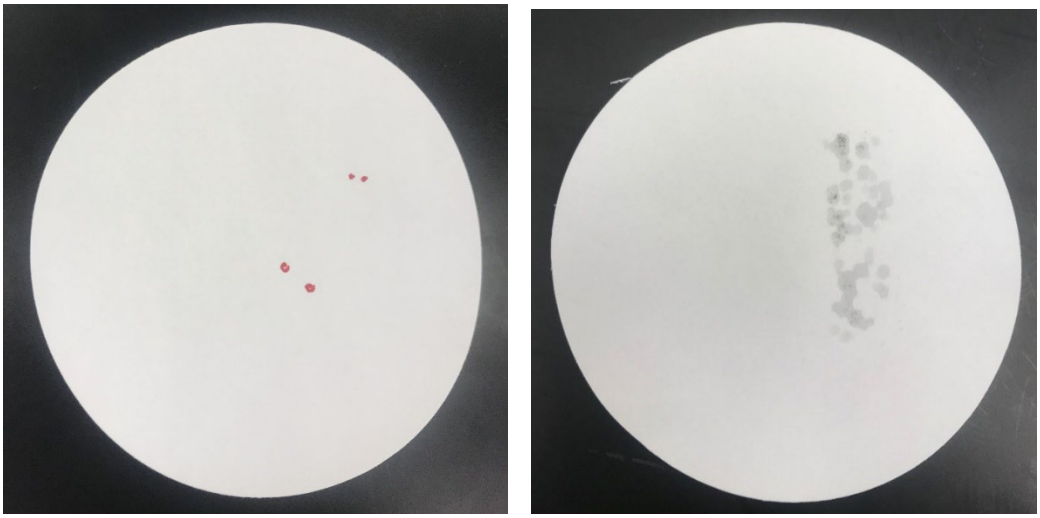


Figure 64. Examples of blotting results. **(Left)** Example of an acceptable result; the red marks indicate spots on the paper where blots were observed prior to evaporation. **(Right)** Example of a poor blot; note the size and quantity of the damp spots on the paper.

6. Dispose of the blotting paper in a biohazard container.
7. Quickly seal the loaded chip with appropriate film (specified by the protocol) using a film-sealing roller. Make sure that the chip is securely sealed to avoid well-to-well contamination and evaporation.

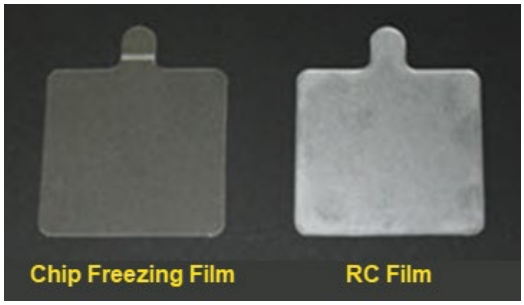


Figure 65. Chip Freezing and RC Films required for the dispenses. The Chip Freezing Film (left) has three layers and a green dot sticker on it. The RC Sealing Film (right) has a translucent backing. Please follow the instructions in each subprotocol regarding preparation and handling of the films.

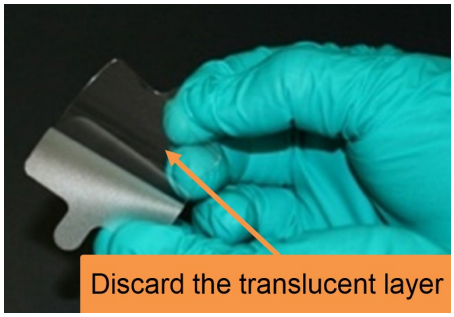


Figure 66. Preparing and adhering RC Film. RC Film is composed of two layers: one clear and the other translucent. When sealing a chip, remove the translucent layer and discard it. Put the remaining layer on the chip.

Figure 67. Sealing the chip with film and a film-sealing roller. The chip is being sealed while placed in the Nanodispenser Chip Centrifuge Spinner.

IMPORTANT: When applying film to the chip, sufficient pressure must be applied to activate the silicone adhesive. It is highly recommended that you do multiple swipes of the roller in both directions to ensure a good seal.

8. Centrifuge as described below.
   a. Place your chip on the Nanodispenser Chip Centrifuge Spinner.
   b. Counterbalance with a second chip inside a second spinner or the Chip Balance included with the Shasta system.

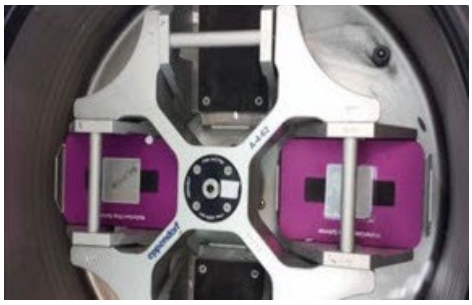


Figure 68. Centrifuge set up.

   c. Centrifuge according to the instructions in the prevalidated application. For custom applications, our initial recommendations are listed in Table 6; see the Shasta Single Cell Advanced Features User Manual for more guidelines for developing custom experiment requirements on the Shasta system.

Table 6. Initial recommendations for centrifuge guidelines by dispense type for custom applications. Settings should be adjusted as needed during the experimental design.

Dispense type	Centrifuge temperature	Time	Centrifuge speed
Sample	22°C	5 min	300*g*
Reagent or index	4˚C	3 min	3,220*g*

G. Discard the 384-Well Source Plate

After completing each dispense mapped for a source 384-well plate, remove the source plate from the plate nest and properly dispose of it.

X. Protocol: Scanning Chip for Single Cells or Nuclei

NOTE: This protocol is only necessary when the sample source is single cells or nuclei. We recommend not performing this protocol if your starting sample is a nucleic acid or the protocol does not require imaging (e.g., Shasta Total RNA-Seq), as it provides no benefit.

1. After centrifugation of the chip with the samples is complete, remove the film on the chip and load the Single-Cell chip in the chip nest. If the sample type is cells, prechill the Nanodispenser Chip Holder at –80˚C.
2. Press "(2) Scan chip" to initiate the cell scan workflow.

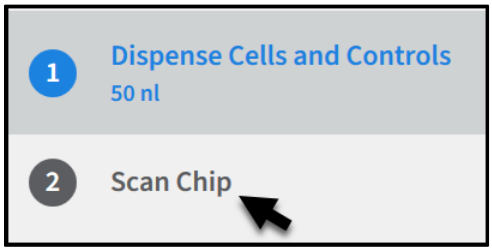


Figure 69. Initiate chip scanning.

The Scan Chip workspace will display (Figure 70).

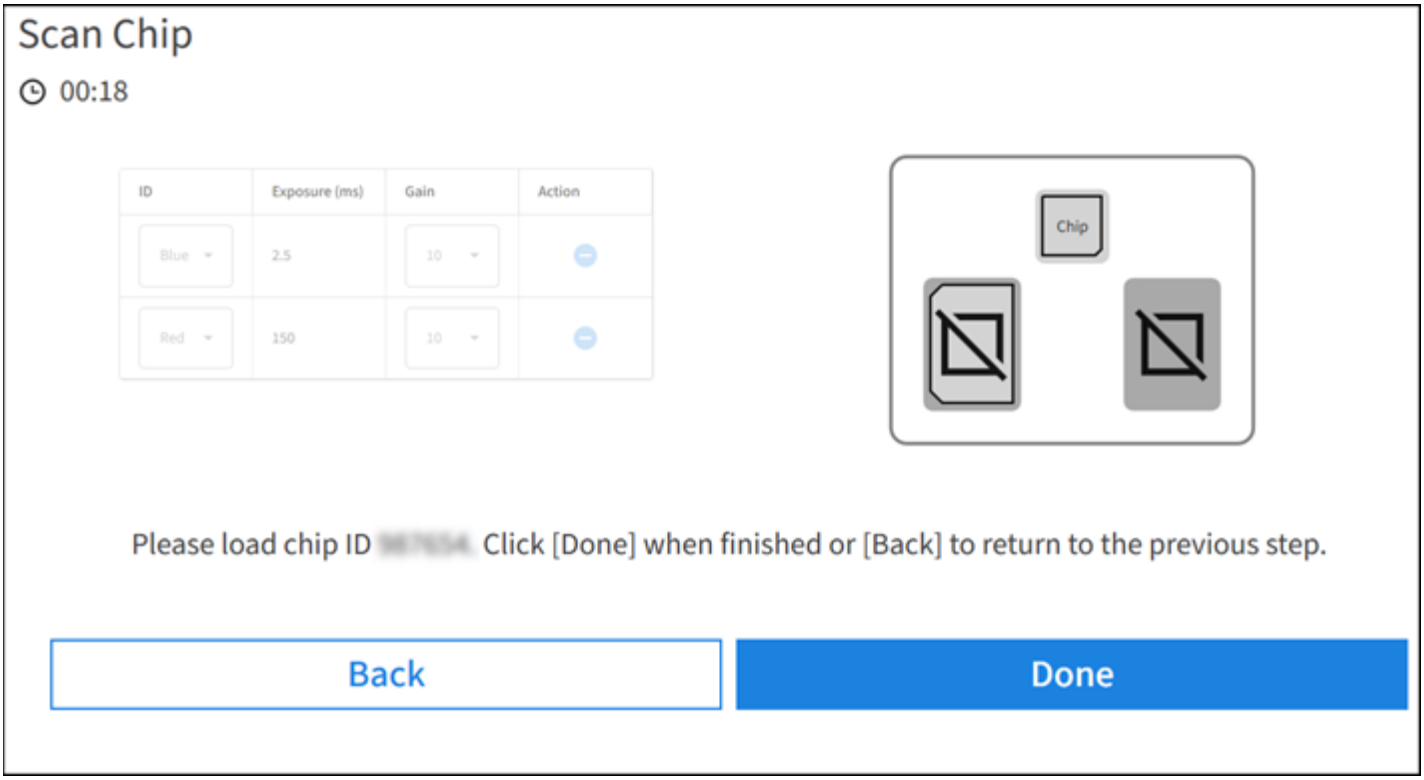


Figure 70. Scan chip view.

3. Follow the prompts on the screen to load the Single-Cell chip (if necessary, Section VII.A) into the chip nest.

NOTE: The scan filter settings on the left-hand side of Figure 70 above are inactive for predefined applications. For more information about this feature, refer to Appendix B.

4. Select the folder to save the image files to (Figure 71).

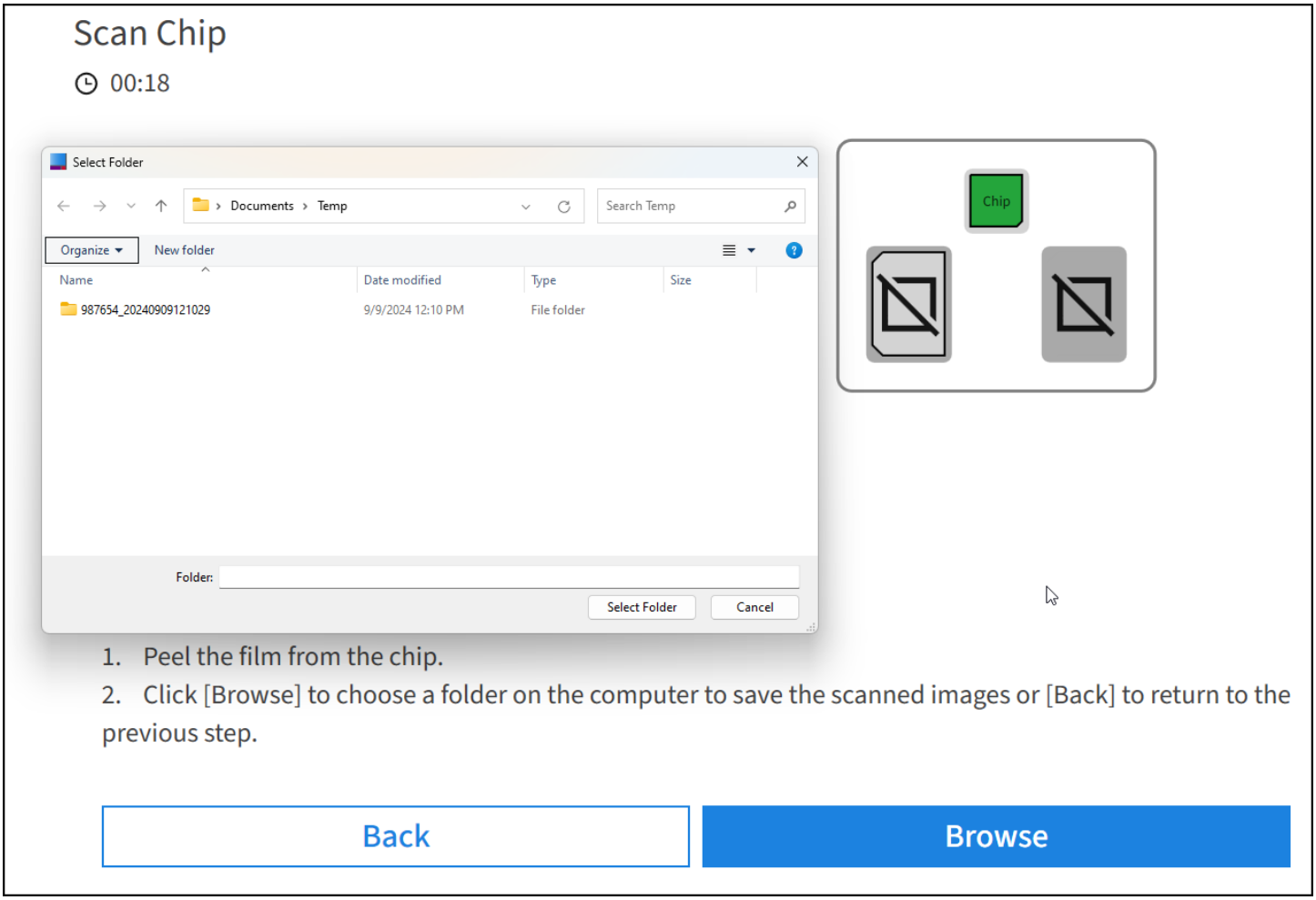


Figure 71. Image save folder browse view.

After selecting the destination directory, the screen will change to display:

- The path to the directory where the output files will be saved.
- An option box will display under the mini-map, [Click here to select a different folder]. Click on the box if you want to change the directory where the files will be saved.
- The largest action button on the bottom will change to [Scan chip].

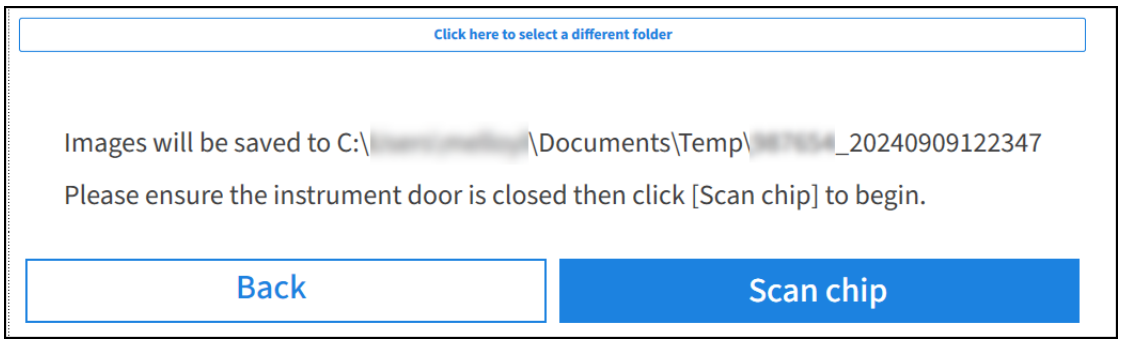


Figure 72. Scan chip options after save folder is selected.

5. Click the [Scan chip] button when you're ready to begin scanning.

   After clicking, the door on the instrument will lock and remain locked until the imaging process is complete. During the dispense process, the software will display an in-progress view complete with the previews of images that are being taken; upon completion, it will display the finished view. It will also automatically start an analysis of the images by CellSelect software. While the analysis is ongoing, you can either wait or navigate to offline steps.

6. Follow the prompts on the screen to walk through the chip scan process.
7. Once the scan is complete, follow the prompts on-screen and remove the chip from the chip nest.
8. Seal the chip with Chip Freezing Film from the Single-Cell Loading Kit (Cat. No. 640206) and a film-sealing roller.
9. Store the chip, temporarily or long term, determined by the following guidelines:

   - When using cells as the sample, the chip will need to be frozen to lyse the cells. Refer to Section XI, "Freeze the Chip" for this procedure.
   - If freezing is not required for the application, such as using nuclei or nucleic acids as your sample source, but you need to pause, the sealed chip can be stored at 4˚C for a short time.

10. Candidate detection by CellSelect is triggered automatically once the scan is finished; a well list and a filter fill will be generated after the candidate selection process is completed.
11. (Optional) Manually inspect the selected nanowells in CellSelect software to exclude or include one or more candidate wells. Please refer to the Shasta CellSelect Software User Manual for more information about this process.

    During the inspection process, it is recommended that the chip be stored at –80˚C or 4˚C per the guidelines in Step 9 (above).

12. If necessary, close only the Shasta CellSelect Software GUI and image viewer, **NOT** the CELLSTUDIO software.

NOTE: This is a safe stopping point in the procedure if the chip is in the freezer.

XI. Protocol: Freezing and Thawing a Sample on a Single-Cell Chip

- If the sample input is cells, the chip should be frozen to lyse them. Freeze the chip at –80˚C for 30 min or longer.
- If appropriate for the sample type, the chip should also be frozen at –80˚C if it needs to be stored for a long time.

A. Protocol: Freeze a Chip

1. Prechill the Nanodispenser Chip Holder at –80˚C, either prior to executing a dispense step (Section VIII) or a chip scan (Section X).
2. When ready to freeze the chip, ensure the chip is sealed with Chip Freezing Film from the Single-Cell Loading Kit (Cat. No. 640206)
   a. Remove the liner from only one side of the Chip Freezing Film (the side that does not have the green dot sticker).

b. Apply the exposed sticky side of the film to the chip, sealing carefully with the plate seal applicator (Figure 67, above). Make sure that the film has adhered completely and evenly on the chip.

IMPORTANT: When applying film to the chip, sufficient pressure must be applied to activate the silicone adhesive. It is highly recommended that you do multiple swipes of the roller in both directions to ensure a good seal.

c. Remove the side with the green dot sticker.

3. Put the sealed chip into the prechilled Nanodispenser Chip Holder.
4. Put the chip holder and chip into a –80˚C freezer.

B. Protocol: Thaw a Frozen Chip

1. Remove the Single-Cell Chip Holder containing the Single-Cell chip from the freezer. Thaw the chip in the chip holder until it reaches room temperature (about 10 min).

   NOTE: Cells require a minimum of 10 min of thawing to lyse.

2. Take the chip out of the chip holder.
3. Use a Kimwipe to dry any liquid on the chip surface.
4. Centrifuge the chip at 3,220*g* (minimum 2,600*g*) for 3 min at 4°C.
5. Keep the chip at 4°C and use the chip as soon as possible.

XII. Protocol: Running the PCR Reaction on the Single-Cell Thermal Cycler

The procedure for this step is protocol dependent.

- If running a prevalidated application, refer to the user manual for that app. Application-specific user manuals are available for download at takarabio.com.
- If creating a new custom application, refer to the Shasta Single Cell Advanced Features User Manual for guidelines on designing a thermal cycler program.

XIII. Protocol: Extracting Library from the Chip

After completing all dispense steps on the Shasta Single Cell, extract the resulting library from the chip.

Before You Start

- Set the centrifuge(s) used for spinning the chip to 4°C.
- You will need a Single-Cell Collection Kit (Cat. No. 640212) for this procedure.
- If the previous step of the application is running a PCR reaction, remove the chip from the thermal cycler.

Procedure

1. Centrifuge the chip at 3,220*g* for 3 min at 4°C.
2. Open the Single-Cell Collection Kit and label the Collection Tube with the engraved chip number. Assemble the collection module by attaching the Collection Tube to the Collection Fixture, as shown in Figure 73.

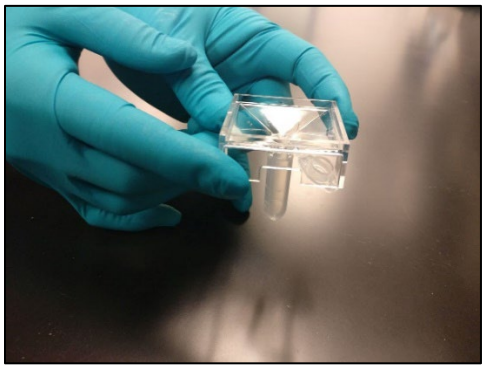


Figure 73. Assembling the collection module.

3. Carefully peel the film from the chip (Figure 74). First, anchor one corner of the chip with your finger. Then, using the tab on the film, apply even pressure until the film is removed.

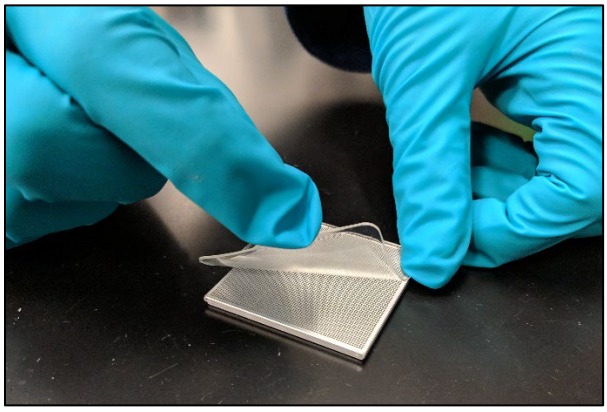


Figure 74. Removing the film from the chip.

4. With the nanowells facing down, place the chip into the assembled collection module (Collection Tube plus Collection Fixture; Figure 75). Surface tension will hold the liquid in the nanowells.

Figure 75. Placing the chip into the collection module.

5. Seal the chip and the top of the collection module with a supplied Collection Film (Figure 76).

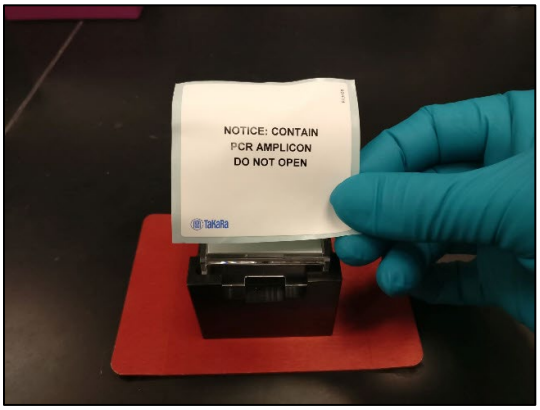


Figure 76. Securing the collection module with Collection Film.

6. Using a balance or blank chip, assemble another collection module. Centrifuge both collection modules at 3,220*g* (minimum 2,600*g*) for 10 min at 4°C.
7. Carefully remove the Collection Tube containing the extracted library (Figure 77).

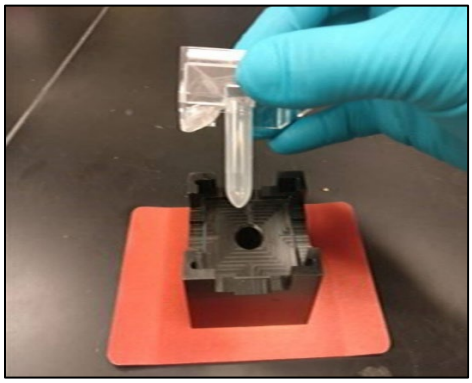


Figure 77. Removing the Collection Tube containing the eluate.

8. Attach the second supplied Collection Tube to the Collection Fixture and seal the entire module. Discard the module in a biohazard waste bin. DO NOT discard the initial collection module containing the balance or blank chip; retain it as a balance module for future extractions.
9. The collection tube containing the eluate can be stored at an appropriate temperature, if needed.

NOTE: This is a safe stopping point in the procedure if the collection tube is frozen at –20°C.

XIV. Instrument Maintenance

CAUTION: There are no user-serviceable parts inside the instrument. The servicing of internal parts should be performed by a qualified Takara Bio service technician.

A. Clean the Shasta Dispensing Platform

It is recommended that the dispense platform area of the Shasta instrument be wiped down occasionally or as needed to keep the dispense platform area clean and free of debris. If heavily used, this should be done at least once a week; otherwise, every two weeks should be sufficient.

1. If there is a 384-Well Source Plate in the plate nest, remove and properly dispose of it.
2. Visually inspect the dispensing platform for any debris. Moisten a cloth with 70% isopropyl alcohol then wipe down the dispensing platform (chip nest, source plate nest, trough, and the areas on the base platform immediately surrounding them). Refer to Figure 78.

WARNING: Take care not to bump the tip nozzles while wiping down the dispensing platform. This could put the tips out of alignment and result in poor dispenses in the future. If this occurs, please contact your authorized Takara Bio service technician or field_support@takarabio.com.

WARNING: Ensure that no liquid drips below the platform. Do not spray the dispensing platform directly.

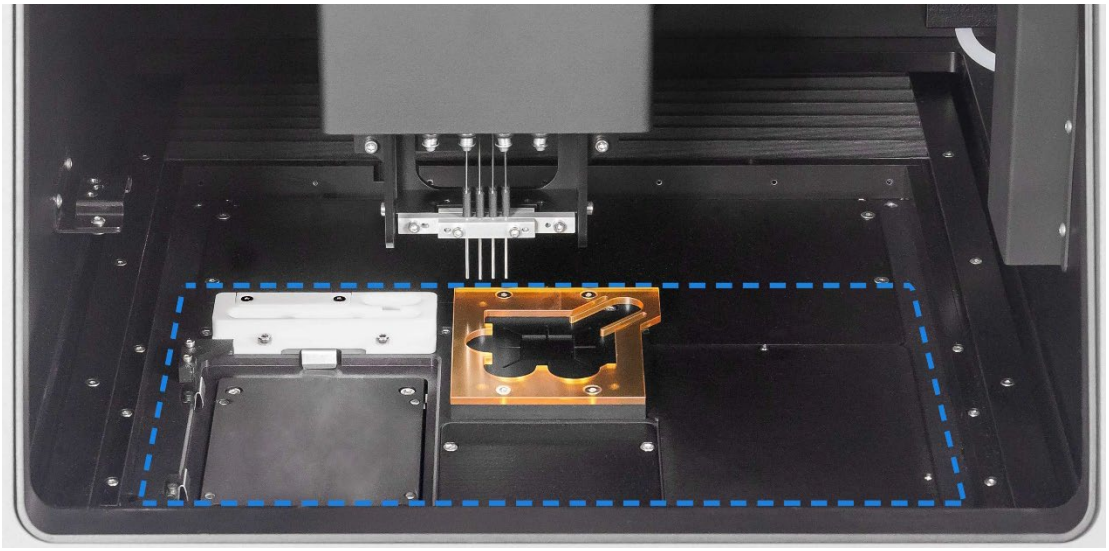


Figure 78. Area of the Shasta system to clean after the end of dispenses for the day. The area to clean is indicated by the blue-dashed rectangle.

B. Daily Maintenance

Daily Maintenance procedures help ensure optimal instrument operation and reduce interruptions in workflow.

1. How to Disconnect and Reconnect the Tubing Lines from the Caps

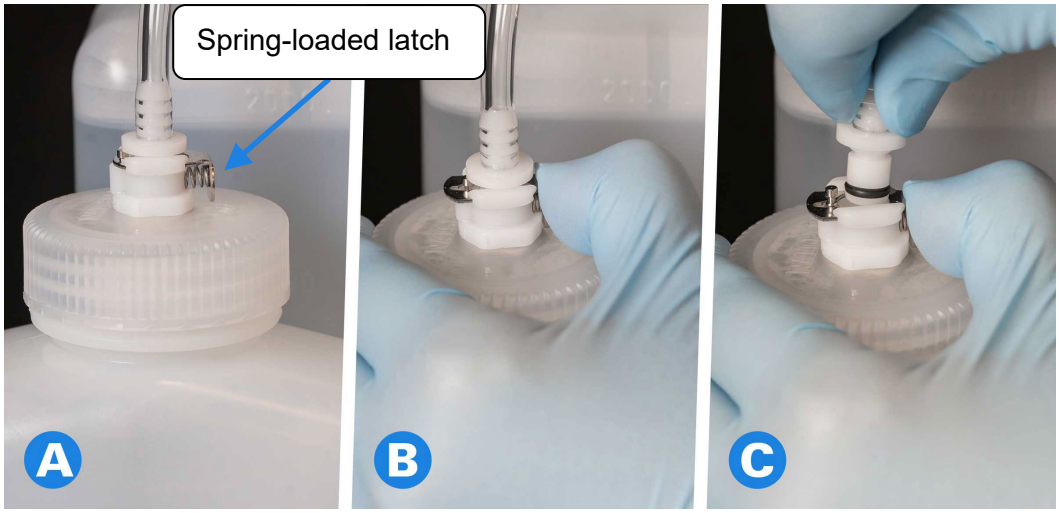


Figure 79. Visual aid for how to remove the tubing connector from the water source bottle, humidifier source bottle, and the waste container.

The procedure below describes the process of disconnecting the tubing fitting from the bottle caps for the water source bottle, humidifier source bottle, and waste container, as demonstrated in Figure 79.

To disconnect the tubing from the bottle cap:

1. Using your thumb, push in the spring-loaded latch at the side of the connector (Figure 79, Panel A). Figure 79, Panel B shows how the latch will look when it's properly pressed.
2. While holding in the latch, grasp the tubing at its connector and pull up to remove the connector from the bottle cap (Figure 79, Panel C).
3. Release the pressure on the latch.
4. Remove the bottle from the instrument. The tubing will remain in place attached to the instrument.

To reconnect the tubing to the bottle:

1. Insert the appropriate connector back into the port located on the cap.
2. Press the connector down until it latches

2. Water Source Bottle Refill Procedure

It is recommended to refill the bottle with deionized, filtered water if the water level is <800 ml. Use the following procedure and refer to Figure 8 if needed to identify each of the bottles.

1. Fill a clean secondary container with deionized, filtered water.
2. Disconnect the humidifier bottle via the fitting at the top of the bottle using the procedure in the previous section ("How to Disconnect and Reconnect the Tubing Lines from the Caps").

To make room to remove the water source bottle, place the humidifier bottle next to the machine on the benchtop.

3. Disconnect the water source bottle and remove the bottle from the instrument through the gap made from removing the humidifier bottle. Place the bottle on the benchtop next to the machine.

Figure 80. Removing the water bottle from the Shasta instrument.

4. Unscrew the cap of the water bottle and leave the cap slightly ajar. The refill water can be poured through this gap.
5. Fill the water source bottle to capacity (2 L) and screw the cap back on.
6. Place the water source bottle on the water scale and reconnect the tubing labeled "Water" to the cap fitting.
7. Place the humidifier source bottle back on the humidifier scale and reconnect the tubing labeled "Humidifier" to the cap fitting.

   Optional: If there is concern about bubbles in the water source tubing, go to **User Utilities** and click [Water prime] to prime tubing with water and flush out bubbles. See Appendix A, Section D for more details.

3. Humidifier Source Bottle Refill Procedure

It is recommended to refill the humidity source bottle if it is filled less than half capacity. Follow these steps to refill the bottle:

1. Fill a clean secondary container with deionized, filtered water.
2. Disconnect the humidifier bottle via the fitting at the top of the bottle and place the bottle next to the machine on the benchtop.
3. Unscrew the cap of the humidifier source bottle and leave the cap slightly ajar. Fresh water can be poured through this gap.

4. Fill the humidifier source bottle per the figure below:

Figure 81. Visualization of the recommended fill volume of the humidifier source bottle. The water level should be below where the bottle begins to curve to the top.

5. Screw the humidifier cap back on the humidifier bottle.
6. Place the humidifier source bottle back in its proper position on the scale and reconnect the tubing labeled "Humidifier" to the cap fitting.

4. Bleach Source Bottle Refill Procedure

Check the bleach bottle and replace sodium hypochlorite solution if either of these conditions are true:

- The volume of bleach in the bottle is <200 ml
- The bottle was last refilled >7 days ago

Follow these steps to replace the solution:

1. Prepare a secondary bottle with 500 ml of fresh 0.2% reagent-grade sodium hypochlorite.
2. Disconnect the bleach bottle via the fitting at the top of the bottle (Figure 82) by twisting the fitting to unlock it.
3. Pull the fitting out and place the bottle next to the machine on the benchtop.

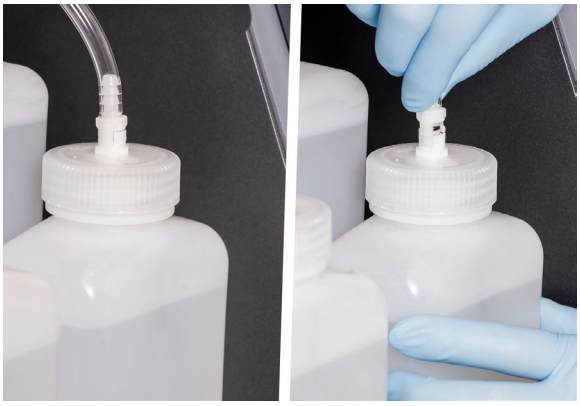


Figure 82. Disconnecting the bleach bottle via the line fitting.

4. Remove the cap from the bleach bottle and properly dispose of any residual sodium hypochlorite solution.
5. Place the bottle back onto the bench, leaving the cap ajar. Fresh solution can be poured through this gap.
6. Refill the bleach bottle with the fresh sodium hypochlorite solution prepared in Step 1.
7. Screw the cap of the bleach bottle back on.
8. Reinsert the fitting into the cap and twist until it locks to ensure a good seal.
9. Place the bleach source bottle back in its proper position on the bleach scale and connect the tubing labeled "Bleach" onto the bleach bottle cap fitting.
10. Go to **User Utilities** and click [Bleach prime] to prime the bleach tubing and flush out bubbles. See Appendix A.C, "Bleach Prime", for more details.

5. Emptying the Waste Container

Dispose of the contents of the waste container if it is filled to more than half capacity. Follow these steps to empty the container.

1. Locate the cap on the waste container and press the quick-release buttons to release the tubing one-by-one from the cap (Figure 83).

Figure 83. Disconnecting the tubing from the waste container cap. The blue arrow is pointing in the image to the tubing connectors which attach to the cap.

2. Carefully set the tubing aside and transport the waste container to the disposal location.
3. Unscrew the cap and properly dispose of the contents.
4. Screw the cap back on and reinstall the container by plugging the tubing fittings back into the ports in the cap.

C. System Shutdown Procedure (<1 month)

If the Shasta instrument will be shut down for less than 1 month, the following procedure can be executed:

1. Check that all nanowell chips and source plates have been removed from the stage module.
2. Follow the Shasta instrument cleaning procedure described above in Section XIV.A, "Clean the Shasta Dispensing Platform".

3. Disconnect the water and humidifier, and bleach bottles following the instructions in the refill procedures above.
4. In **User Utilities**, run the [Bleach prime] (Appendix A, Section C) followed by the [Water prime] (Appendix A, Section D).
5. Dispose the contents of the water, humidifier, and bleach bottles.
6. Reconnect the water, humidifier, and bleach bottles.
7. Safely dispose of the contents of the waste bottle using the procedure from Section XI.A.

D. System Shutdown Procedure (>1 month)

If the Shasta instrument needs to be shut down for longer than 1 month, follow the procedure in Section B., "System Shutdown Procedure (<1 month)" (above), then perform the following additional steps.

8. Exit the CELLSTUDIO software (see Section V.A.7).
9. Turn off the Shasta instrument by toggling the power switch on the back of the system.

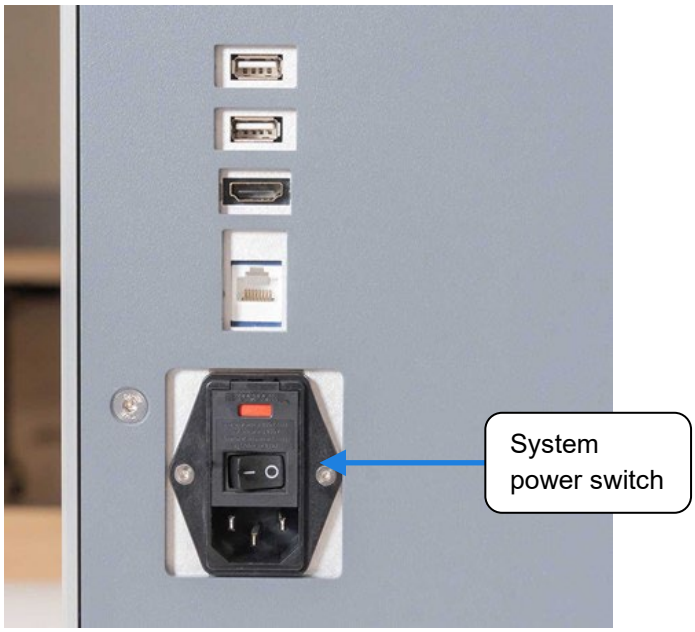


Figure 84. Location of the system power switch.

E. Restarting the Shasta from a Complete Shutdown

1. Turn on the Shasta instrument by toggling the power switch in the back of the system.
2. Refill humidifier, water, and bleach source bottles per Section XIV.B (2–4), "Daily Maintenance".
3. Start up the CELLSTUDIO software by touching the touch power button for > 1 second.
4. Run the Daily Warmup. Refer to Appendix A.A for more details.

F. Annual Preventative Maintenance

The Shasta Single Cell should be examined and calibrated once every six months by a Takara Bio service engineer.

Appendix A. User Utilities

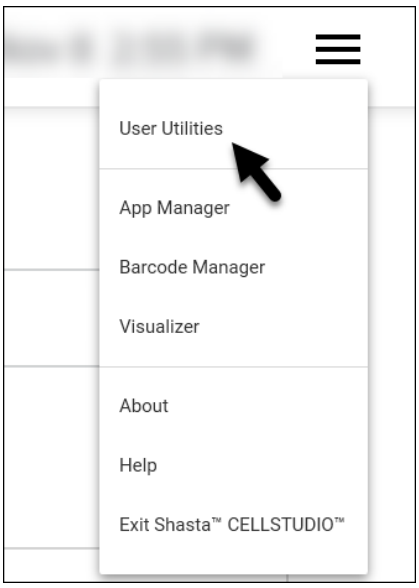


Figure 85. How to access *User Utilities* through the menu.

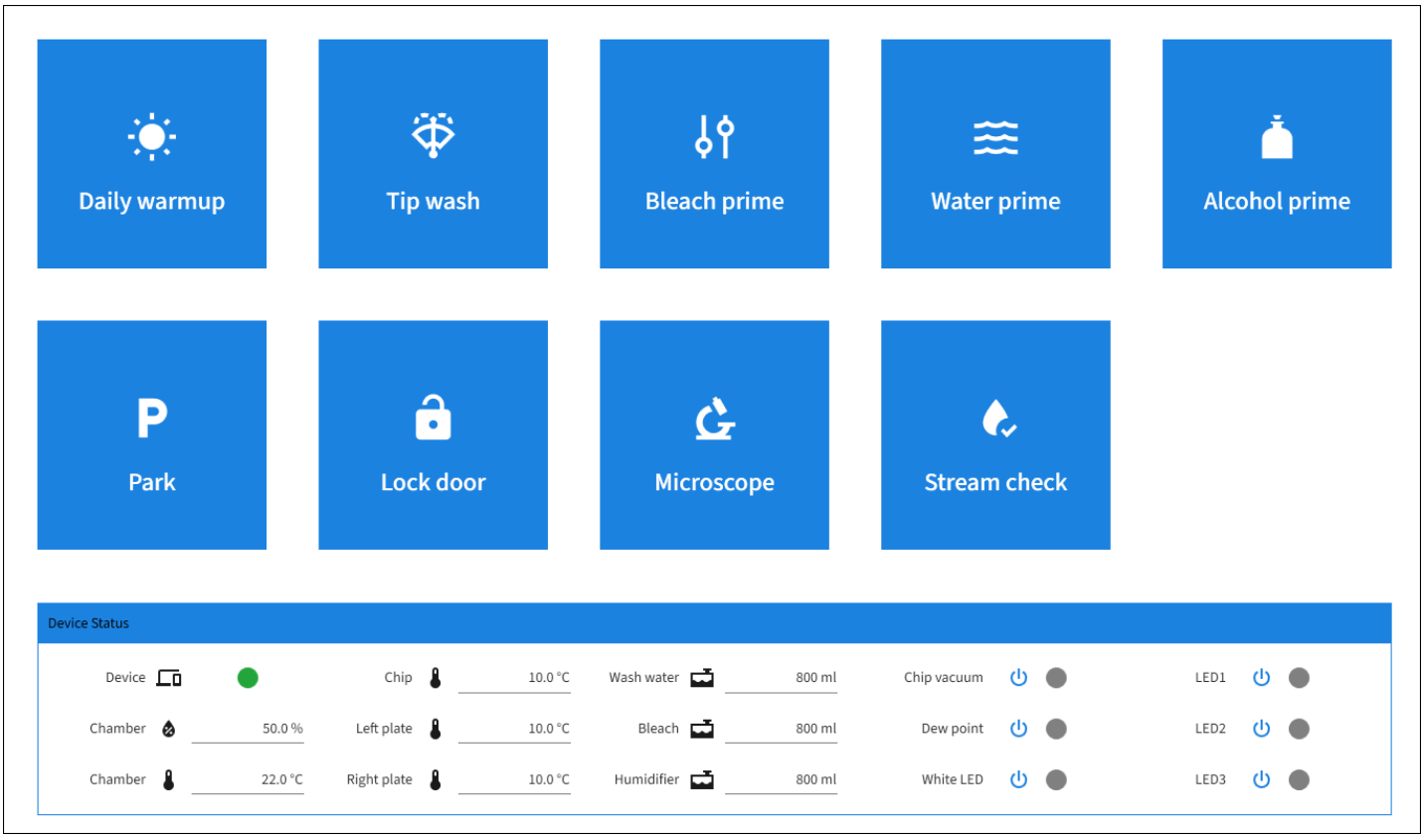


Figure 86. The *User Utilities* menu display.

The User Utilities menu is meant to provide users flexibility to manually run any commonly performed system functions on demand or to access the microscope for optimizing scan settings for their specific cell types and dyes.

See the sections below for a brief explanation of the most used options shown in Figure 86.

A. Daily Warmup

The Daily warmup:

- initializes all hardware,
- primes the water tubing,
- primes the bleach tubing,
- performs an alcohol tip prime, and
- performs a tip wash procedure.

NOTE: The last three actions can also be performed individually through the "Tip wash", "Bleach prime", "Water prime", and "Alcohol prime" options of the User Utilities menu, as described in Sections B–E.

The reminder to run the daily warmup will be displayed on the Action Center once per day but can also be re-run from the User Utilities using the [Daily warmup] button.

Prior to running the daily warmup, the following actions should be taken:

1. Check the three fluidic source bottles (Section XIV.B, "Daily Maintenance") and refill as necessary.
2. Prepare a 384-well source plate with 100 µl of reagent-grade alcohol (or equivalent) in wells A1–D2.
3. Place the source plate into the plate nest of the instrument and close the door.
4. Click the [Daily warmup] button to initiate the warmup sequence.
5. Follow the prompts on the screen to complete the warmup.

After the daily warmup is complete, remove the source plate and discard the alcohol.

B. Tip Wash

The tip wash protocol cleans the tips with bleach and rinses out the tips. Tip washes are automatically performed before and after every aspirate/dispense cycle, but additional tip washes can be manually initiated here.

C. Bleach Prime

When the button is pressed, the user will be asked to confirm when the bleach was replaced last (the date displayed will be the last recorded date). The user can either confirm and prime the fluidic lines or click the [Cancel] button to return to the User Utilities view (Figure 87). Once confirmed, bleach flows from the bleach bottle through the fluidic line to the tip wash trough for a preset amount of time.

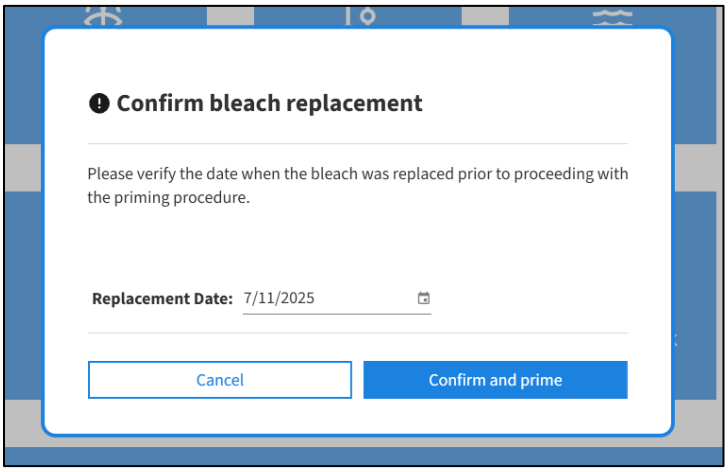


Figure 87. Confirm bleach replacement view.

NOTE: Bleach priming should be performed after the bleach solution is replaced to flush out the old bleach and replenish the bleach trough in the tip wash trough with fresh bleach.

D. Water Prime

This action causes water to flow from the water bottle through the fluidic line to the tip wash trough for a preset amount of time.

This button can be optionally used after replacing the Water source bottle to get rid of bubbles in the line.

E. Alcohol Prime

Alcohol priming helps clean the tips and remove trapped air bubbles, which improves dispense quality. The Alcohol prime is run as part of the daily warmup but can be optionally repeated from the User Utility menu.

Prior to the alcohol prime, the following actions should be taken:

1. Prepare a 384-well source plate with 100 µl of reagent grade alcohol (or equivalent) in wells A1–D2.
2. Place the source plate into the source plate nest and close the door.
3. Click the [Alcohol prime] button to initiate the alcohol prime sequence.

After the process is complete, remove and safely discard the source plate and alcohol.

F. Park

The [Park] button is used place the stage back into the default ("park") position. When parked, the chip and source plate are accessible, and the tips are raised high.

G. Lock/Unlock Door

This toggle button manually locks or unlocks the door.

H. Microscope

This view allows user to visually inspect the cells. This is useful in confirming or exploring the gain and exposure settings needed for custom cell stains.

For more information about this utility, see the Shasta Single Cell Advanced Features User Manual.

I. Stream Check

The stream check is a tool that can be used to check if the tips are clogged or if the streams are at an extreme angle relative to the syringe, changing directions, or collecting as a droplet on the tip.

Actions to be taken prior to running stream check:

1. Open the door.
2. Click [Stream check].

WARNING: To avoid injury from or damage to any moving part, the user should at no point reach into the instrument or stage area during this process.

3. Observe the streams coming out of the tips; they should be relatively straight in relation to the tips and not changing directions. If the streams do not look straight, perform an alcohol prime (Section E) to flush the tips out.
4. After the alcohol prime, re-run the stream check to determine if it addressed the issue.

   If the problem persists, please contact your authorized Takara Bio service technician or field_support@takarabio.com.

Appendix B. Modifying the Scan Filters During Imaging for an Experiment Based on a Custom Application

NOTE: This section only applies to custom applications; the functionality is not available for prevalidated apps.

When imaging the sample during a custom application workflow (Section X, "Protocol: Scanning Chip for Single Cells or Nuclei"), the scan filter values defined in the application display grayed out but can be modified by clicking the [Edit] button.

Figure 88. Editable scan filter settings during imaging step of an experiment based on a custom application.

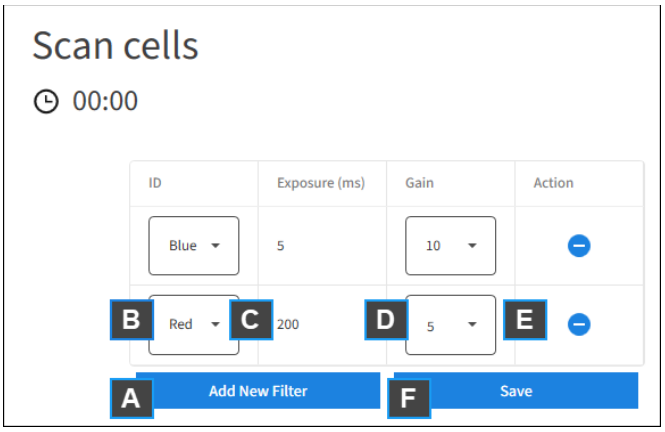


Figure 89. The scan filter settings when editing the values during the imaging step of a custom application workflow.

In the editing view, you can take one or more of the following actions. The letters correspond to the labeled areas in Figure 88.

[A] [Add New Filter]—this will add a new row to the table.

[B] Change the color of LED used for the scan—select a new color from the dropdown menu in the "ID" column of an existing row. Each color can only be associated with one row or configuration.

Table 7. Excitation and emission information for the available color channels.

Color channel	Excitation λ (nm)		Emission λ (nm)	
	Center	Width	Center	Width
Blue	377	50	447	60
Green	485	20	536	40
Red	544	24	641	75

[C] Change the exposure value—the exposure is the length of time in milliseconds the camera sensor spends capturing the image for each well. The exposure time for a given color channel should be in the range of 2–500 ms, although values up to 2,000 ms are possible. To set or edit the value, click on the cell to activate the input box (Figure 89, left) and type in the numerical value desired. Press **[Enter]** on your keyboard to commit the value to the configuration (Figure 89, right).

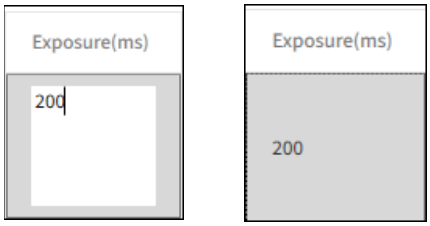


Figure 90. The imaging filter "Exposure(ms)" input box. **(Left)** The input box is shown (white background) after the cell in the column is clicked on and a number can be typed into the field. **(Right)** The cell after committing the change.

[D] Change the gain value—a new gain value can be selected from the dropdown menu. Gain represents the amount of amplification applied to the signal coming from the camera sensor, i.e., the camera sensitivity. Select a value from the drop-down menu of either '5', '10', or '15'. The recommended initial value is '10'.

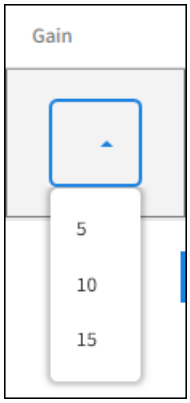


Figure 91. The imaging filter "Gain" drop-down menu.

[E] Delete an existing filter—click the button in the "Action" column.

IMPORTANT: There is no confirmation prompt and no method to undo the deletion. Please use wisely.

[F] [Save] when done with any changes.

For more information on how to add or delete a filter in a custom application, refer to the Shasta Single Cell Advanced Features User Manual, Section III.B, App Manager "Details: Workflow Step ".

Appendix C. Advanced Filter File Configuration

A filter file is automatically generated by CellSelect software after analysis if the chip is imaged. But users might find a need to either edit or manually create a filter file, such as in the case of using nucleic acids as an input—since imaging is not done on nucleic acids, analysis is not performed by the CellSelect software.

The following information describes the structure of a filter file, if editing or manual creation is desired.

A. Filter File Characteristics

- Since the filter file is usually specific to a chip, its file name must start with a five- or six-digit number that represents the chip ID.
- The contents of the file are comma-separated values, so the file name extension should be `*.csv`.
- There is no header row to the contents.
- There are only two possible values in the comma-separated list: 0 and 1.
  - 1 tells the Shasta instrument to dispense to the corresponding well on the Single-Cell chip.
  - 0 means do not dispense to this well.
- The dimensions of the filter file must be large enough to cover the dispense pattern of the selected app (i.e., 72 x 72).
- During the process of dispense, if the chip ID in the filter file name does not match the barcode ID of the chip, you will get a warning message, but you can choose to ignore it and continue.

B. How to Manually Create a Filter File

1. The easiest way to create a filter file is by using MS-Excel or another spreadsheet program. Either:
   - Load an existing filter file created by CellSelect software and modify it.

     -or-
   - In a blank spreadsheet, fill a square of 72 x 72 rows and columns with ones and zeroes (1 and 0). In MS-Excel, this is the range A1:BT72.
2. Save the spreadsheet in CSV format with a `*.csv` file extension.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	1	1	1	1	1	1	0	0	0	0	0	0	
2	1	1	1	1	1	1	0	0	0	0	0	0	
3	1	1	1	1	1	1	0	0	0	0	0	0	
4	1	1	1	1	1	1	0	0	0	0	0	0	
5	1	1	1	1	1	1	0	0	0	0	0	0	
6	1	1	1	1	1	1	0	0	0	0	0	0	
7	0	0	0	0	0	0	0	0	0	0	0	0	
8	0	0	0	0	0	0	0	0	0	0	0	0	
9	0	0	0	0	0	0	0	0	0	0	0	0	
10	0	0	0	0	0	0	0	0	0	0	0	0	
11	0	0	0	0	0	0	0	0	0	0	0	0	
12	0	0	0	0	0	0	0	0	0	0	0	0	
13													

Figure 92. Example of a partial manually created filter file in MS-Excel. Reagents dispensed with this filter file applied will only dispense into the 6 x 6 grid (range A1:F6) containing values of values of '1'.

NOTE: CELLSTUDIO software's dispense algorithm is optimized based on assumptions about the filter file:

- The file represents well contents that are candidates.
- The number of candidate wells, in turn, are dispensed based on Poisson statistics.

Appendix D. Sequencing Data Analysis Guidelines

After library generation and sequencing, the `<chipID>_WellList.txt` file should be exported from the Experiment Summary page (Section XI) and used to demultiplex the data using our bioinformatic software, Cogent™ NGS Analysis Pipeline (CogentAP). More information about CogentAP can be found at the bioinformatics resources portal at takarabio.com.

For customers wanting to use bioinformatics pipelines other than CogentAP, the structure of the file is described in the Shasta CellSelect Software User Manual, Appendix C, Section A ("Wells Data Table"). An excerpt of an example file is shown in Table 8.

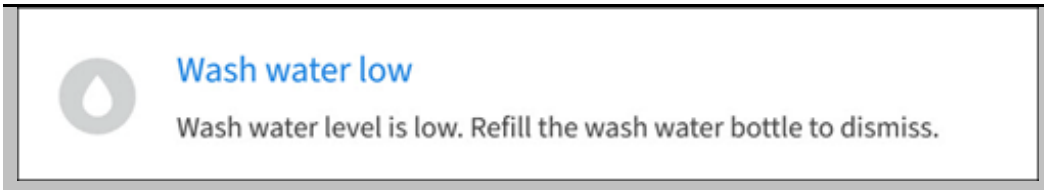
Table 8. Excerpt of an example `<chipID>_WellList.txt`.

Row	Col	Candidate	For dispense	Sample	Barcode
0	0	yes	yes	A1	TGACCGAT+TCAGATTG
0	1	yes	yes	A1	TCTAGGTT+TCAGATTG
0	2	yes	yes	A1	CTGGTCTT+TCAGATTG
0	3	no	no	A1	GTCGTTCT+TCAGATTG
0	4	yes	yes	A1	AGAGTTCT+TCAGATTG
0	5	yes	yes	A1	AGCTGAAT+TCAGATTG
0	6	yes	yes	A1	GACGTATG+TCAGATTG
0	7	no	no	A1	CATAATGG+TCAGATTG
0	8	yes	yes	A1	ATTCAAGG+TCAGATTG
0	9	yes	yes	A1	TGATTCCG+TCAGATTG
0	10	yes	yes	A1	GCAACTAG+TCAGATTG
0	11	no	no	A1	TACGCGAG+TCAGATTG
1	0	yes	yes	A1	TGACCGAT+CTATCGTT
1	1	yes	yes	A1	TCTAGGTT+CTATCGTT
1	2	yes	yes	A1	CTGGTCTT+CTATCGTT

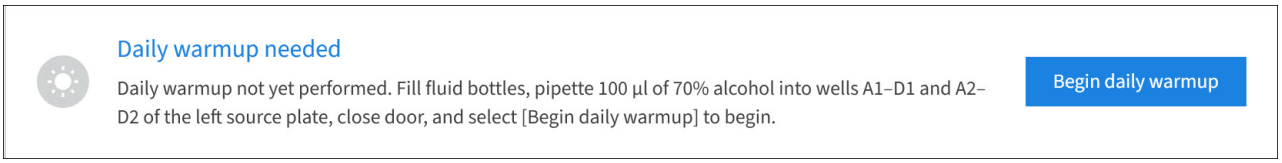
Appendix E. Troubleshooting Guide

Table 9. ***Action Center*** **alerts.**

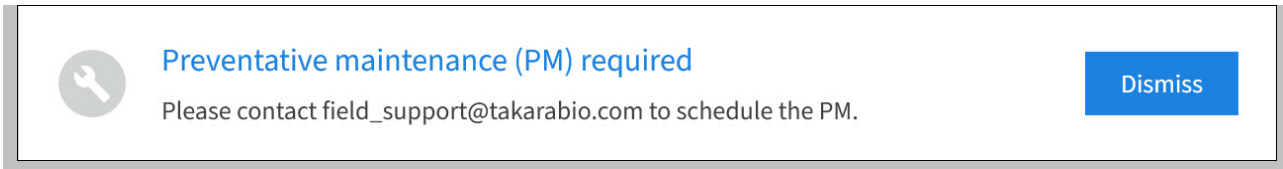
System not ready indicates to the user that not all the action alerts have been resolved yet. This can be skipped if the alerts present are not essential to the system use.



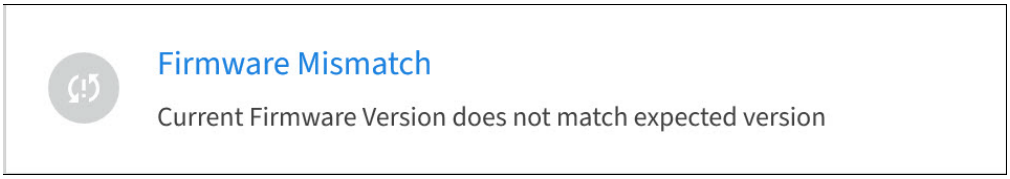
Low Fluid Level Alerts If any of the source fluid bottles (Water, Humidifier, Bleach) are running low on fluid, the low water warnings will be displayed. After refilling the source bottles with respective fluids and placing the bottles back on the scales, the alert message should automatically disappear.



Daily Warmup should be completed daily for optimal system performance. This will home all motors, prime fluid lines and perform a tip wash. It is recommended to run the daily warmup immediately prior to the first dispense of the day.

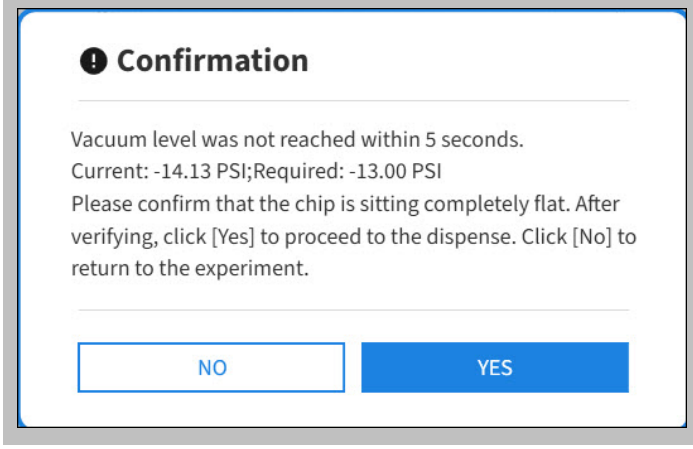
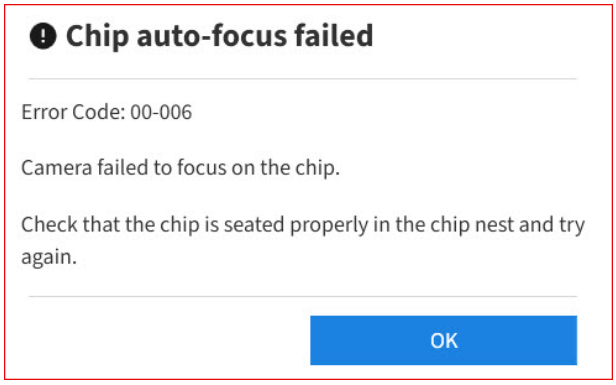
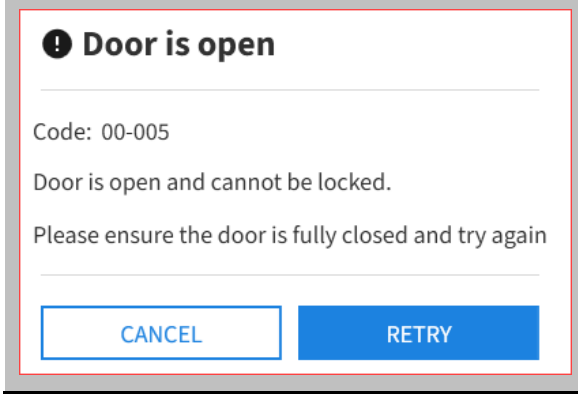


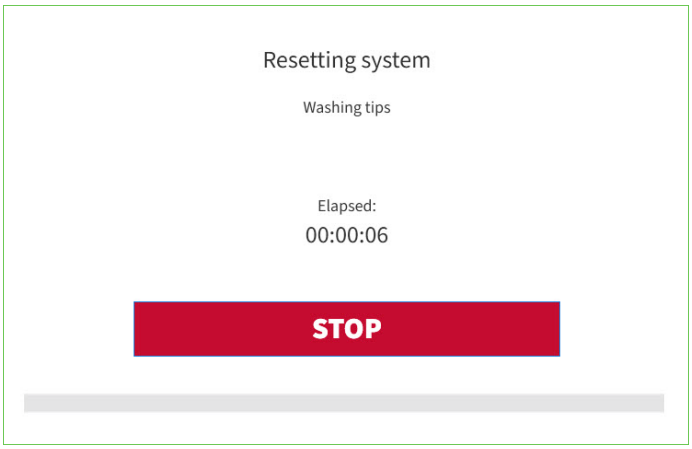
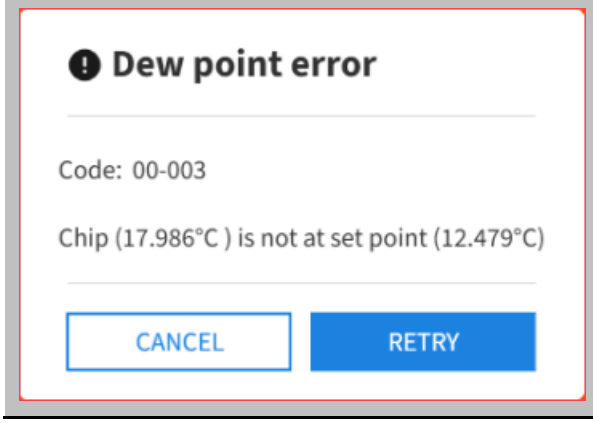
Preventative maintenance (PM) is recommended to be performed once every six months. This notification will appear nine months after the last preventative maintenance appointment to remind users that it should be performed soon. Please contact your authorized Takara Bio service technician or field_support@takarabio.com to arrange for the service visit. This warning may be dismissed but proceed to use at your own risk.



Firmware Mismatch may occur in a rare situation if the instrument firmware gets out of sync with current software version, potentially after a system update. Please contact your authorized Takara Bio service technician or field_support@takarabio.com to report this error.

Table 10. Potential CELLSTUDIO pop-up messages.

Message displayed	Possible explanation	Solution
Chip vacuum, or "Vacuum level was not reached within 5 seconds" message. 	May occur if the chip is not sitting flush inside the chip nest or if there is debris under the chip	Please check that the chip is in good contact with the chip nest base and that the chip nest surface is free of debris. If this error continues, please contact your authorized Takara Bio service technician or field_support@takarabio.com as there could be a leak in the vacuum hardware. Generally, it is safe to proceed if the chip has been verified to be sitting fully flat inside the chip nest.
Chip auto-focus failed message, "Camera failed to focus on the chip". 	May also display if the chip is not seated properly (fully flat inside the chip nest), as the camera may not be able to properly image the wells.	Reseat the chip in the nest and click [OK] to try again. If the error persists, contact your authorized Takara Bio technician or field_support@takarabio.com
Door is open, "Door is open and cannot be locked." 	The door to the instrument is open or not completely closed when starting a dispense	Open the door all the way and close it again, then retry the dispense.

Message displayed	Possible explanation	Solution
"Resetting system", Washing tips 	This message displays after the system encountered an unexpected state, such as after stopping a workflow step in-progress.	This message only displays while executing the recovery process after user acknowledgement. Wait until the reset completes and follow the prompts on screen as needed. If the initial cause prompting the reset persists, contact your authorized Takara Bio technician or field_support@takarabio.com.
Dew point error, "Chip is not at set point" 	The chip nest fails to reach the dewpoint temperature	Click [Retry] to have the system check the dew point again. If this error persists, contact your authorized Takara Bio technician or field_support@takarabio.com as there could be an issue with the dewpoint control system.

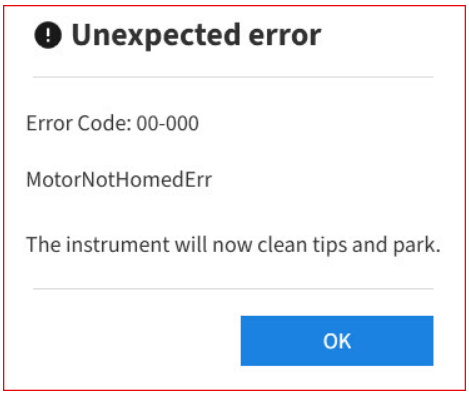
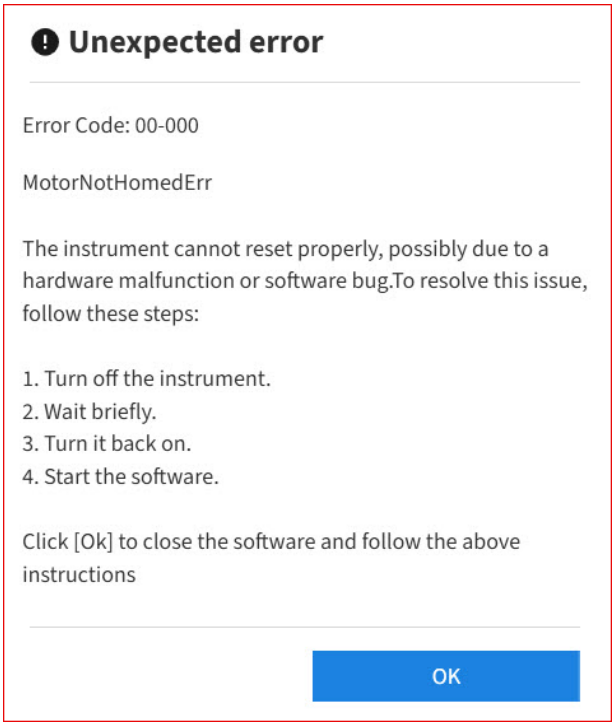
Message displayed	Possible explanation	Solution
Motor Not Homed, "MotorNotHomedErr"   	This will only occur if the instrument did not boot properly in the expected 'home' position	After clicking [OK], the system will restart CELLSTUDIO software and home the motor of the Single Cell instrument after the reboot. Follow the four steps from the bottom image to continue.

Table 11. Instrument and workflow troubleshooting guide.

Problem	Possible Explanation	Solution
Large damp spots visible on the blotting paper. Visible liquid on the surface of the chip after dispense. 	An indication that dispense was unsuccessful.	If this is occurring with a prevalidated application, contact your field support scientist or email field_support@takarabio.com. If seen during development of a custom application, you may need to make the reagent more or less concentrated and/or dispense a different volume to accommodate for the final concentration in the reaction.
Water is seen under the front right corner of the instrument. 	In the very rare occasion that there is a leak in the humidifier module, the system has been designed to route the fluid to a safe location at the front right side of the instrument. This may create a small puddle of water in the location of the circle on the image to the left.	Stop using the instrument and contact your authorized Takara Bio service technician or field_support@takarabio.com.

Appendix F. Instrument Certification and Standards Information

The Shasta system fulfills the following requirements:

UL 61010-1:2012 R4.16, UL 61010-2-081:2015, CAN/CSA-C22.2 NO. 61010-1-12 + GI1 + GI2, CAN/CSA-C22.2 NO. 61010-2-081:15, EN 61010-1:2010.

Safety specifications are also met under the following environmental conditions, which are in addition to those stated in the operating conditions:

- **Installation Category (overvoltage category) II, according to IEC 60664-1.** The Installation Category defines the level of transient overvoltage in which the instrument is designed to withstand safely. It depends on the nature of the electricity supply and its means of overvoltage protection. For example, in CAT II, which is the category typically used for instruments in hospital, research, and industrial laboratories, the expected transient overvoltage is 2,500 V for a 230-V supply and 1,500 V for a 120 V supply.
- **Pollution Degree 2, according to IEC 60664-1.** Pollution Degree 2 assumes that normally only nonconductive pollution (e.g., dust) are present in the operating environment, with the exception of occasional conductivity caused by condensation.

Both the Installation Category (overvoltage category) and the Pollution Degree affect the dimensioning of electrical insulation within the instrument.

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This document has been reviewed and approved by the Quality Department.