

Takara Bio USA, Inc.

ICELL8® cx Single-Cell System User Manual

Cat. Nos. 640188, 640189, 650001
software v2.0
(122419)

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I. Introduction

A. Thank You for Your Order!

Congratulations on the purchase of your ICELL8 cx Single-Cell System (referred to as ICELL8 cx system, instrument, or unit in this manual). The ICELL8 cx system is designed to load samples into chips and then perform imaging.

B. What's New for 2.0

ICELL8 cx CELLSTUDIO™ v2.0 Software, the open architecture software for the ICELL8 cx system, further expands on the flexibility of the platform. Enhancements to workflow customization include:

- New application manager and application editor—allows researchers to design their own ICELL8 cx system dispense flows easily using a software interface
 - Choose from a number of sample source options, such as cells or nucleic acids
 - Configure the number of wells in your dispense pattern: from a 12 x 12 grid (144 nanowell) up to a 72 x 72 grid that utilizes an entire ICELL8 cx chip
 - Import barcode identification files into your application: either from Takara Bio prevalidated chemistries or create your own
 - Select your own reagents and/or indexes, from a range of dispense volumes
 - Decide the order of those dispense steps – rearrange, delete, and add as needed
- Improved barcode handling—barcodes defined in the application manager are automatically forwarded to ICELL8 cx CellSelect® v2.0 Software (referred to as CellSelect software)—no more need to select them a second time
- A cell re-suspension feature (e.g., “Pause” function)—this allows users to re-suspend samples or reagents that may settle quickly—improving the uniformity of cell distribution and increasing the number of single cells present in the chip
- Applications designed by Takara Bio are still standard options within the software, making it as easy as ever to take advantage of our ready to use prepackaged kits for experiments full-length transcriptome analysis, TCR, and more.








C. About this Manual

This manual provides instructions for the safe operation and maintenance of the ICELL8 cx system:

Symbols and Conventions

The following symbols and conventions (Table 1) are used throughout this manual.

Table 1. User manual symbols and conventions

Symbol	Description
	DANGER: Indicates a hazardous situation that could result in death or serious injury.
	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 symbol.
	Indicates the presence of a biological hazard. Proceed with caution. This symbol may appear next to either a WARNING or CAUTION symbol.
	Indicates the presence of a mechanical or pinch hazard. Proceed with caution. This symbol may appear next to either a WARNING or CAUTION symbol.
	IMPORTANT: Provides information on proper system operation.
NOTE:	NOTE: Provides helpful ancillary information to support the use of the system.

D. Technical Support

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

E. ICELL8 cx Instrument Safety Information



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. The covers protect the user from moving parts 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 ICELL8 cx only inside an appropriate building. Do not operate the ICELL8 cx outside or in wet environments.

Instrument use:



WARNING: Use of the ICELL8 cx Instrument 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 ICELL8 cx 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.



WARNING: The machine is powered by two cords. The instrument is off only when the power switch is off, and the ethernet power cord is disconnected. See image below.

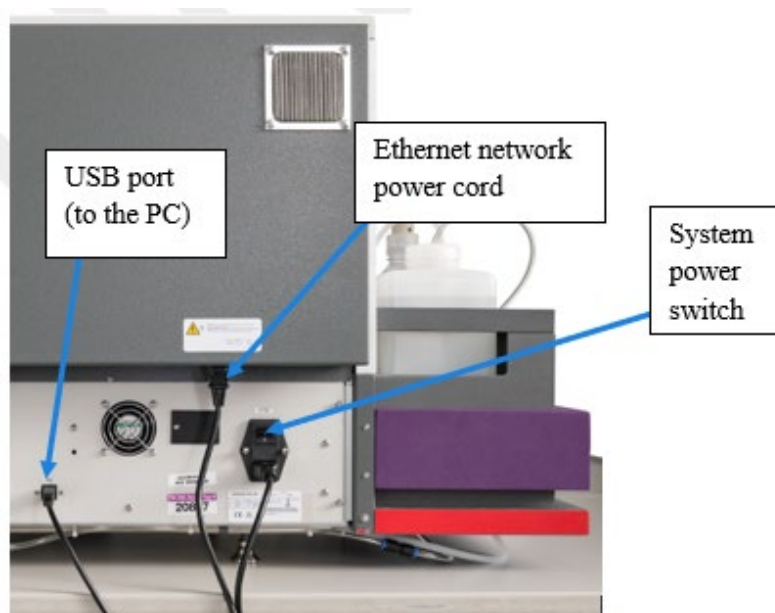


Figure 1. The ICELL8 cx Instrument.

Certification and standards information:

The ICELL8 cx fulfills the following requirements: EN 61010-1:1993 + A2:1995/IEC 61010-1:1990 + A1:1992 + A2:1995.

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.

Moving the system:



WARNING: If you need to move the system after it has been installed, please contact Takara Bio technical support.

Warning labels on the instrument:

Please note the warning label on the instrument.



WARNING: This system contains moving parts. Keep hands away from the system while the instrument is in use.

II. System Description: Component Overview

The complete ICELL8 cx Single-Cell System (Cat. No. 640188, 640189*) includes the components listed below.

ICELL8 cx Single-Cell System (Cat. No. 640188, 640189*)	
Component	Quantity per kit
ICELL8 cx Instrument	1
ICELL8 cx CELLSTUDIO v2.0 Software	
ICELL8 cx CellSelect v2.0 Software	
ICELL8 cx Thermal Cycler*	1
Regional Power Cord*	5
Universal Power Strip	1
ICELL8 cx Accessory Kit	1
2 x ICELL8 cx Centrifuge Chip Spinner	
1 x ICELL8 cx Centrifuge Balance	
1 x ICELL8 cx Cold Block	
1 x Nanodispenser Blotter	
2 x ICELL8 Chip Holder	
1 x Magnetic Tube Stand	
1 x Plate Seal Applicator	
ICELL8 cx Installation Kit	1
1 x Imitation Master Mix with UV Dye & Rox (45 ml)	
1 x Nanodispenser Alignment Chip	
1 x Nanodispenser Chip Alignment Film (Pack of 10)	
1 x Blotting Paper (Pack of 10)	
3 x 384-Well Source Plate Seal	
3 x 384-Well Source Plate	
2 x Chip Balance	
1 x Handheld Magnifier with UV Illumination	
2 x ICELL8 Extraction Fixture – L	
ICELL8 cx Loading Kit**	3
2 x Blotting Paper	
1 x Optical Imaging Film	
1 x TE Sealing Film	
1 x SmartChip™ Intermediate Film	
ICELL8 Collection Kit – L**	3
1 x Collection Fixture – L	
2 x Collection Tube (2.0 ml)	
1 x Collection Film	
ICELL8 384-Well Source Plate and Seal**	1
5 x 384-Well Source Plate	
5 x 384-Well Source Plate Seal	
ICELL8 cx Blank Chip - 250 nl FB**	3
ICELL8 Blank Chip Reagent Kit**	3
2 x 1X ICELL8 Fiducial Mix (25 µl)	
2 x 100X Second Diluent (15 µl)	

ICELL8 3' DE Reagent Kit**	2
5 µl Control K-562 RNA (1 µg/µl)	
5 µl MgCl ₂ (1 M)	
65 µl 5X First-Strand Buffer	
35 µl SeqAmp™ PCR Buffer (2X)	
30 µl SMARTScribe™ Reverse Transcriptase (100 U/µl)	
10 µl SeqAmp DNA Polymerase	
20 µl Triton X-100 (10%)	
5 µl ICELL8 3' DE Oligo Mix	
10 µl DTT (100 mM)	
60 µl GC Melt (5 M)	
25 µl dNTP Mix (25 mM each)	
15 µl Second Diluent (100X)	
25 µl ICELL8 Fiducial Mix (1X)	
15 µl RNase Inhibitor (40 U/µl)	
10 µl Primer P5 (5 µM)	
500 µl Nuclease-Free Water	
ICELL8 cx 3' DE Chip**	2

*640189 is the ICELL8 cx Single-Cell System for Japan. It does not include a thermal cycler, and therefore only includes four power cords.

**This component is also sold separately.

III. System Description: Instrument Overview



Figure 2. The ICELL8 cx Instrument (external view). This image is for Cat. No. 640188. Note: A thermal cycler is not included in Cat. No. 640189.

The ICELL8 cx Instrument includes the following main components:

- A. Desktop computer and monitor
- B. Water reservoir and electronic scale
- C. Wash bottle
- D. Humidifier (not visible) and humidifier reservoir
- E. Waste container

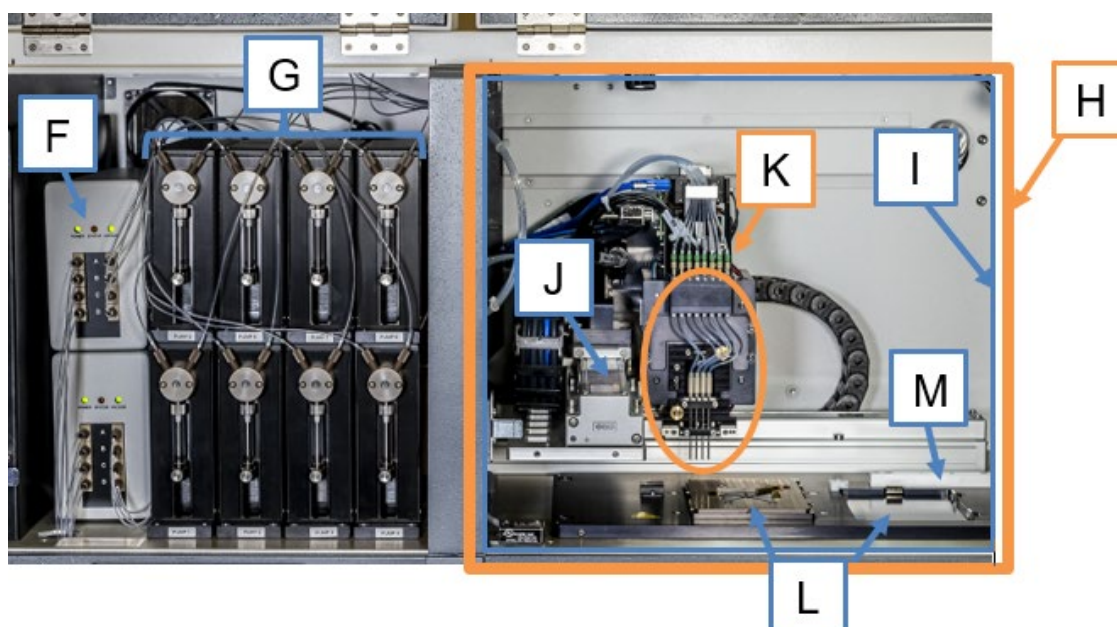


Figure 3. The ICELL8 cx Instrument (internal view).

Behind the front access doors are the following components:

- F. Degassers
- G. Syringe pump bank
- H. Environmental chamber
- I. cx stage module
- J. XY transitional axes
- K. Tip nozzle assembly & optics module
- L. Chip nest (left) & 384-well plate nest (right)
- M. Trough

Water reservoir and electronic scale

The water reservoir contains deionized Milli-Q water (or equivalent) that occupies all fluid paths in the ICELL8 cx Instrument. The liquid is used to draw reagents into and dispense out of the tips. The water reservoir sits on an electronic scale that monitors the water level so that users can make sure there is enough water prior to starting a chip-dispense operation.

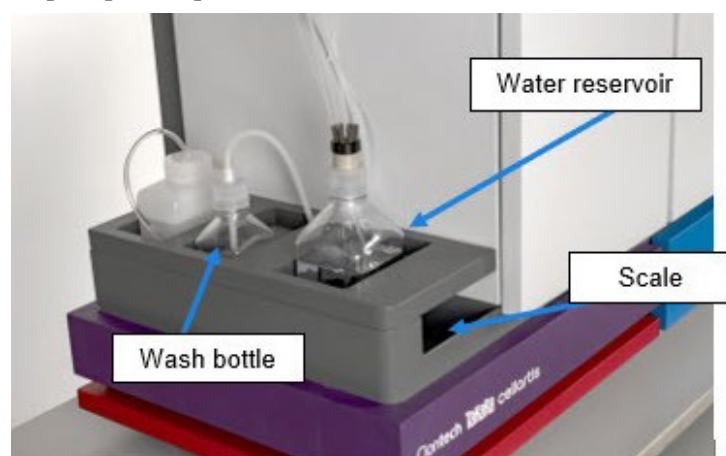


Figure 4. Water reservoir seated on the electronic scale.

Wash bottle

The wash bottle is the source for the wash well in the trough. It should be filled with a 0.2% sodium hypochlorite solution, which is used for cleaning the tip nozzles after dispenses to prevent cross-contamination.

Humidifier and humidifier reservoir

The humidifier is used to maintain the relative humidity levels in the environmental chamber to minimize reagent evaporation during the dispensing process. A sensor embedded in the platform base is used to monitor the enclosure temperature, relative humidity, and chip temperature. The system adjusts the humidity and chip temperature so that the chip is at or near the dew point temperature, which minimizes evaporation during sample dispensing. The humidifier is behind the back cover of the ICELL8 cx unit (Figure 5, below).

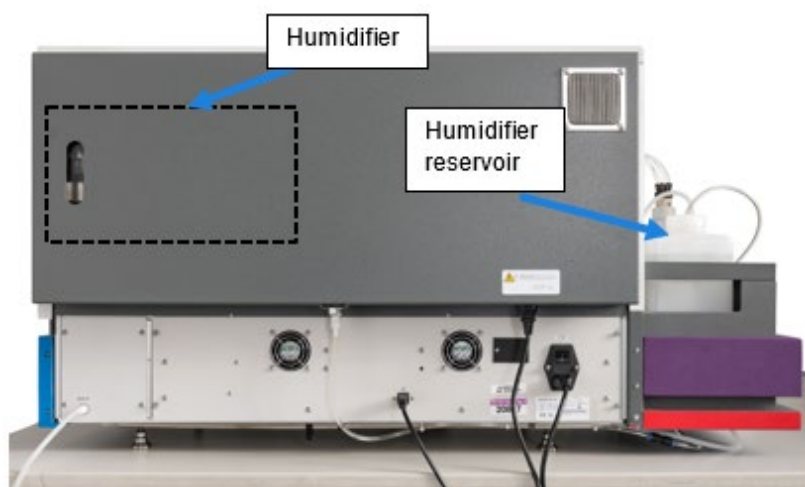


Figure 5. Humidifier unit. The dotted-line box indicates the approximate location of the humidifier assembly under the back cover.

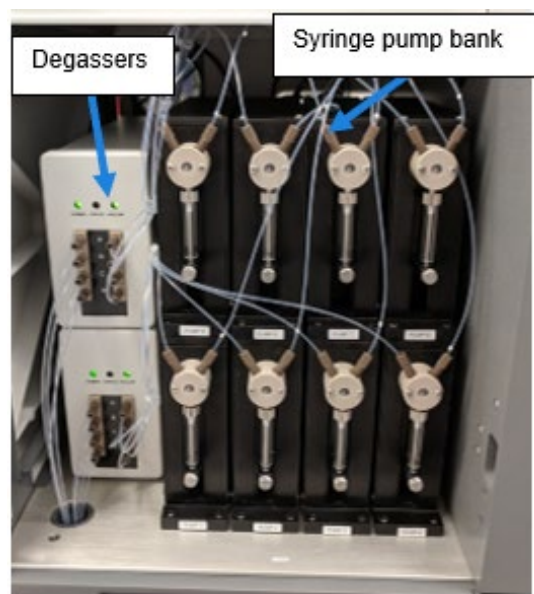


Figure 6. Degassers and syringe pump bank.

Degassers

The degassers sit to the left of the syringe pump bank. Their purpose is to remove dissolved gas from the system fluid, helping the system maintain good dispense quality.

Syringe pump bank

The syringe pump bank consists of eight hydro-pneumatic systems that drive the aspiration and dispensing of samples and reagents in the ICELL8 cx Instrument. A system of tubing connects the tip nozzle assembly and the syringe pumps. The syringe pumps are used in tandem with solenoid valves to generate discrete droplets during a dispense sequence.

Waste container

The waste container collects the fluid that is discarded through the tip nozzles into the rinse and waste wells of the trough.

Stage module

The stage module houses the XY transitional axes, tip nozzle assembly and optics module, chip and source plate nests, and trough. The XY transitional axes transport the tip nozzle and optics module around the stage. When the door to the stage module is closed, it creates an environmental chamber that encompasses the stage module to maintain 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 reads the chip barcode. 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 for cleaning. After a sample is dispensed, the optics module scans the chip to generate a map (a.k.a. filter file) of the wells that contain single cells. The dispensing process is repeated for all downstream reagents, but they will only be dispensed to the wells that are flagged in the filter file.

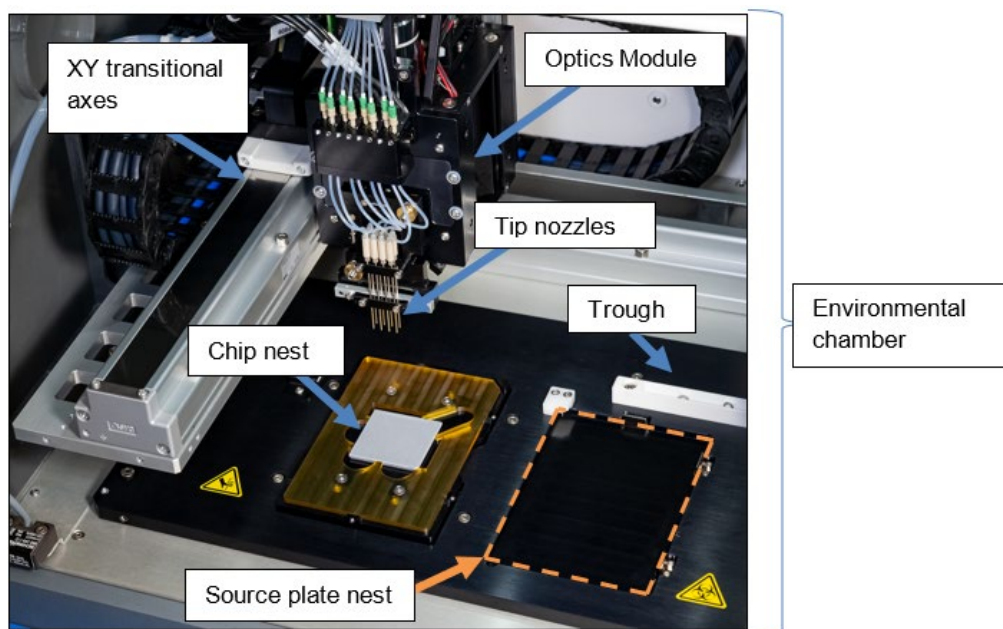


Figure 7. ICELL8 cx stage module. The environmental chamber refers to the stage module area and the chassis enclosing it.

Trough

The trough consists of three wells. From left to right they are the “Rinse”, “Waste”, and “Wash” wells. The rinse well is 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.

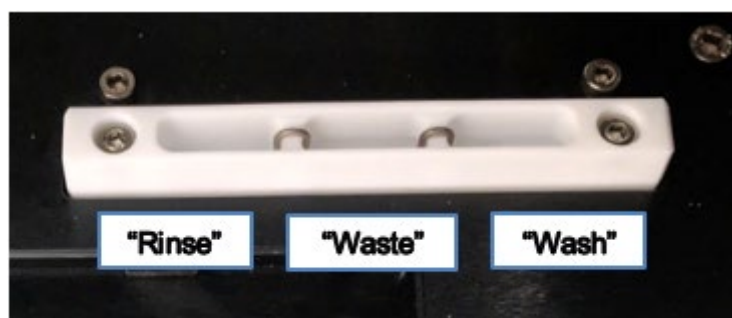


Figure 8. The trough component.

A. ICELL8 cx Specifications and Lab Requirements

Table 2. ICELL8 cx specifications and lab requirements

Category	Specification
Dispense volume	35 nl, 50 nl, or 100 nl per nanowell
Software	ICELL8 cx CELLSTUDIO v2.0 Software (preinstalled)
Computer	Enterprise-level Windows 10 PC (included with system)
Power requirements (for different electrical grid types)	120 VAC/60 Hz mains: four 120 V, 15 or 20A circuits 220–240 VAC/50 Hz mains: four 10A outlets 100 VAC/50–60 Hz mains: four 15A outlets
Fuses	Dispenser: 4 Ah, 250 V
Environmental conditions	Ambient temperature: 15–30°C Relative humidity, noncondensing: 30–70% Altitude: <2,000 m from sea level Pollution Degree: 2 or less
Dimensions	Desktop computer tower: 7" W x 14.5" H x 17.5" D (18 cm x 37 cm x 44 cm) ICELL8 cx Instrument: 42.5" W x 23" H x 23" D (108 cm x 58 cm x 58 cm)
Bench space	Bench space including clearance for ICELL8 cx module and desktop computer: 70" W (minimum) x 38" H (minimum) x 30" D (178 cm x 97 cm x 76 cm) <i>Note: Bench space must be capable of supporting 200 pounds (91kg)</i>
Floor space	Waste container: 10" W x 10" H x 10" D (25 cm x 25 cm x 25 cm)
Weight	200 pounds (91 kg)

B. Setup and Installation

Your Takara Bio Service Engineer will unpack and install your ICELL8 cx Instrument and explain the basic operation of the system. They will use material from the ICELL8 cx Installation Kit to qualify the instrument after installation and will leave any reusable and/or remaining materials at your site. Table 3 (below) lists the ICELL8 cx Installation Kit components.

Table 3. ICELL8 cx Installation Kit components

Component
Nanodispenser Alignment Chip
Nanodispenser Chip Alignment Film
Imitation Master Mix with UV Dye & Rox (45 ml)
Blotting Paper (Pack of 10)
384-Well Source Plate (3)
384-Well Source Plate Seal (3)
Chip Balance (2)
Handheld Magnifier with UV Illumination
ICELL8 Extraction Fixture – L (2)

NOTE: To avoid contaminating your PCR, do not install the ICELL8 cx Instrument in an area that could contain high-copy DNA or amplicons from previous PCR experiments.

C. Required Reagents and General Lab Equipment

Reagents for ICELL8 cx 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 system tubing
- Refer to application-specific user manuals for details about required samples and reagents

General Lab Equipment

- Secondary containers (e.g., 1-L or 500-ml bottles) for refilling reservoirs and for alcohol priming
- Kimwipes or similar dry wipes for cleaning the stage module
- Refer to application-specific user manuals for any other required equipment

IV. System Description: SmartChip Technology

SmartChip technology distinguishes Takara Bio's platform from other systems. Each chip has a 72 x 72 array of nanowells and can accommodate up to 5,184 reactions in a single run.

The ICELL8 cx unit transfers samples, controls, and reagents from the 384-well plate to the chip. After dispensing, the chip can be processed for cell analysis (Figure 9).

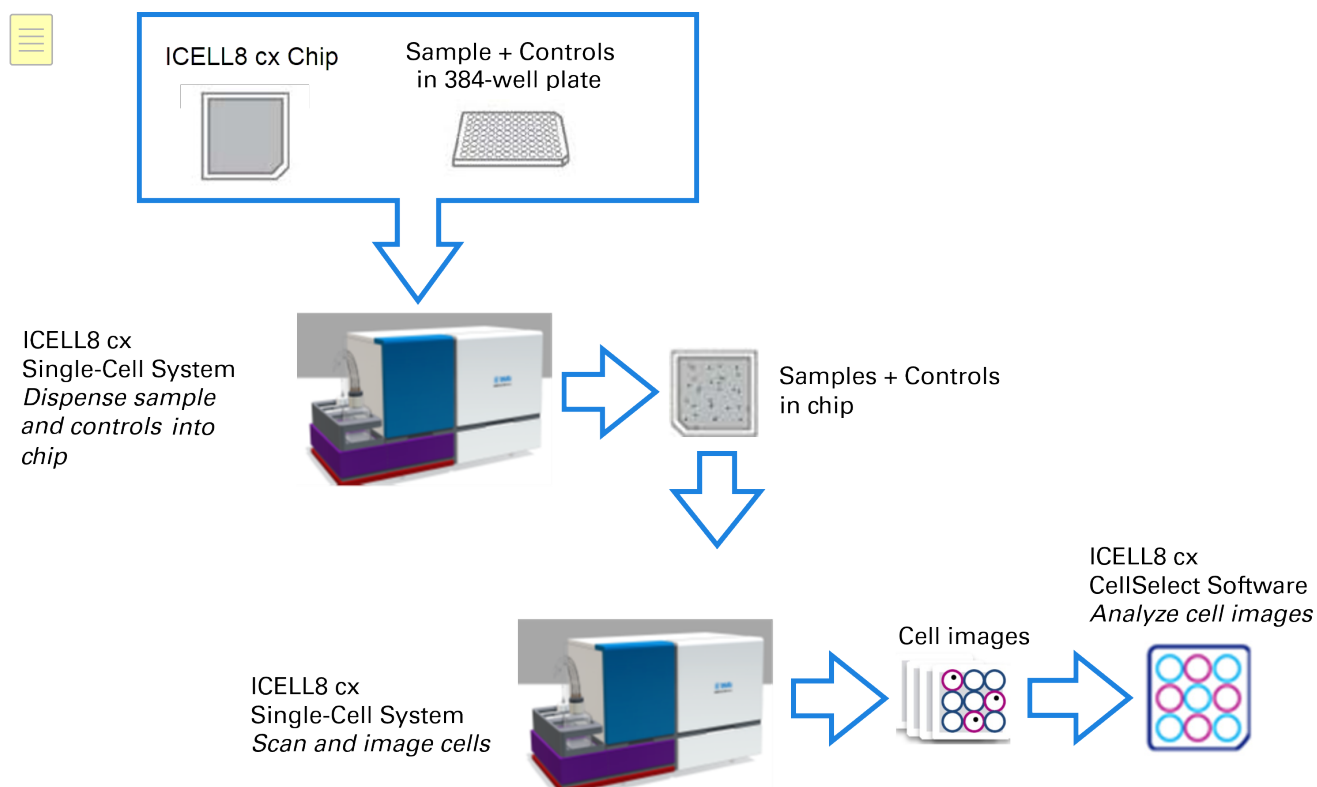


Figure 9. ICELL8 cx cell scan workflow.

V. System Description: CELLSTUDIO & CellSelect software

A. CellSelect Software Overview

ICELL8 cx CellSelect v2.0 Software (referred to as CellSelect software) analyzes images generated with the ICELL8 cx Instrument 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

More information about CellSelect software can be found in the user manual “ICELL8 cx CellSelect v2.0 Software User Manual” available for download at takarabio.com.

B. CELLSTUDIO Software Overview

The ICELL8 cx Single-Cell System comes preinstalled with ICELL8 cx CELLSTUDIO v2.0 Software (referred to as CELLSTUDIO software). CELLSTUDIO software is your flexible, customizable interface with the dispensing system of the ICELL8 cx Instrument, from preparation and maintenance of the instrument to implementation of the SmartChip technology described above in [Section IV](#).

C. CELLSTUDIO Application Manager

The application manager is a feature of CELLSTUDIO software that allows for the creation, modification, and management of application workflows for use with the ICELL8 cx Single-Cell System. The prevalidated applications defined by Takara Bio, such as single-cell full-length transcriptome analysis, come configured within CELLSTUDIO software by default. By using the application manager, additional workflow options become available for selection and use on the main CELLSTUDIO software interface.

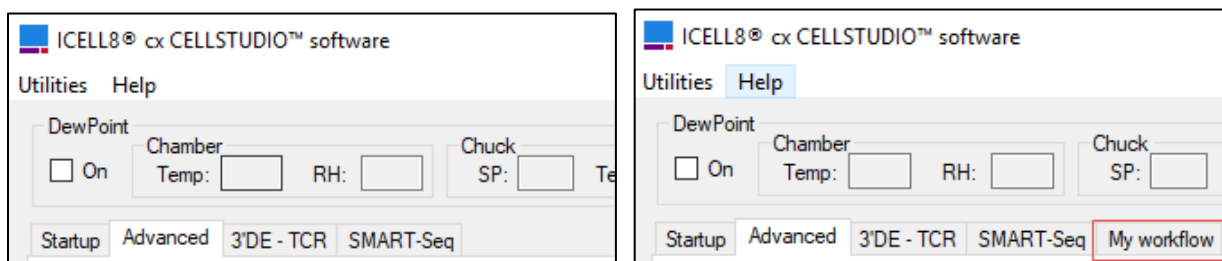


Figure 10. The tabs on the CELLSTUDIO software interface. (left) The 3'DE-TCR and SMART-Seq® tabs are default applications. **(Right)** When custom applications are defined through the application manager, those workflows are created as new tabs to the right of the defaults. The red box highlights a custom app added to the menu tab options.

The application manager can be accessed from within the CELLSTUDIO software from the **Utilities > Application Manager...** menu option.

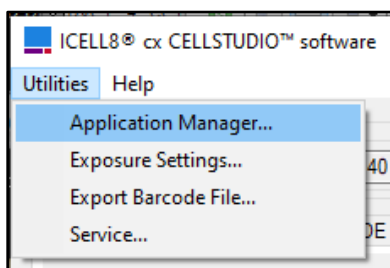


Figure 11. How to access the CELLSTUDIO application manager.

The *Application manager* window will pop up.

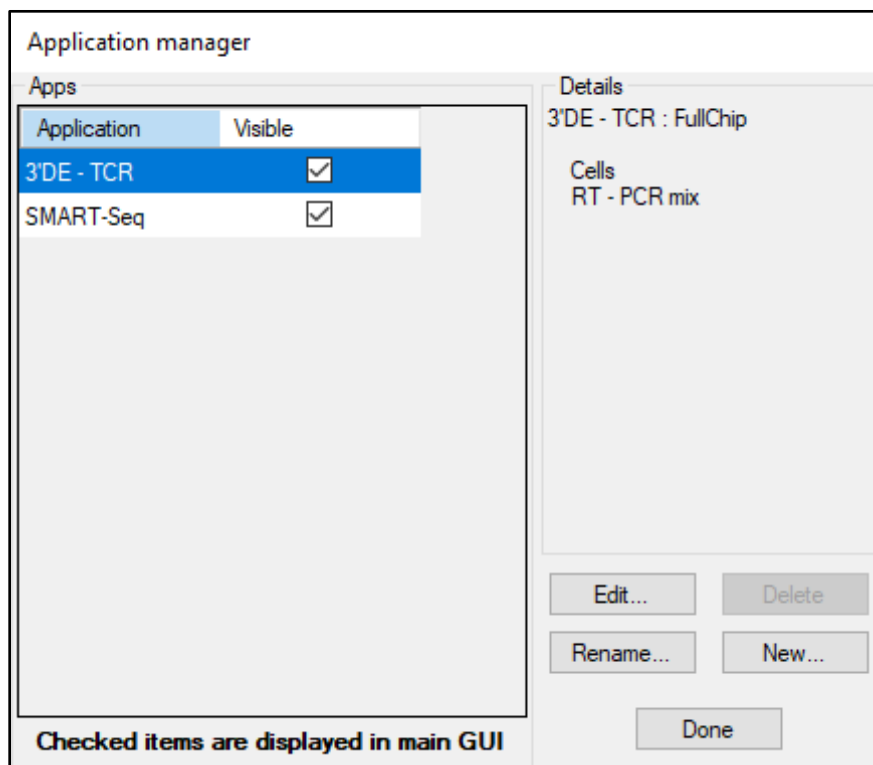


Figure 12. The default view of the *Application manager* window.

The functions of this interface are listed and described below.

1. Apps

The *Apps* window lists all the applications currently configured in the CELLSTUDIO software. In Figure 12 (above), the two applications listed—*3'DE - TCR* and *SMART-Seq*—are the default Takara Bio applications which are prevalidated. Any applications created through the application editor ([Section VII.A](#)) would also be listed here.

The application tabs displayed in the main CELLSTUDIO window can be modified by the check box in the “Visible” column of the application manager.

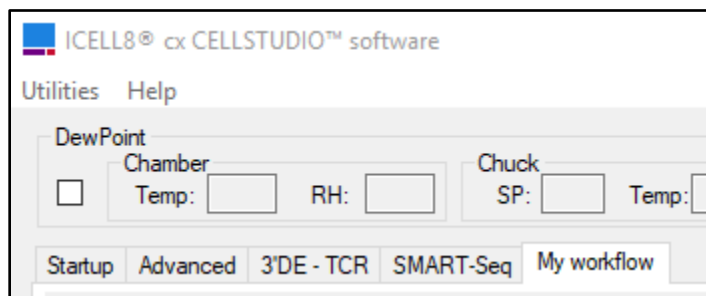


Figure 13. A partial view of the main CELLSTUDIO window. The tabs listed along the bottom edge of the images are two default CELLSTUDIO functions and three applications (e.g., the two predefined apps and a custom app, *My workflow*).

Applications currently visible as tabs in the main window can be hidden by unchecking the box next to the Application name, as shown in the images in Figure 14. Conversely, an application not currently available on the main window can be made available for use on the main window by checking the “Visible” box.

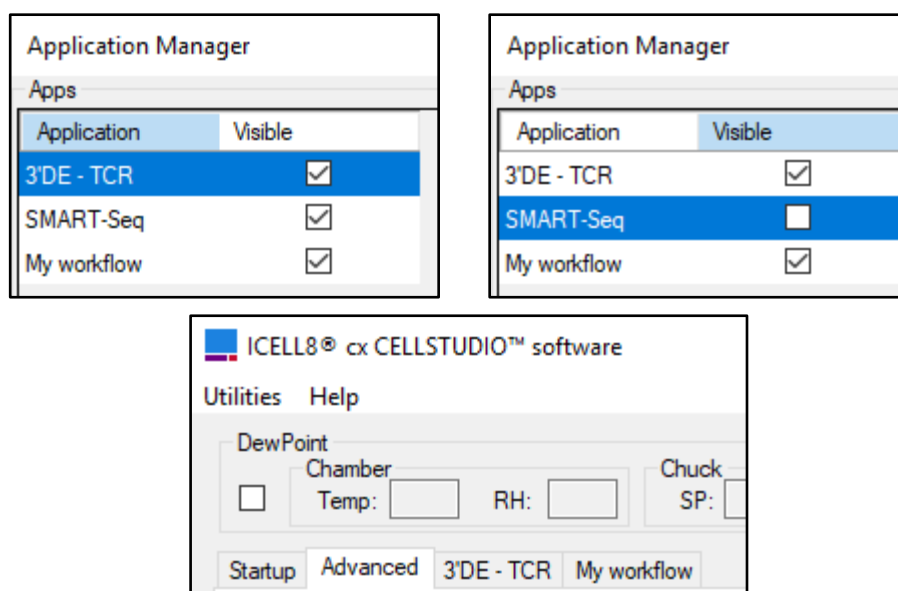


Figure 14. Example of hiding an application from the main CELLSTUDIO window. (Top left) All three applications listed in the application manager have the “Visible” box checked. (Top right) The SMART-Seq application is unchecked. (Bottom) The view of the main window after clicking [Done] in the application manager. Note the *SMART-Seq* tab displaying in Figure 13 is now missing from the bottom image.

2. Details

The *Details* pane of the *Application Manager* window provides a brief summary of the configuration of the selected workflow. Figures 15 & 16 illustrate examples:

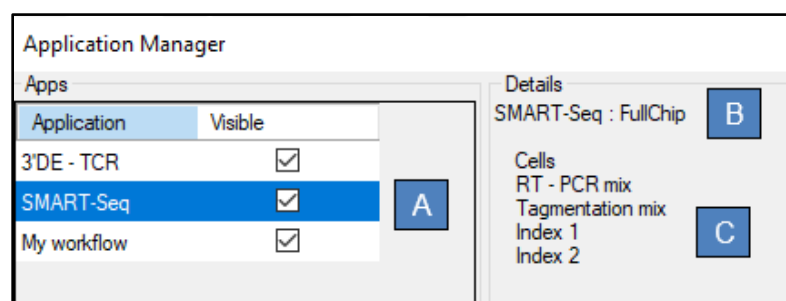


Figure 15. A partial view of the *Apps* list and *Details* pane of the *Application Manager* window for a predefined application.

- The name of the application.
- The dispense pattern defined for the workflow in the *Properties* window of the application editor. (See [Section VII](#) for more information about this window.)

NOTE: The text ‘FullChip’ indicates a 72 x 72 pattern.

- The list of dispense steps. The step names are either the ones defined in the defaults or the names as configured by a user for a custom application. The steps are listed in the order of sample source, reagents 1–6, and index 1–2.

A second example, showing a custom workflow and a 48 x 48 dispense pattern is illustrated below:

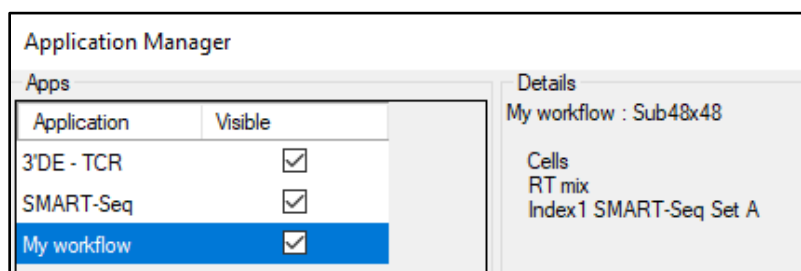


Figure 16. A partial view of the *Apps* list and *Details* pane of the *Application Manager* window for a custom application.

3. [New...]

The [New...] button is clicked to create a new custom application for use in the ICELL8 cx system. This is covered in detail in [Section VII](#), creating a custom application.

4. [Edit...]

NOTE: The default 3'DE - TCR and SMART-Seq applications cannot be overwritten if edited.

Existing applications can be modified by selecting the application name in the Apps list with a left mouse-click and then clicking the [Edit...] button.

The default applications CAN be used as a template for creating derivations of the selected application. Select the app and then the [Edit...] button. When saving, users will be prompted to save the workflow with a new name (i.e., as a new application).

The *Application Editor* window will display. See [Section VII](#) for more information on how to modify a workflow.

5. [Delete]

NOTE: The default 3'DE - TCR and SMART-Seq applications cannot be deleted.

Custom applications can be deleted from the application manager if no longer needed or to reduce the number of applications defined.

Select a workflow with a left mouse-click, then click the [Delete] button.

6. [Rename...]

NOTES:

- Application names may only contain alphanumeric characters, underlines, hyphens, and/or spaces. No other special characters are allowed.
- The default 3'DE - TCR and SMART-Seq applications cannot be renamed.

Existing custom applications can be renamed (i.e., how the application is listed in the Apps list of the application manager and in the workflow tabs of the main CELLSTUDIO interface) by selecting the application name in the Apps list with a left mouse-click and then clicking the [Rename...] button.

The *Rename* window will pop up (Figure 17). Type in the new name into the text field and click [OK] to accept, or [Cancel] to make no change.

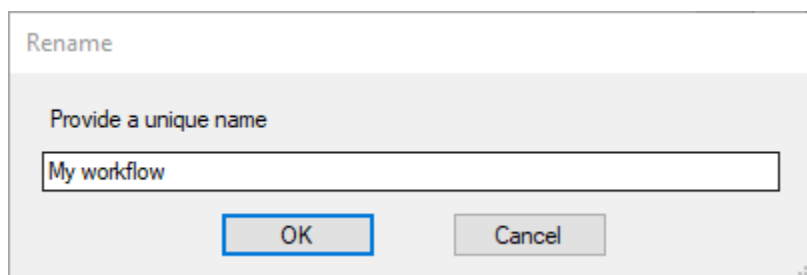


Figure 17. The *Rename* pop-up window.

7. [Done]

Clicking this button will return you to the main CELLSTUDIO window and save any changes made in the application manager.

D. CELLSTUDIO *Advanced* Tab

The CELLSTUDIO *Advanced* tab contains general functions that are required to maintain the instrument and prepare for a run in the *Manual Control* section.

- The *Filter configurations* section is used to choose the image capture settings for particular cell types or fluorescence conditions.
- The *Cell re-suspension* section is used to set specific cell dispense configurations for any application.

Changes made to the *Filter configuration* and *Cell re-suspension* sections are global settings that apply to any application. These settings will default to the ones chosen by the last cell dispense and scan and should be actively changed if needed.

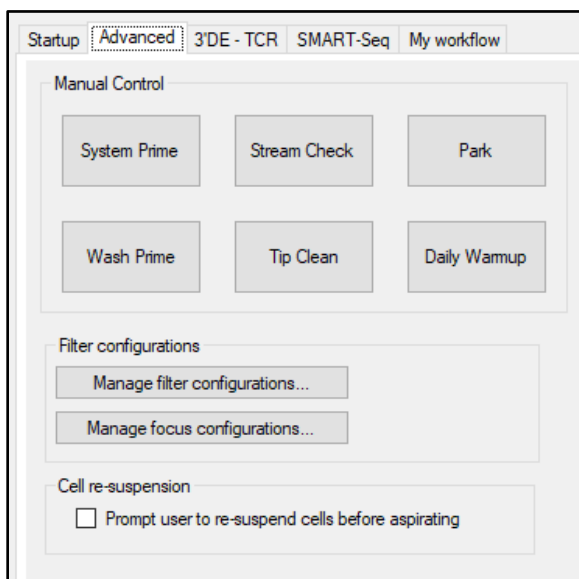


Figure 18. The *Advanced* tab interface.

Manual Control

- [System Prime] – Primes system tubing. Used for purging residual bubbles trapped inside the fluidic lines. The function charges the 250-µl syringes with water from the reservoir bottle and discharges to the waste trough for multiple cycles.
- [Stream Check] – Checks for partially clogged tip nozzles. During the stream check, the tip array is positioned on top of the rinse trough, and a stream of water is ejected from each tip nozzle. With the doors open, look to see if the stream of water is ejected at an angle from any of the tip nozzles, which is an indication of a partially clogged nozzle. If so, perform the [Daily Warmup] procedure several times and repeat the [Stream Check]. If the problem persists, contact Takara Bio technical support at technical_support@takarabio.com.
- [Park] – Moves the tip array to a safe position.
- [Wash Prime] – Refills the wash buffer in the wash trough. This is used when preparing the system for use ([Section VIII.B](#)), might be used during a tip clean procedure ([Section VIII.E](#)), and used during instrument shutdown ([Section XII.B](#)).
- [Tip Clean] – Washes the tip nozzle array. This is done during system warmup ([Section VIII.C](#)), after each dispense ([Section X](#)), and when cleaning the system ([Section X.H](#)). See [Section VIII.E](#) for more information.
- [Daily Warmup] – Homes transitional stages and syringes, primes wash solution, and purges residual bubbles trapped inside the fluidic lines. The function charges the 250-µl syringes with water from the reservoir bottle and discharges to the waste trough for multiple cycles. See [Section VIII.C](#) for more information.

Filter configurations

- [Manage filter configurations...] – This provides an option to quickly review and modify the available filter configurations for image capture when scanning the chip. The functionality is similar to **Utilities > Exposure Settings** described in [Appendix E](#). The difference is that image capture is disabled, you won't be able to snap images to see the effects of any changes.
- [Manage focus configurations...] – This provides detailed options on how chip scanning is to be performed for the various chip types. Users should not change these settings unless instructed to do so by Takara Bio support personnel. **Any changes made here will be reset when the program is restarted.**

Cell re-suspension (“Pause” function)

This option is global and affects all apps with a “Cells” dispense step (labeled “Dispense Cells and Controls”). If selected (i.e., the box is checked), it introduces pauses during the cell dispense steps with a prompt to re-suspend the cells (Figure 19) before each aspiration. Resuspending the cells improves the uniformity of cell distribution and increases the number of single cells present in the chip. The cells can be resuspended in the source plate, or a new suspension can be added to the source plate.

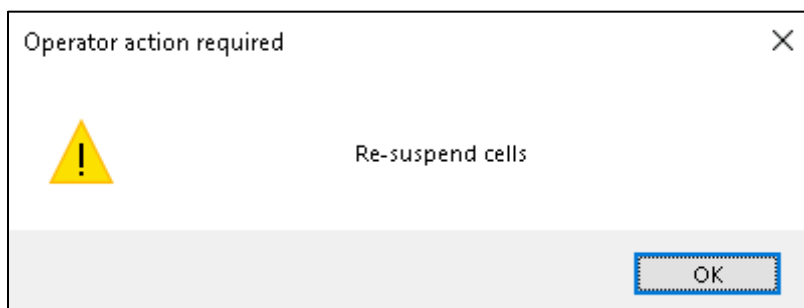


Figure 19. Cell re-suspension pop-up window reminder.

If you use this function during cell dispense but want to remove the pause for other dispenses or applications, you will need to go into the *Advanced* tab and deselect this option.

VI. Protocol: Quick Reference Guide

Table 4 below is a quick reference guide for the general workflow of dispensing samples into the chip, scanning cells, and dispensing downstream reagents. Print this guide for easy reference in the laboratory. Each step is described in detail in the sections following the quick reference guide.

Table 4. Quick reference guide

1. (Optional) Create a new CELLSTUDIO application ([Section VII](#))
2. Prepare the ICELL8 cx system ([Section VIII](#))
3. Prepare the sample source plates ([Section IX](#))
4. Dispense samples into the chip ([Section X.A](#))
5. Blot and centrifuge the chip ([Section X.D](#))
6. (If applicable) Scan chip for single cells and freeze the chip ([Section X.E](#))
7. Dispense reagents into the chip ([Section X.F](#))
8. Run your reaction on a thermal cycler ([Section X.G](#))
9. Clean the ICELL8 cx system ([Section X.H](#))
10. Extract library from the chip ([Section XI](#))

VII. Protocol: Creating a New CELLSTUDIO Application

This protocol is written as a reference guide to create or edit application workflows within the CELLSTUDIO software. For detailed information about designing an application tailored to your sample and experimental goals, refer to [Appendix G](#).

NOTE: If you are performing one of Takara Bio's prevalidated applications, refer to the user manual specific to that application (available for download at takarabio.com) and proceed to [Section VIII](#).

A. The Application Editor

The application editor is the CELLSTUDIO software functionality that allows for the creation or modification of applications. It is accessed through the application manager ([Section V.C](#)).

1. From the main CELLSTUDIO software interface, select **Utilities > Application Manager...**

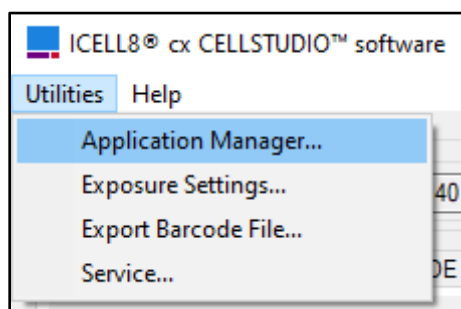


Figure 20. Selecting Utilities > Application Manager... in the menu.

The *Application manager* window will pop up.

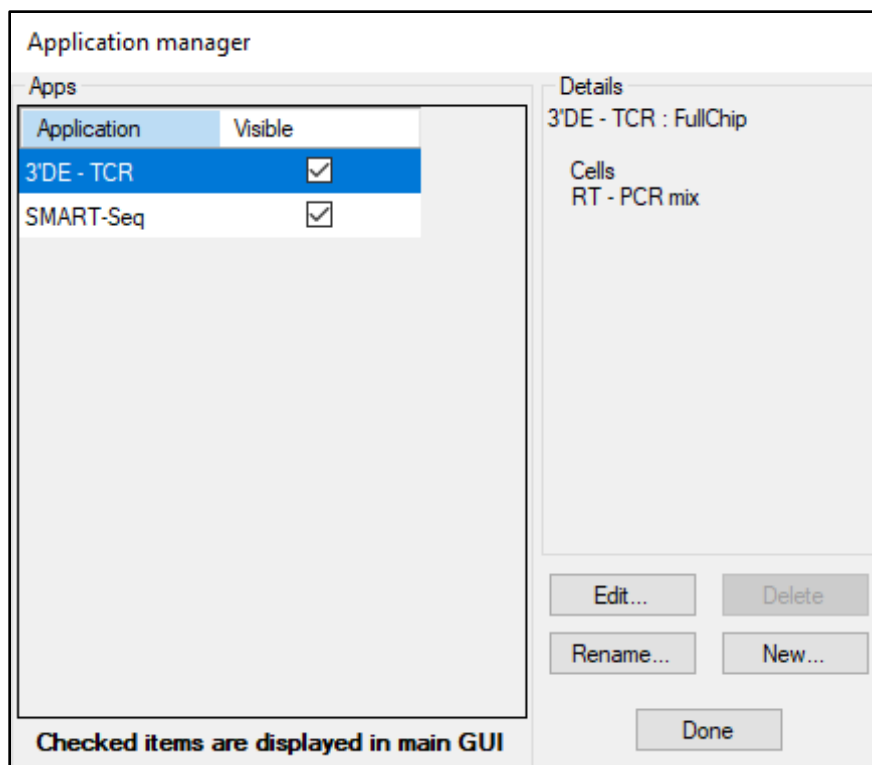


Figure 21. Default Application manager window.

2. Click on the [New] button to create a new application. The default *Application Editor* window will pop up.

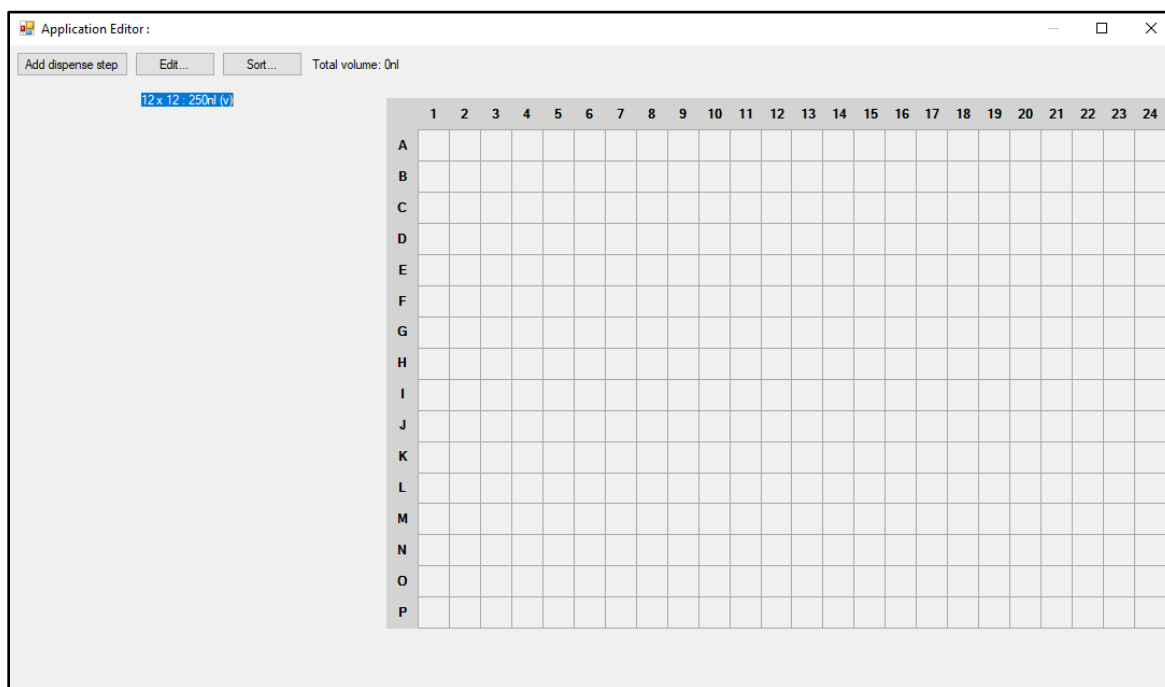


Figure 22. Default *Application Editor* window.

The window layout includes:

- Three menu buttons in the top-left corner
- Two informational text strings to the right and under the menu buttons
- An area on the left where dispense steps will be listed
- A template map for a 384-well plate, which will serve as the source plate map for samples, reagents, and primers that will be dispensed based on the application constructed

Zooming in on the buttons and informational text, a brief description of each can be found below:

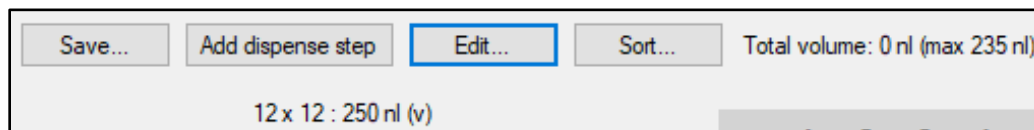


Figure 23. *Application Editor* window, focusing on the buttons and informational text.

- a. [Save...] – Allows you to save the application, either when creating a new one or editing an existing one (see [Section VII.E](#)).
- b. [Add dispense step] (see [Section VII.C](#)).
- c. [Edit...] – Used as both the first step in creating a new application workflow and to change existing ones (Editing an existing workflow is covered in [Section VII.D](#)).
- d. [Sort...] – Used to change the order of the application workflow dispenses (see [Section VII.D](#)).

- e. Total volume – The value here is a running sum of the total nanoliters configured by each of the dispense steps with a note of what the maximum volume can be based on what chip size is configured in the *Properties* ([Edit] menu) (see [Section VII.C](#)).
- f. The text ‘12 x 12 : 250nl (v)’ is a display of the dispense pattern (12 wells by 12 wells), ‘250 nl’ is the volume of the chip well, and ‘(v)’ is the shape of the well (options will be ‘v’ or ‘flat’) (see [Section VII.B](#)).

B. *Properties* Window

Click the [Edit] button to bring up the *Properties* window and begin configuring the workflow.

The *Properties* section contains option settings for a number of items:

- The configuration of the application for the pattern to dispense to the ICELL8 cx chip
- The folder containing the barcode files that should be imported into the software to assign to each well location on the chip
- Whether index primers will be used
- The type of ICELL8 cx chip that is being used

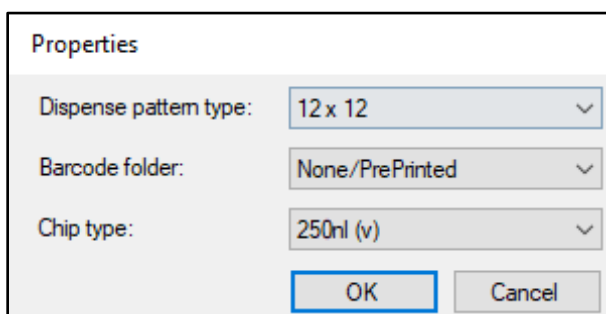


Figure 24. The application editor's *Properties* window.

Details about each field are listed below.

- a. Option: “Dispense pattern type”

This represents the largest area of the chip the application will dispense to, delineated by a square grid of wells. The preset options are displayed in the figure below and range from a 12 x 12 grid up to 72 x 72 (the full chip).

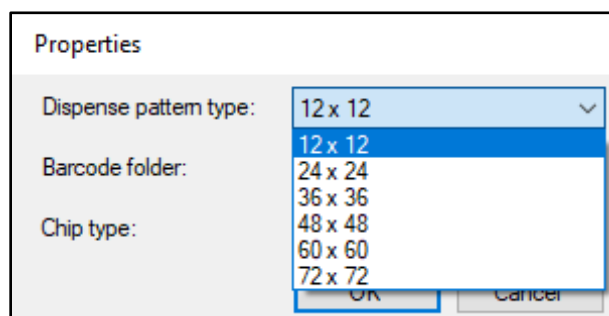


Figure 25. The *Properties* > “Dispense pattern type” drop-down menu expanded.

These patterns also correspond to the potential creation of barcode files from templates provided with the software, as the number of barcodes should be equal to or greater than the number of wells in the dispense pattern. For more information about barcode files, see the next section and [Appendix C](#).

- b. Option: “Barcode folder”

NOTE: See [Appendix C](#) for more information related to barcode files and creating custom barcode files for use within CELLSTUDIO software applications. If using custom primers, the barcode files should be created and configured in *Properties* before proceeding to [Section VII.C](#).

Entries in the drop-down menu are defined by the default of ‘None/PrePrinted’; the rest are the list of subfolders contained in the directory C:\ProgramData\Takara\ICELL8 cx\Barcodes (the barcode folder parent).

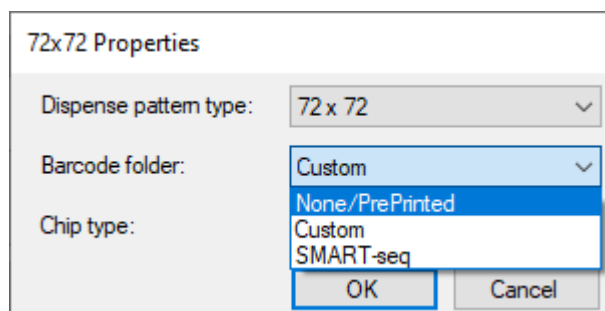


Figure 26. The application editor’s *Properties* > “Barcode folder” drop-down menu expanded. All options after ‘None/PrePrinted’ correspond to index barcode options.

- ‘None/PrePrinted’ – this is the default option and should be selected if the application doesn’t include an index dispense step. This applies to apps that do not use barcodes at all or applications that utilize preprinted chips which already have barcodes (e.g., the ICCELL8 cx 3’ DE Chip [Takara Bio, Cat. No. 640199] used in the 3’DE application or the ICCELL8 cx TCR Chip [Takara Bio, Cat. No. 640200] used in the TCR application).
- Barcode folders – these may include configured barcode files shipped with the prevalidated products, such as the ‘SMART-seq’ application, or ‘Custom’ barcode files created following the guidelines in [Appendix C](#).

Index barcode options may be one layer or nested, depending on the configuration in the directory structure—i.e., whether the index files are stored directly within the barcode folder in the drop-down list (single-layered) or within a subfolder of the listed barcode folder (nested).

- If the index files are directly under the drop-down list barcode folder, selecting the barcode folder at this step will apply that set of indexes to all dispenses performed using that application. No additional steps are needed by users when running the application in the dispense steps.
- If the selected barcode folder is nested, such as the predefined ‘SMART-seq’ option, the user will be prompted at the beginning of the dispense workflow to choose which

index subset files to use. Figure 27 shows an example of this; the ‘SMART-seq\’ prefix represents the barcode folder from the *Properties* window, while ‘SetA’ and ‘SetB’ are subfolders of SMART-seq application.

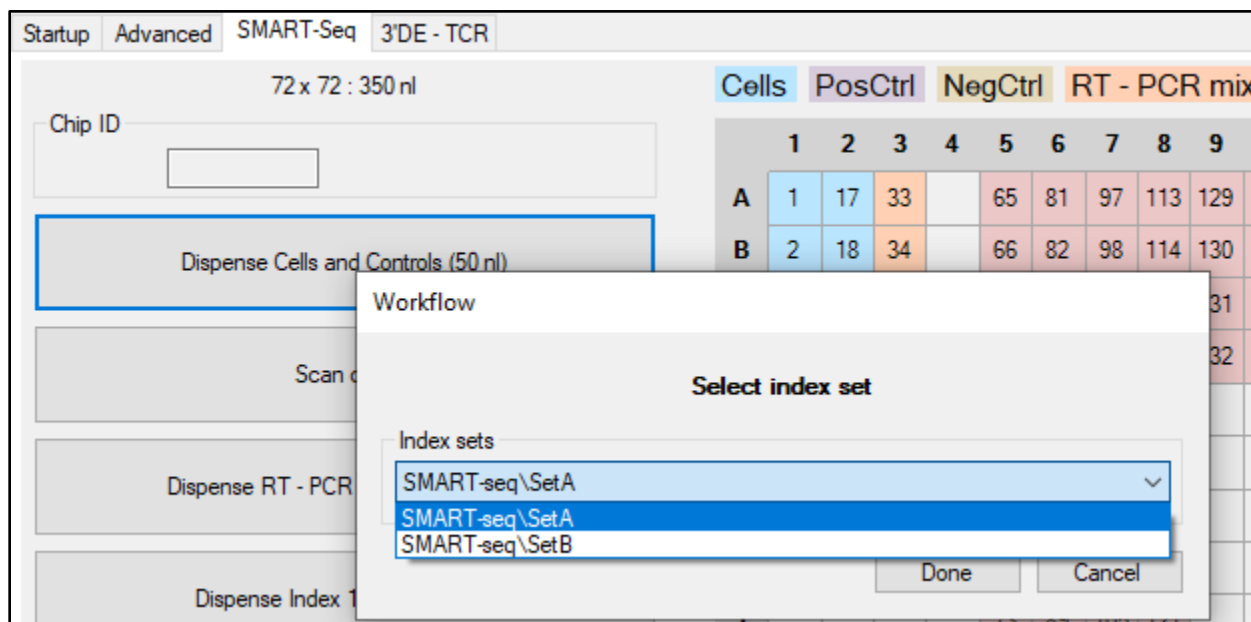


Figure 27. Example selection prompt of an index subset during the sample dispense step. The ‘SMART-seq’ barcode folder is selected for the application properties, so the user is prompted to choose either the ‘SetA’ or ‘SetB’ subsets in that folder.

NOTES:

- An application CAN reference a barcode folder that has barcodes defined for a larger pattern than the one defined in the application. Only the required barcodes will be dispensed.
- An application should NOT reference a barcode folder that has fewer barcodes defined than is required for the pattern. An error message will display if this occurs.

Example:

An application with a 48 x 48 dispense pattern CAN reference a barcode folder for 48 x 48, 60 x 60, or 72 x 72 barcodes, but NOT barcode folders for 12 x 12, 24 x 24, or 36 x 36 barcodes.

c. Option: “Chip type”

The drop-down menu is populated with all available volume and shape configurations of the ICELL8 cx chips which can be used on the ICELL8 cx system.

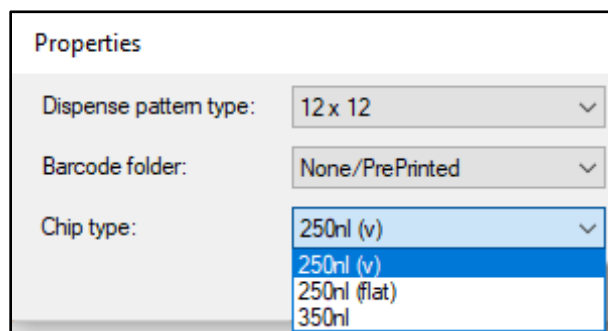


Figure 28. The *Properties* > “Chip type” dropdown menu, expanded.

The following table lists which option should be selected based on the catalog number of the chip being used.

Table 5. Chip type option descriptions mapped to the official product name and catalog number

Chip type	Product name	Cat. No.
250 nl (v)	ICELL8 250v Chip	640183
250 nl (flat)	ICELL8 cx Blank Chip - 250 nl FB	640193
	ICELL8 cx 3' DE Chip	640199
	ICELL8 cx TCR Chip	640200
350 nl	ICELL8 350v Chip	640019

3. Configure the three options for the design of your dispense workflow and click [OK] to return to the application editor window.

C. *Dispense type* Window

Once the Properties options are configured, begin adding dispense steps using the [Add dispense step] button. The *Dispense type* window will pop up.

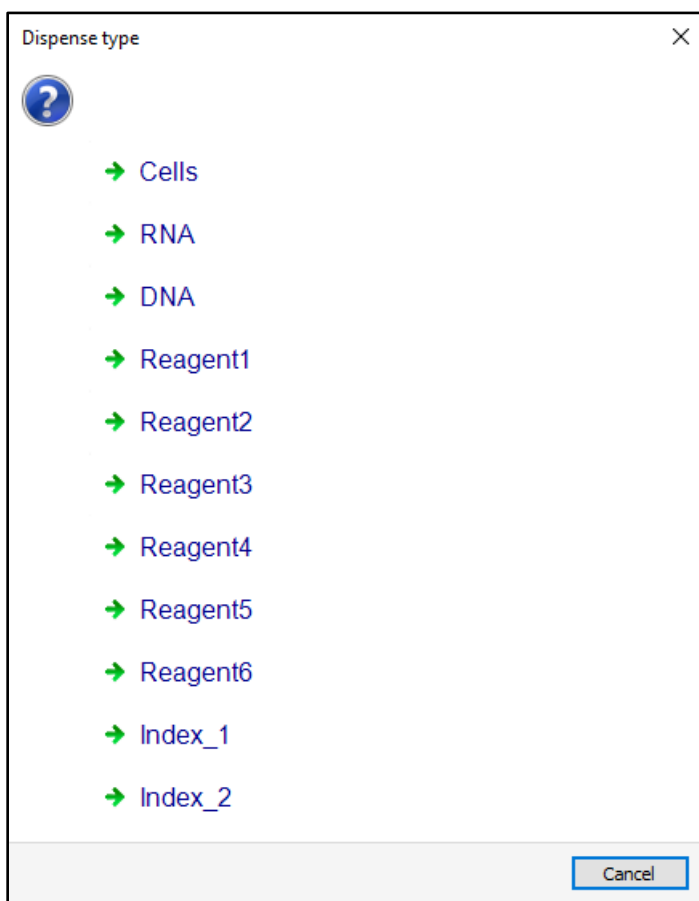


Figure 29. The initial *Dispense type* window.

The dispense type options can be grouped into three categories: sample, reagents, and indexed primers (barcodes).

1. Sample: Cells, RNA, DNA

The form the sample being tested is in. Only one of these three options is generally selected.

NOTE: If starting with cells and planning to do imaging, the sample dispense step needs to be the first.

Selecting one of the three options will bring up the *<sample-type> properties* window, where *<sample type>* will be the sample type selected (examples below).

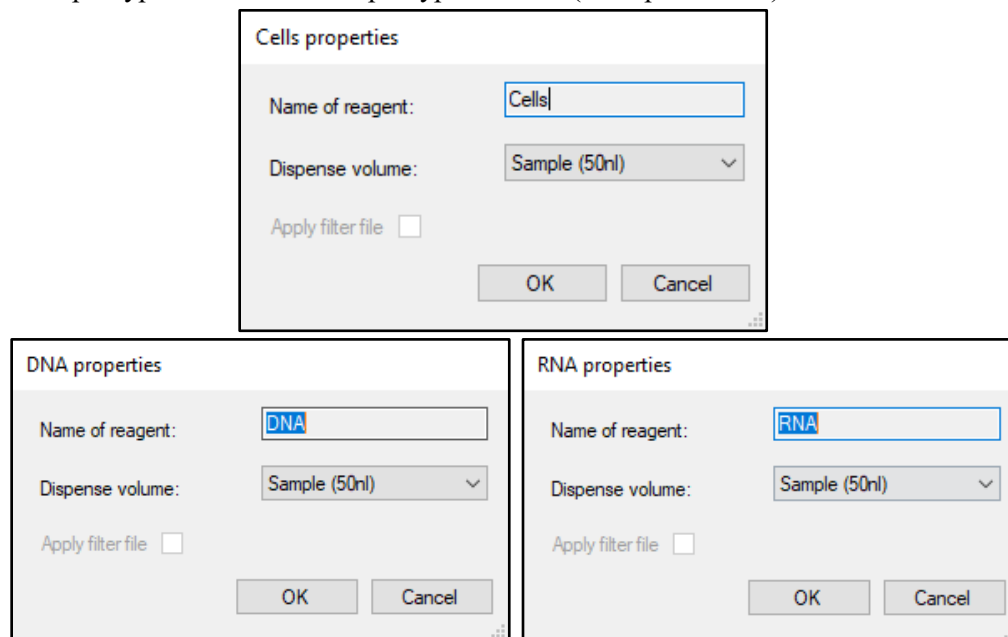


Figure 30. The three sample *properties* windows in the application editor dispense [Add dispense step] workflow.

The three sample *properties* windows are nearly identical. A description of the options in the *Cells properties* is used as an example below.

- “Name of reagent” – Cannot be edited for the sample properties. This will display as illustrated in the screenshots of Figure 30.
- “Dispense volume” – Has two options, 35 and 50 nl (default is 50 nl). This is the volume of sample that will be dispensed from the source plate into the ICELL8 chip wells.

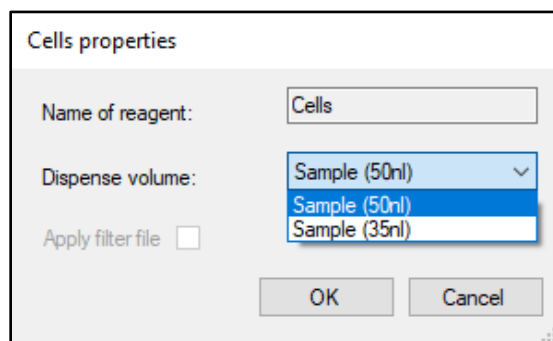


Figure 31. The “Dispense volume” drop-down menu of the *Cells properties* option.

- Once the dispense volume is selected for the sample, click [OK] to add the cell dispense step to the workflow configuration.

After being added, the *Application Editor* window will be updated to a display similar to the following. The list below describes elements of the updated display.

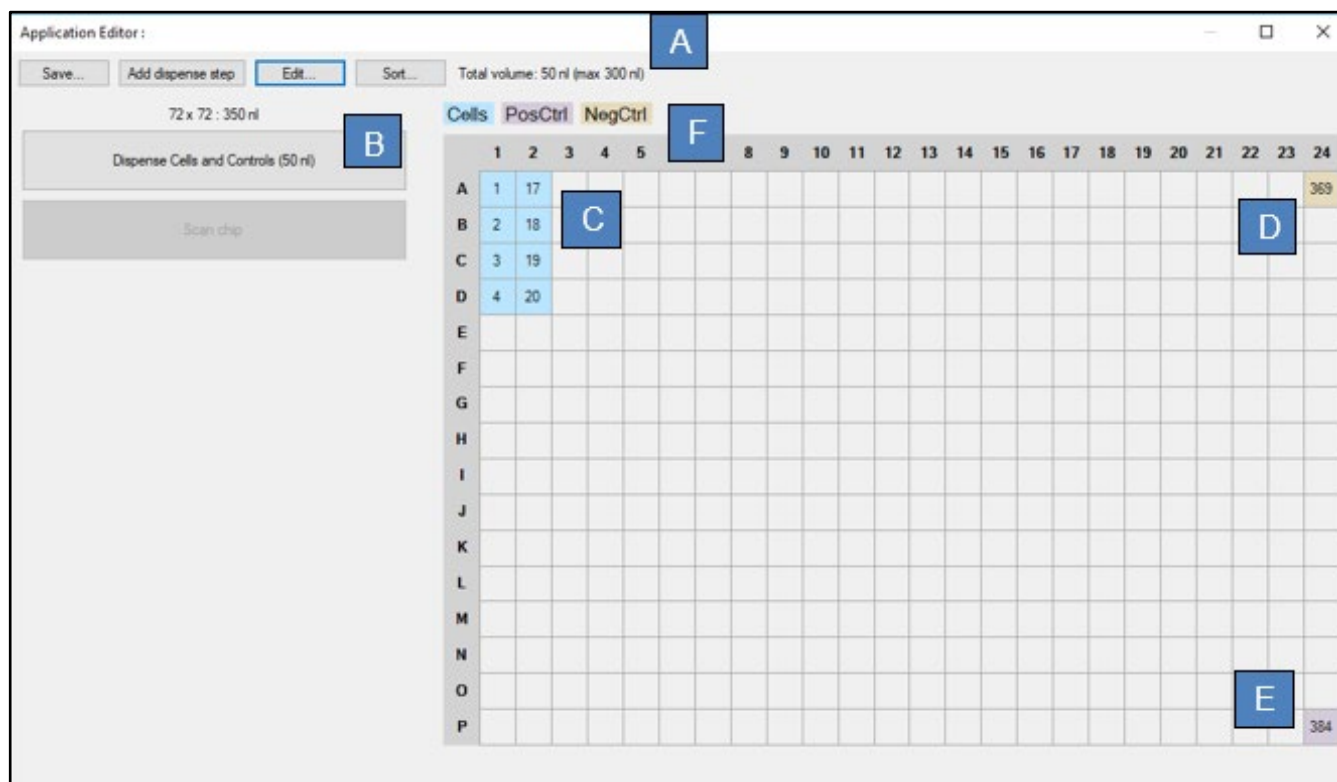


Figure 32. The updated *Application Editor* window after the sample dispense step is added. The blue-box labels correspond to the list below and are not shown in the actual interface.

- A. The total dispense volume is updated to include the volume of the sample dispensed (e.g., 50 nl in the example in the figure).
- B. The new workflow step(s) are added to the column to the left of the 384-well plate map, labeled with the type of step it is and the volume being dispensed.

NOTE: The “Scan chip” step is automatically added when cells are the sample source and cannot be deleted without deleting the cells sample option.

- C. The well location on the plate where the sample will be dispensed from are added to the map, assigned a color code, and labeled by the well number. Well numbers are assigned starting in the top, left corner of the 384-well plate (A1), then down the column (B1–P1). Numbering shifts right to the next column (2) and repeats in this fashion until well #384 (P24).
- D. The negative control is designated for well #369 (A24).
- E. The positive control is assigned to well #384 (P24).

- F. The color code legend for the plate map assignments. If the mouse hovers above one of the legend labels, the numbers in the corresponding wells will be underlined and bolded as a way of highlighting them.

	Cells	PosCtrl	No	
	1	2	3	4
A	<u>1</u>	<u>17</u>	33	
B	<u>2</u>	<u>18</u>	34	
C	<u>3</u>	<u>19</u>	35	
D	<u>4</u>	<u>20</u>	36	

Figure 33. The results of clicking on one of the legend labels for the source well plate map. The well numbers corresponding to where that sample/reagent/oligo solution should be located on the 384-well plate are indicated by bold and underlined font.

2. Reagent1–6

There can be up to six reagents dispensed into the sample wells. Selecting any of the Reagent options in the list will bring up the *Reagent<#> properties* window, where <#> will be the reagent number selected (e.g., Reagent4 in the figure below).

Reagent4 properties

Name of reagent:

Dispense volume: ▼

Apply filter file ☒

OK Cancel

Figure 34. The *Reagent<#> properties* window in the application editor [Add dispense step] workflow.

All *Reagent<#> properties* windows are identical in layout. A description of the options can be found below:

- “Name of reagent” – Can be customized in the text field. (E.g, ‘RT mix’) Names may only contain alphanumeric characters, underlines, hyphens, and/or spaces. No other characters are allowed.

- b. “Dispense volume” – Can be selected from the prepopulated drop-down list; the options are ‘35nl’, ‘50nl’, or ‘100nl’. This is the volume of the reagent that will be dispensed into the ICELL8 cx chip wells.

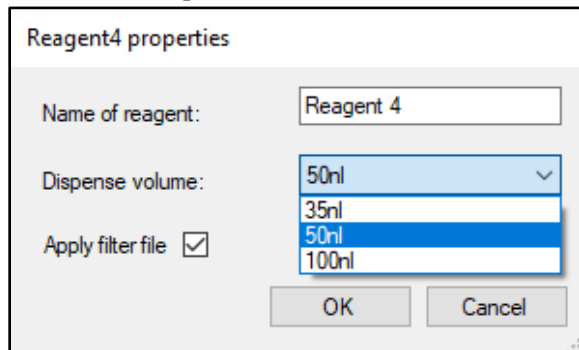


Figure 35. The “Dispense volume” drop-down menu of the *Reagent<#> properties* option.

- c. Apply filter file – This file can be created as a result of the imaging and triage process of CellSelect software or created manually when imaging is not required, such as with nucleic acids as the starting sample or when single-cell isolation is not important (for more information, see [Appendix D](#)).

If this option is checked (selected), the dispense step will display as (<volume> filtered) in the Application Editor window, where <volume> is the dispense volume configured here.

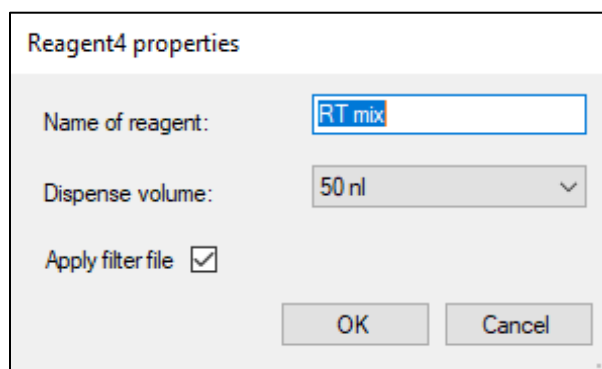


Figure 36. Example of a configured reagent dispense in the application editor [Add dispense step] workflow.



IMPORTANT: If using a 60 x 60 or 72 x 72 dispense grid without a filter file (see the *Properties* window, [Section VII.B](#), above), the configured dispense volume must be 35 or 50 nl. Do not use 100 nl.

If a filter file is used, 100 nl can be selected for any dispense grid size.

- d. Click [OK] when finished to add the dispense to the workflow. Figure 37 shows what the *Application Editor* window would look like after this step.

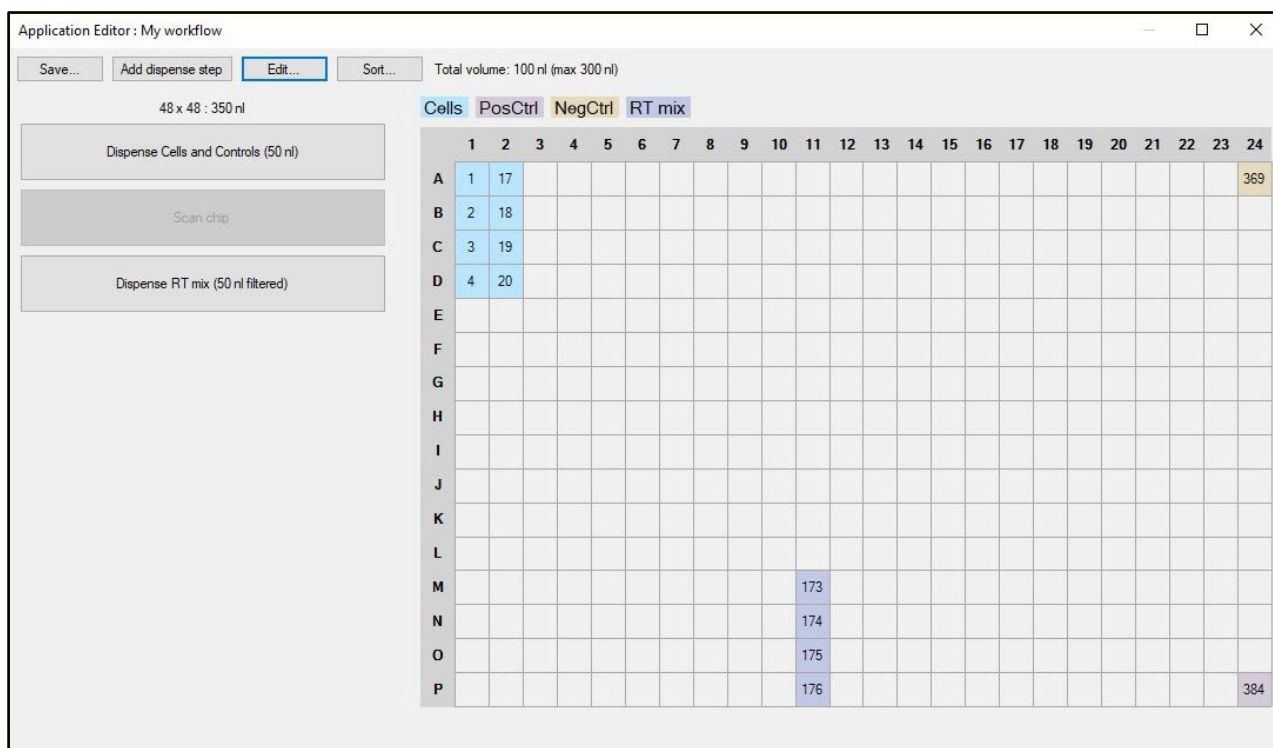


Figure 37. Reagent dispense from Figure 36 displayed in the *Application Editor* window. The dispense step has been added to the workflow list on the left and displays as ‘(50 nl filtered)’ as a 50 nl dispense was selected and “Apply filter file” option was checked in the configuration properties. The reagent reference was added to the legend, and the reagent source wells to the map (173–176).

3. Index_1–2

If index primers will be added, select **Index_1** or **Index_2**.

NOTE: Adding an Index dispense step requires a barcode file to be specified in the [Edit...] window ([Section VII.B](#), above).

If no barcode file is specified in the **Application editor > Properties** window and an Index dispense is added, the “Total volume” text will turn red and a warning icon will appear next to it, as in Figure 39 (below). To correct, click the application editor [Edit...] button to configure a barcode file.

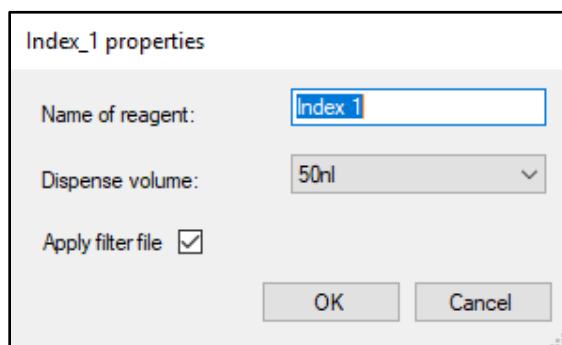


Figure 38. The *Index_<#> properties* window in the application editor [Add dispense step] workflow.

Configuration of these options is identical to that of the reagents. Refer to the Reagents dispense step (Section VII.C.2) prior to this section.

After adding a primer index dispense, the *Application Editor* window looks similar to this:

Application Editor : My workflow

Save... Add dispense step Edit... Sort... Total volume: 150 nl (max 300 nl) ⚠

48 x 48 : 350 nl

Dispense Cells and Controls (50 nl)

Scan chip

Dispense RT mix (50 nl filtered)

Dispense Index 1 - SMART-Seq Primer Set A (50 nl filtered)

	Cells	PosCtrl	NegCtrl	RT		
	1	2	3	4	5	6
A	1	17			65	81
B	2	18			66	82
C	3	19			67	83
D	4	20			68	84
E					69	85
F					70	86

Figure 39. Index dispense added through the window in Figure 38, displayed in the *Application Editor* window. The dispense step has had a custom named applied and added to the workflow list on the left.

Repeat the steps in this section (Section VII.C) as necessary for each reagent in the workflow.

If the total of dispensed sample and reagents exceeds the maximum recommended volume for the chip well, the “Total volume” and its value text will turn red with a warning indicator icon. Mousing over the warning icon will pop-up text with the reason for the warning message, such as in Figure 40.

Application Editor :

Save... Add dispense step Edit... Sort... Total volume: 250 nl (max 235 nl) ⚠

48 x 48 : 250 nl (flat)

Dispense Cells and Controls (50 nl)

Scan chip

Dispense RT mix (100 nl)

Dispense Index 1 - SMART-Seq Primer Set A (100 nl)

Total volume 250 nl is more than the usable well capacity: 235 nl

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
A	1	17	33	49	65	81	97	113									
B	2	18	34	50	66	82	98	114									
C	3	19	35	51	67	83	99	115									
D	4	20	36	52	68	84	100	116									
E					69	85	101	117									
F					70	86	102	118									
G					71	87	103	119									

Figure 40. The warning icon next to the red text drawing attention to the “Total volume” display. Hovering the mouse cursor over the icon will pop up text explaining the reason for the warning.



CAUTION: The software will not prevent the wells from being overfilled if this warning is ignored or overridden. Please modify the chip size, dispense volumes, or the number of reagents being dispensed in the workflow to prevent loss of your sample material or damage to the ICELL8 cx system.

D. Editing Application Configuration

Changes can be made to steps that were previously defined, either via an existing application or in-progress by clicking on the dispense step, then clicking [Edit...] to change the top-level definitions previously configured, or the [Sort...] button to change the order of dispense steps without having to delete and reconfigure the existing ones.

1. To edit the parameters or delete a dispense step

1. Left mouse-click on the step. The properties window for the step will automatically pop up.
2. If editing the information (name, volume, or application of a filter file), make the desired change(s).
3. Click [OK] to save the change(s) Or [Delete this...] to delete the step from the workflow.

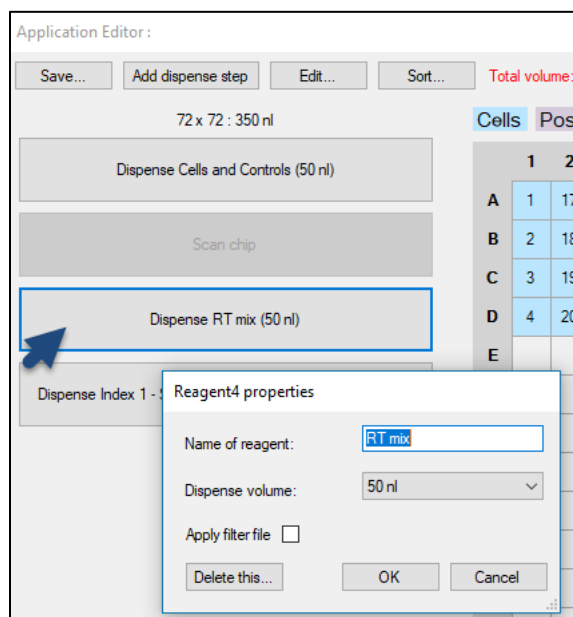


Figure 41. Example window editing a dispense step in the *Application Editor* window.

A dispense step can also be quick deleted by right mouse-clicking the parameter to be deleted and then selecting **Delete**.

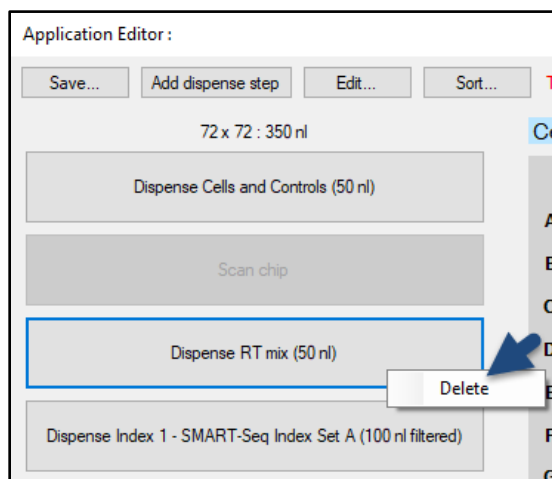


Figure 42. Deleting a dispense step in the *Application Editor* window by right mouse-click.

2. To edit the *Properties* of the application

The [Edit...] button can be used after beginning to configure the workflow ([Section VII.B](#)) to change the high-level configuration of the application.

Example:

In Figure 40 (above), the workflow was initially configured with the 250 nl (flat) chip size selected, but the total volume of the reagents is 250 nl. Clicking [Edit...] again (Figure 43, left), the 350 nl chip size could be selected. Clicking [OK] to apply the update resolves the volume warning message (Figure 43, right).

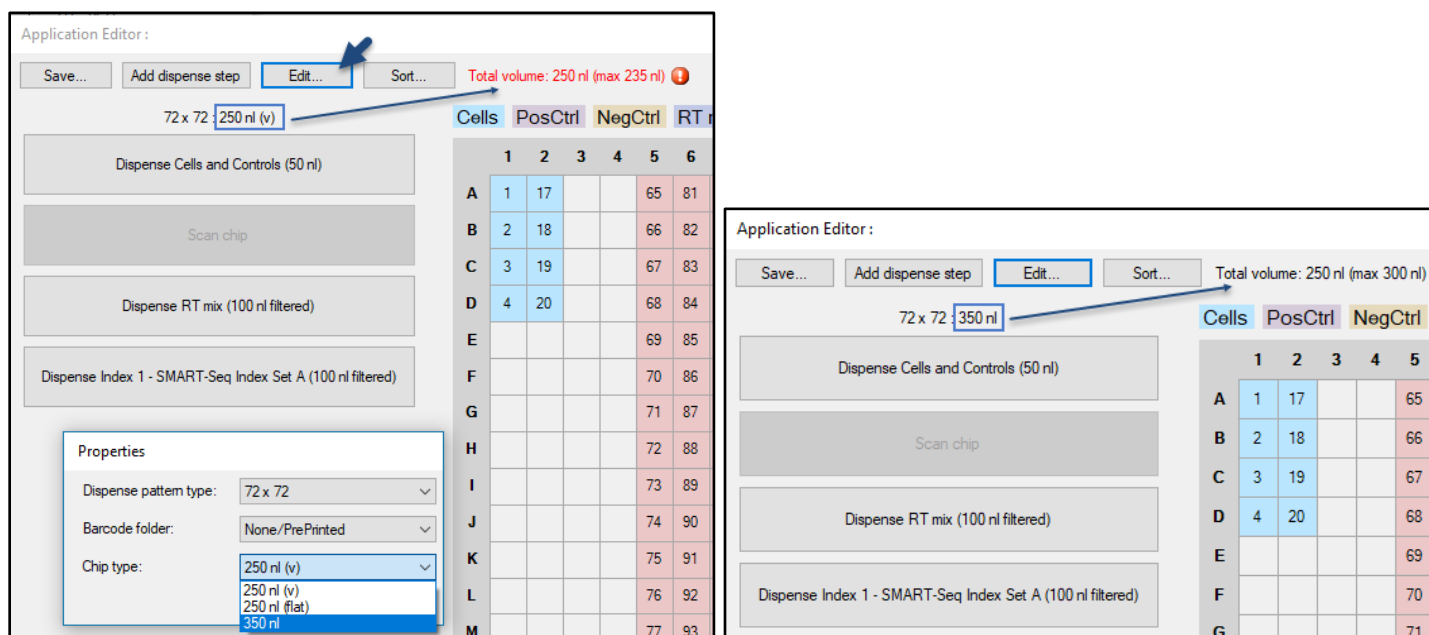


Figure 43. Using the chip information display and the [Edit...] button to resolve an overfill configuration in the *Application Editor* window. **(Left)** The application dispense volumes exceed the maximum volume recommended for a 250 nl chip. The “Total volume” display turns red to alert the user to this fact. **(Right)** Changing it to a 350 nl chip resolves the issue and the warning message.

3. To change the order of dispense steps

The [Sort...] button can be used to rearrange the order of dispense steps that have already been defined in the application. Clicking this button brings up the *Change Order* window with a list of the currently configured dispense steps. The order of dispense can be changed by clicking on the given dispense step and clicking the [Move up] or [Move down] buttons to move the step to an earlier or later position in the workflow, respectively.

Clicking [OK] will save any changes made and will then be reflected in the *Application manager* list.

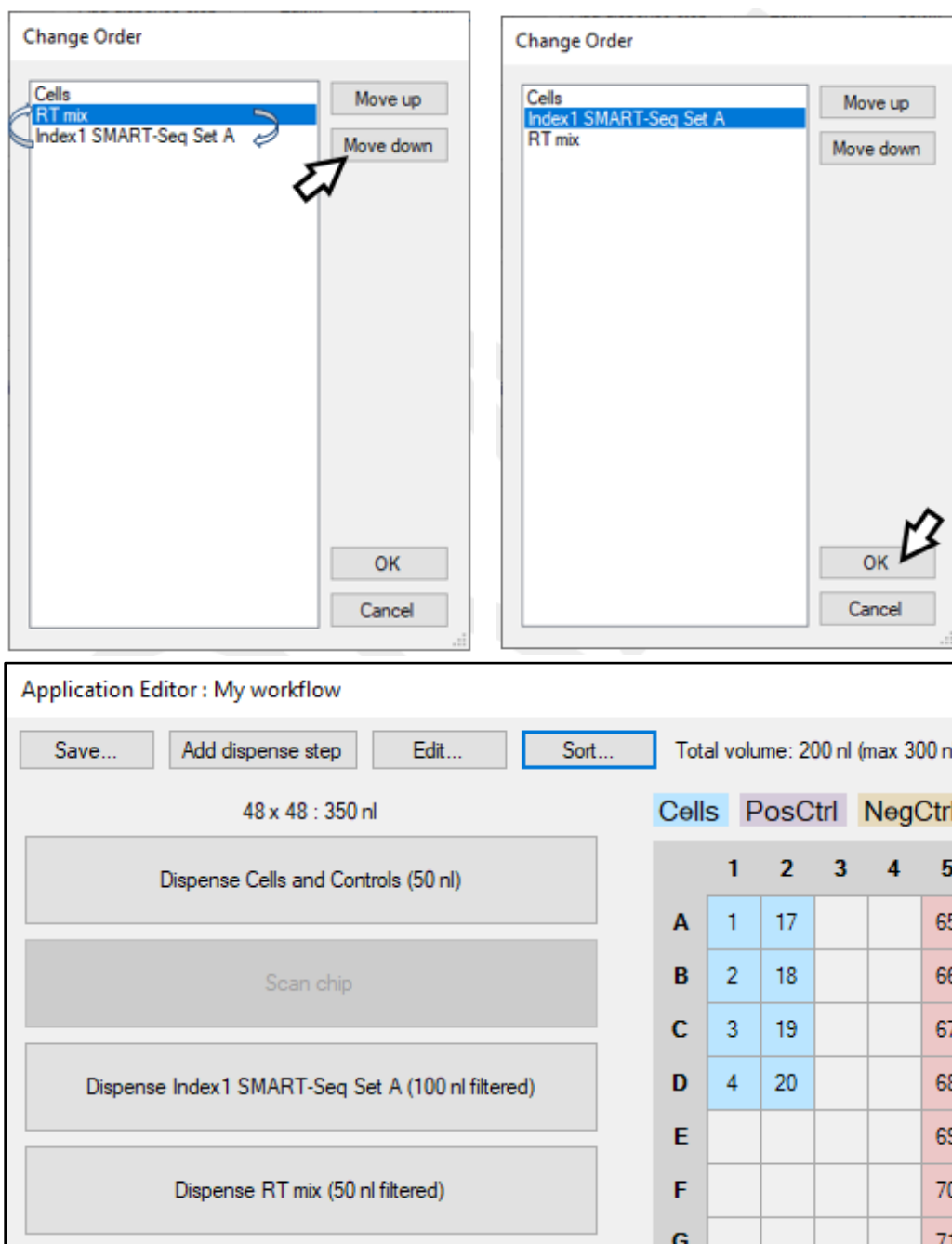


Figure 44. Walkthrough of the application editor [Sort...] function. Dispense steps can be reordered through a simple [Move up]/[Move down] list interface. Compare the bottom image with the order in Figure 43.

E. Save a Custom Application

Once the workflow is configured to your liking, it can be saved in the system for future use.

1. Saving a new application or through editing a default application

The [Save...] button will not quit you out of the application editor, so it can be used to make incremental saves during the process of defining the workflow.

1. Click the [Save...] button in the upper, left-hand corner of the *Application Editor* window. A *Save as ...* window will pop up.
2. Type in the name to identify the application; this is the name as it will appear in the *Application manager* list (see [Section V.C](#)). Names should only include characters, digits, and spaces.

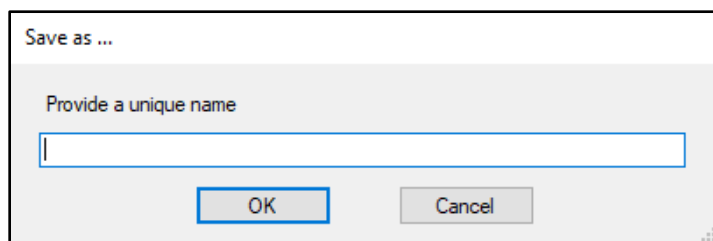


Figure 45. The *Save as...* window for naming a custom application.

3. Click [OK] to save the settings with that application name.

As mentioned in the description of the CELLSTUDIO application manager ([Section V.C](#)), the default applications in the CELLSTUDIO software (i.e., prevalidated Takara Bio workflows) cannot be modified directly. However, if chosen and the [Edit...] button is selected in the application manager the predefined template will load the configuration into the application editor ([Section VII.A](#)). This allows you to use the default application as a template or to create custom variations of the application.

When the [Save...] button is clicked in this scenario, the software will automatically prompt you with the *Save as ...* window, forcing you to save the application with a new name.

2. Using [Save...] when editing a previously defined application

If the [Save...] button is clicked while using the [Edit...] button from the application manager ([Section V.C](#)) to edit a previously defined workflow, the save prompt will change asking you if you want to create a new workflow or to save with the same name (i.e., ‘overwrite existing’) (Figure 46, below).

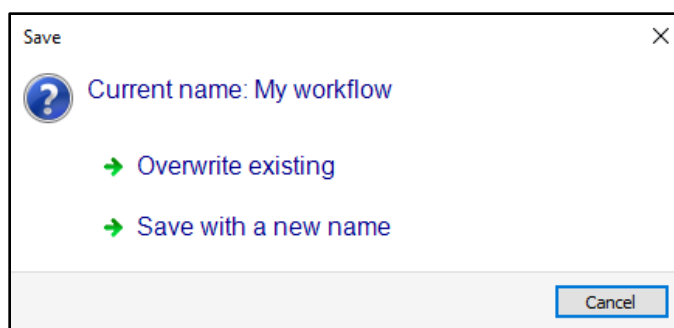



Figure 46. The *Save* window when editing an existing application. ‘Overwrite existing’ will update the existing workflow, while ‘Save with a new name’ creates a new application derived from the existing one and does not update the existing one.

F. Close the Application Editor

When you’re done configuring a new application or modifying an existing application, click on the close window button () to return to the application manager window.

As you do so, if any modification was made to a workflow in the application editor that wasn't saved with the [Save...] button, you will be automatically prompted to save your changes. Which version of the two displayed in Figure 47 depends on whether it is a new workflow (or modifying a default application) or editing a previously defined custom app.

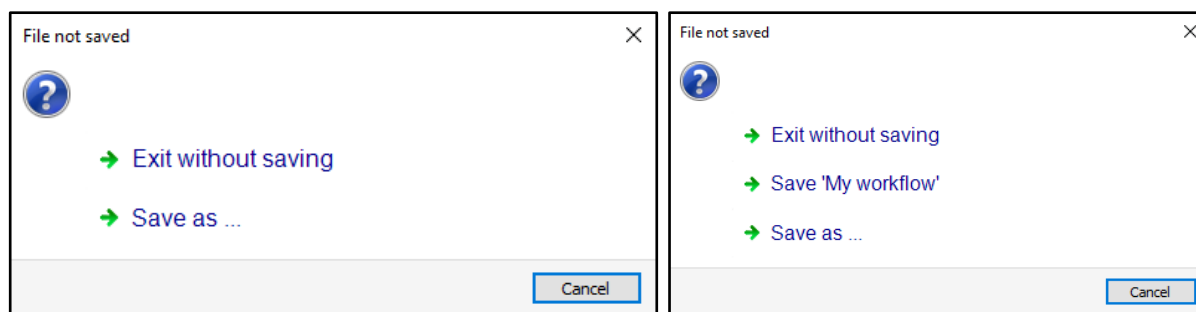


Figure 47. *File not saved* prompts when closing the application editor without doing a [Save...] first. (Left) A new custom application or derivation of a default app. (Right) Editing an existing custom application named 'My workflow'.

Once the close window button is clicked and the *File not saved* prompt is addressed (if necessary), the *Application Editor* window will disappear.

G. Return to the Application Manager

The *Application Manager* window will still be open after the application editor is closed. If a new application was saved, the *Apps* list will be automatically updated to include the new application, and the “Visible” box will be checked ([Section V.C](#), CELLSTUDIO application manager). Verify the “Visible” box is checked to ensure it is enabled for use when using the ICELL8 cx Instrument.

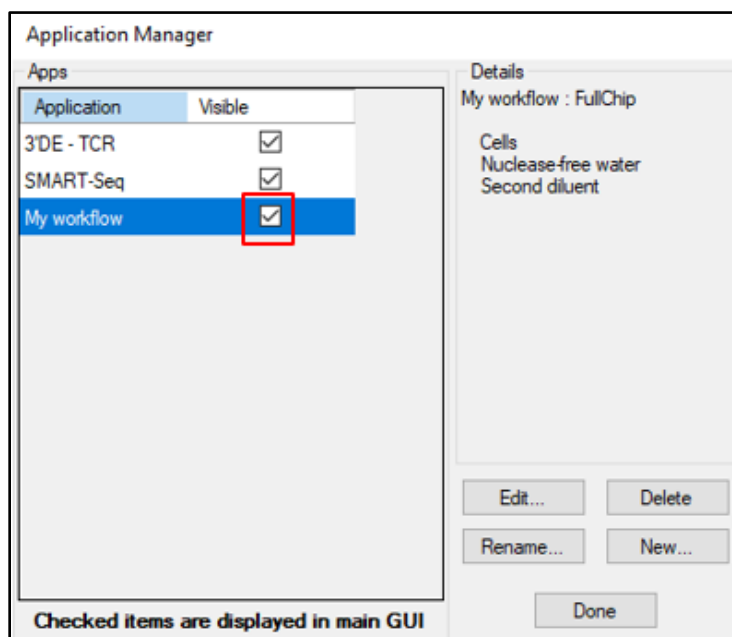


Figure 48. The list of defined applications in the application manager. The red box is to highlight that the example custom application ('My workflow') has a check in the “Visible” column, meaning the application will appear as a tab in the main CELLSTUDIO interface.

VIII. Protocol: Prepare the ICELL8 cx System

IMPORTANT:



- Make sure that the ICELL8 cx Module is connected to the **USB 3.0 ports** on the computer (blue ports), and not the USB 2.0 ports (black ports). Connecting to the USB 2.0 port will result in software errors.
- After powering on the system (step A) it takes 10 min for the system to stabilize to proceed to step B. Plan for this delay in the daily maintenance prior to beginning an application run.

A. Power on the System

1. Power up the system by flipping the switch at the back-left of the ICELL8 cx unit.
2. Start the ICELL8 cx Software on the computer by double-clicking the **ICELL8 cx** icon on the desktop.



Figure 49. ICELL8 cx Software icon.

- a. The XY axes, syringe pumps, and optics module will automatically initialize when the software is connected to the ICELL8 cx module.
- b. While the system is powering up and stabilizing (about 10 mins), the live chamber temperature, chamber relative humidity, chuck setpoint, and chuck temperature are displayed in the boxes below. (Note: temperature is reported in degrees Celsius.)

DewPoint					
<input checked="" type="checkbox"/>	Chamber		Chuck		
	Temp:	23.7	SP:	9.9	Temp: 14.1

Figure 50. Example of DewPoint parameter display.

B. Check and Fill System Containers

Prior to dispensing, click on the *Startup* tab and follow the list to check the system containers.

Startup	Advanced	3'DE - TCR	SMART-Seq
Daily Checklist			
Check water container			Done
Check humidifier water container			Done
Check waste container			Done
Check bleach container			Done
Daily warmup			Start

Figure 51. System startup menu in the ICELL8 cx Instrument software.

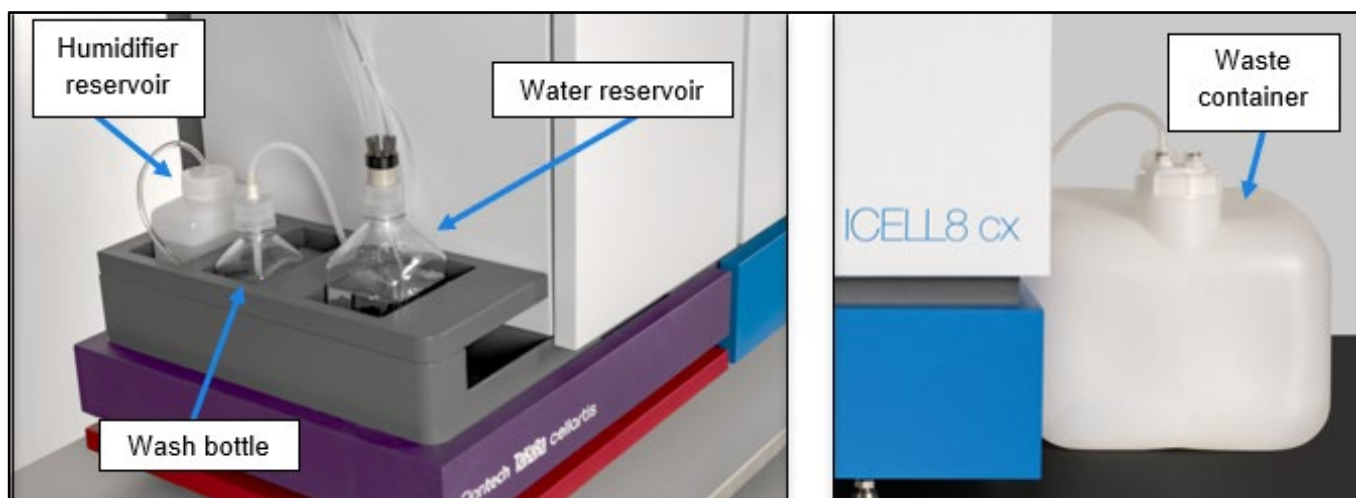


Figure 52. Items to be checked during the instrument startup process.

1. Check the amount of water in the water reservoir. Make sure the water bottle is filled with deionized filtered water with at least 800 ml. Refill as needed following these steps:
 - a. Fill a clean secondary container with deionized filtered water.
 - b. Carefully unscrew the cap of the water-reservoir bottle.
 - c. Slightly lift the cap up with one hand and use your other hand to pour water from the secondary container into the water reservoir bottle.

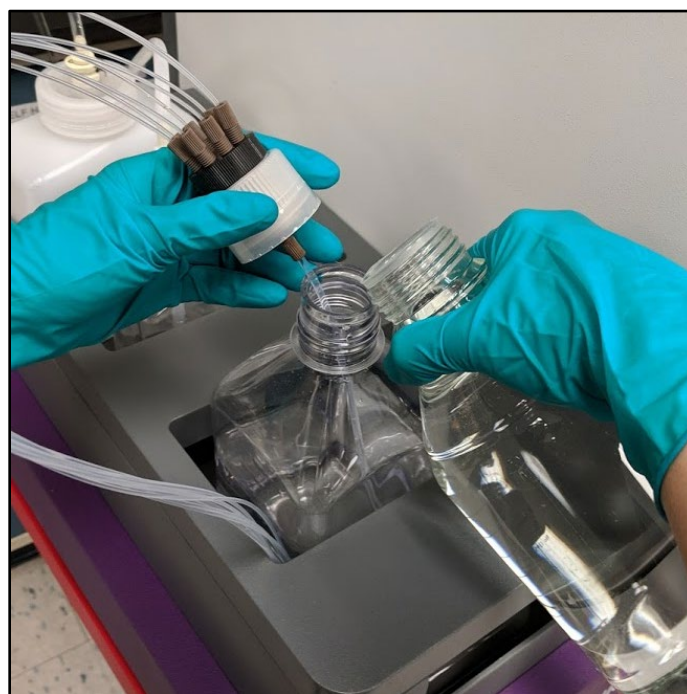


Figure 53. Recommended method of refilling the water reservoir.

- d. After the bottle has been refilled, screw the water reservoir bottle cap back on.
2. Check the humidifier reservoir and refill with deionized filtered water as needed.

Use the same procedure for refilling the humidifier reservoir as described above for the water reservoir.



Figure 54. Recommended method of refilling the humidifier reservoir.

3. Check that the waste container is no more than half-full. If so, dispose of the liquid waste appropriately and reinstall the empty waste container following these steps:
 - a. To uninstall the waste container, first, locate the cap and press the quick-release buttons to release the tubing.
 - b. Carefully set the tubing aside and transport the waste container to the disposal location.
 - c. Unscrew the cap and properly dispose of contents.
 - d. Screw the cap back on and reinstall the container by plugging the tubing back into the port in the cap.
4. Check the wash bottle and replace sodium hypochlorite solution if the volume is <50% or if the solution is more than a week old. Follow these steps to replace the solution:
 - a. Disconnect the quick-connect fitting at the top of the cap and set tubing aside on a clean surface.
 - b. Remove the cap from the wash bottle and place the cap on a clean surface.
 - c. Properly dispose of the old fluid and refill with 500 ml of fresh 0.2% reagent grade sodium hypochlorite.
 - d. Screw the cap of the wash bottle back on and reconnect the quick-connect fitting.



Figure 55. Disconnecting the quick-connect fitting from the wash bottle cap.

- e. Go to the *Advanced* tab and click [Wash Prime] to prime tubing with fresh 0.2% sodium hypochlorite solution.

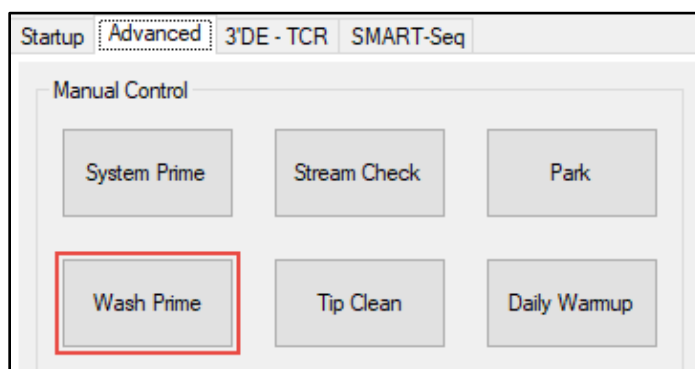


Figure 56. The [Wash Prime] button under the *Advanced* tab of the instrument software.

C. Run the [Daily Warmup]



IMPORTANT: Run the [Daily Warmup] function each day prior to performing any experiments. Failure to do so may result in poor dispensing.

1. After the check of the containers is complete, click the [Done] button for each of the categories:

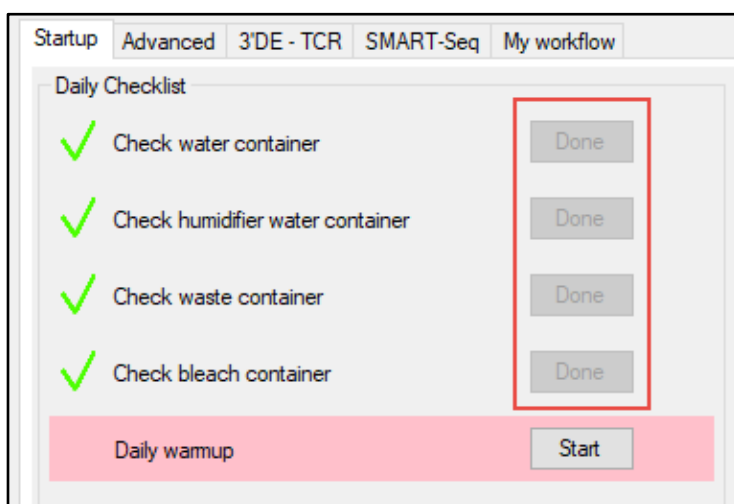


Figure 57. Daily checklist after completing all the check steps.

2. Click the [Start] button next to “Daily warmup”. The warmup process takes roughly 5 min. During this time, the ICELL8 cx will do the following:
 - a. Home the XY axes and initialize the syringe pump bank.
 - b. Perform a [System Prime] to flush the system tubing with fresh water and purge trapped air.
 - c. Perform a [Tip Clean].
 - d. During the warmup, monitor the syringes for trapped bubbles. If bubbles are observed, click [Start] again to repeat the Daily Warmup process up to two times.

NOTE: If large bubbles are still observed in syringes or tubing (Figure 58) after three warmup cycles, we recommend priming the system tubing with isopropyl alcohol. Continue to [Section VIII.D](#) for the priming protocol. If there are no large bubbles observed in the syringes or tubing, skip to [Section VIII.E](#).

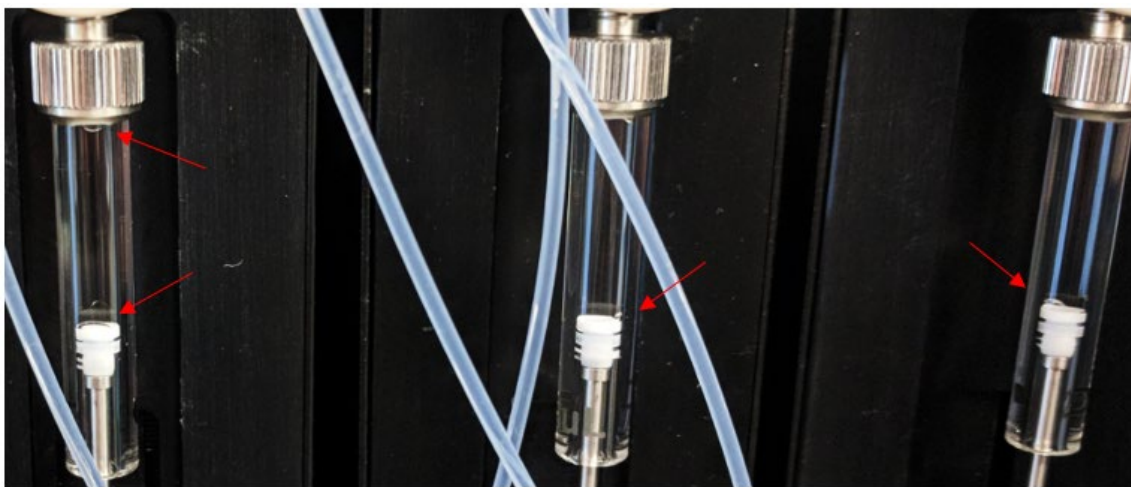


Figure 58. Example of large bubbles in syringes.

D. Perform Alcohol Prime of System Tubing (If Needed)

An alcohol prime of the system tubing should be performed once at the beginning of the workweek and when air bubbles are observed to be trapped in tubing or pump syringes during normal operation.

1. Fill a bottle with >500 ml of 70% isopropanol and another bottle with deionized water for rinsing the tubing.
2. Carefully unscrew the cap of the water reservoir and place the end of the tubing into the isopropanol bottle.
3. Click the [System Prime] button to prime the tubing with isopropanol.

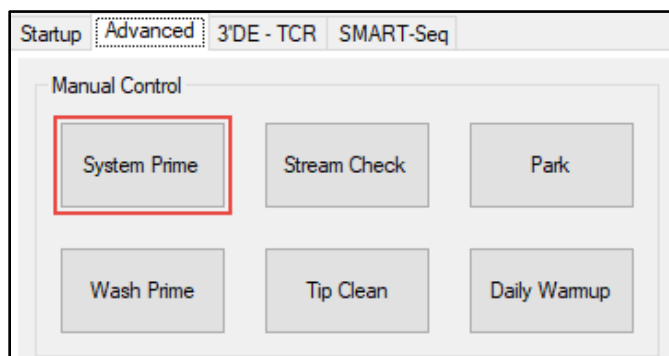


Figure 59. The [System Prime] button under the *Advanced* tab of the instrument software.

4. Upon completion, remove the cap from the isopropanol bottle and submerge the cap tubing in the bottle with deionized water to rinse off the residual isopropanol.
5. Place the clean tubing back into the water reservoir bottle and screw the cap back on.
6. Run the [System Prime] process two more times (twice) while connected to the water reservoir to flush out the isopropanol and replace with water.
7. Proceed to the next section ([Section VIII.E](#)) and run one [Tip Clean] procedure to finish the procedure.

E. Run the [Tip Clean] Procedure

The cleaning process takes ~2 min.

NOTE: This process will also be run 3–4 times after each dispense step ([Section X.C](#) and [Section X.F](#)).

1. Click the *Advanced* tab in the CELLSTUDIO software.
2. Observe the “Wash” well of the trough.

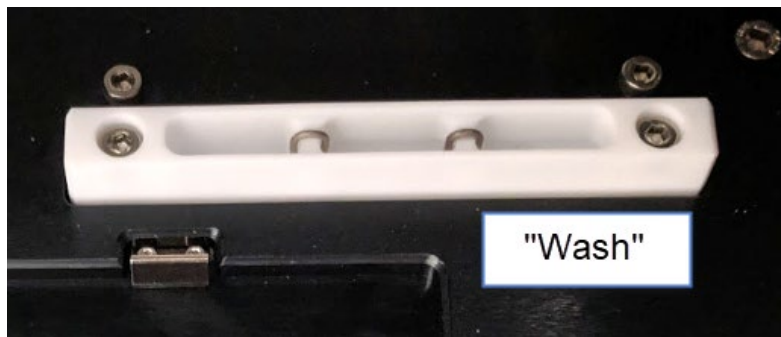


Figure 60. Location of the Wash well of the trough.

3. If the well is dry, click the [Wash Prime] button to fill with wash solution.

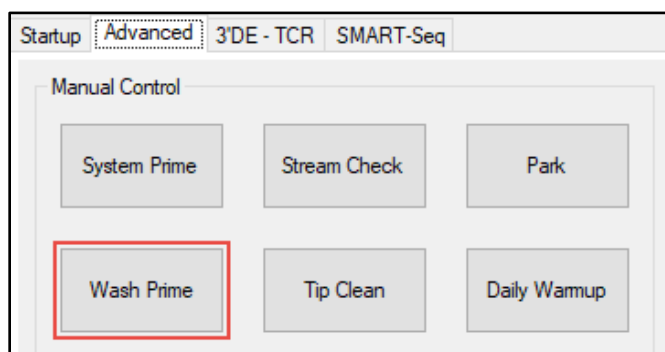


Figure 61. The [Wash Prime] button.

4. Click the [Tip Clean] button to initiate the sequence to wash the inside and outside of the tips.

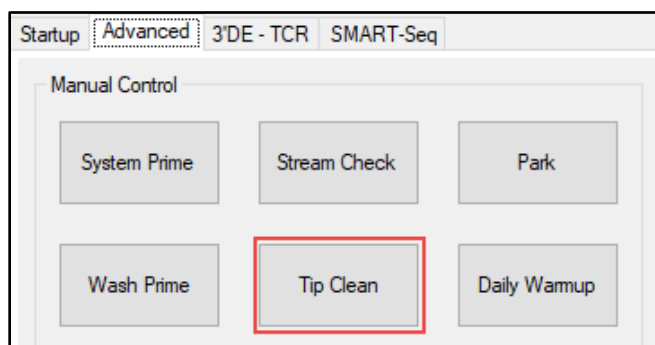


Figure 62. The [Tip Clean] button under the *Advanced* tab.

5. After this step is complete, the system is ready for use.

IX. Protocol: Prepare the sample source plates

A source plate is a 384-well plate containing 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 ICELL8 cx Instrument.

!

IMPORTANT: Only the plates included in the ICELL8 384-Well Source Plate and Seal kit (Cat. No. 640192) are validated for use on the ICELL8 cx system.

!

IMPORTANT: Avoid introducing dust and debris to solutions that will be dispensed with the ICELL8 cx. 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

The SMART-Seq ICELL8 cx application is used to illustrate the basic workflow.

1. Prepare your sample. If you're starting with cells and Hoechst 33342 and propidium iodide are selected for use as dyes, such as in the prevalidated applications from Takara Bio, the guidelines in the [ICELL8 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 devised tailored to your sample source and experimental needs. [Appendix G](#) covers broader considerations for developing a new application for the ICELL8 cx system; [Appendix G.L](#) discusses the use of dyes at a high level.

2. In the CELLSTUDIO interface, select the desired application from the available tabs on the top, left edge of the main workspace (Figure 63, below). The SMART-Seq ICELL8 cx application will be used to illustrate the basic workflow.

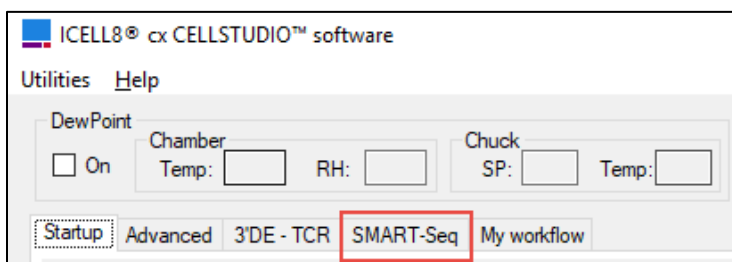


Figure 63. Highlighting the *SMART-Seq* application tab on the top left edge of the application workspace.

3. Pipette the sample (cell suspension or nucleic acids) and experimental controls into a 384-well sample source plate, following the plate map displayed in the workspace for the application selected. [Appendix H](#) includes plate map images that can be printed for reference in the lab, if needed.
 - If using a prevalidated application, please refer to the fill instructions in the associated application-specific user manual available at [takarabio.com](https://www.takarabio.com).
 - If creating or using a custom application, the recommended fill volume per well of the source plate, depending on the dispense volume, are outlined in Table 6.

Table 6. Recommended volume fill of sample, control, reagent, or indexes added to the sample source plate

Dispense type	Dispense volume ¹	Source well volume
Cells or nucleic acid	Sample (35 or 50 nl)	80 µl
Control (pos or neg)	35 or 50 nl	25 µl
Reagent (filtered)	100 nl	85 µl
Reagent (filtered)	35 or 50 nl	50 µl
Reagent (unfiltered)	35 or 50 nl	80 µl
Index	35, 50, 100 nl	20 µl

¹The dispense volume is the amount configured to dispense by the ICELL8 cx system into the ICELL8 chip, as defined in the CELLSTUDIO application.

Cells	PosCtrl	NegCtrl	RT - PCR mix				Tagmentation mix				Index 1	Index 2												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	17	33		65	81	97	113	129	145	161		193	209	225	241	257	273						369
B	2	18	34		66	82	98	114	130	146	162		194	210	226	242	258	274						
C	3	19	35		67	83	99	115	131	147	163		195	211	227	243	259	275						
D	4	20	36		68	84	100	116	132	148	164		196	212	228	244	260	276						
E					69	85	101	117					197	213	229	245								
F					70	86	102	118					198	214	230	246								
G					71	87	103	119					199	215	231	247								
H					72	88	104	120					200	216	232	248								
I					73	89	105	121					201	217	233	249								
J					74	90	106	122					202	218	234	250								
K					75	91	107	123					203	219	235	251								
L					76	92	108	124					204	220	236	252								
M					77	93	109	125					205	221	237	253								
N					78	94	110	126					206	222	238	254								
O					79	95	111	127					207	223	239	255								
P					80	96	112	128					208	224	240	256								384

Figure 64. Example source plate map for loading the 384-well plate with samples, controls, reagents, and/or indexes. The SMART-Seq full-length application is shown here. Using the values from Table 6 (above), 80 μ l of cells are dispensed into each of the eight blue-highlighted wells (1–4, 17–20)—640 μ l total.

4. After filling the wells, seal the plate with a 384-well source plate seal. Do not centrifuge.

! IMPORTANT Use source plates immediately.

X. Protocol: Dispense samples into the chip

A. Place the Chip in the ICELL8 cx Unit

1. Visually inspect the chip nest and clean it if there is any debris.
2. Remove the seal on the chip and place the chip on the chip nest. Make sure the chip fits tightly and lays flat (Figure 65).

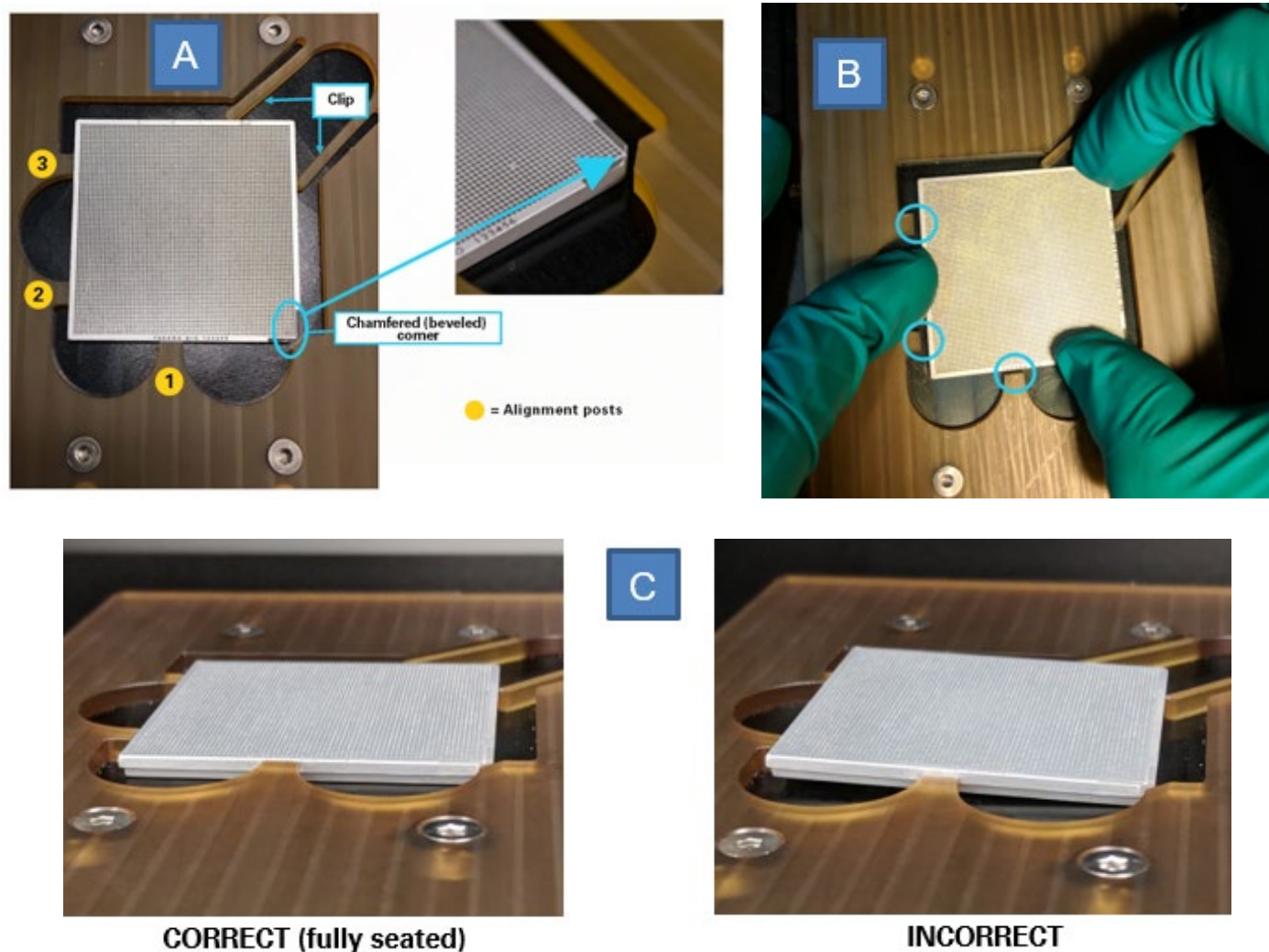


Figure 65. Procedure to place the chip in the chip nest. When loading the chip, it is recommended to wear tight-fitting gloves so as not to interfere with chip placement, and to only touch the edges of the chip, minimizing contact with the wells (top face of the chip). To load the chip, first correctly position the chip by setting it down on top of the chip nest with the chamfered corner oriented to the bottom right and the sides of the top-right corner of the chip contacting the inner ends of the prongs (**Panel A**). Then, gently slide the chip toward the top right into the clip; the arms of the clip will spread out slightly. Once the left and lower sides of the chip clear the alignment posts (**Panel B**), push the chip down into the chip nest and back towards the alignment posts. To ensure the chip is fully against the alignment posts, push down on all corners of the chip and pull the chip towards the alignment posts. (**Panel C**). Visually check to make sure the bottom face of the chip is flat against the black anodized aluminum surface (“chuck”), and the top face of the chip is flush with the surface of the chip nest. It is important to check that the chip is fully seated before dispensation to avoid interference with the dispenser tips.

B. Place the Sample Source Plate in the ICELL8 cx Unit

1. Place the sample source plate on the plate nest with the A1 position in the top-right corner (Figure 66). Press down on the plate to ensure it is sitting flat against the platform.

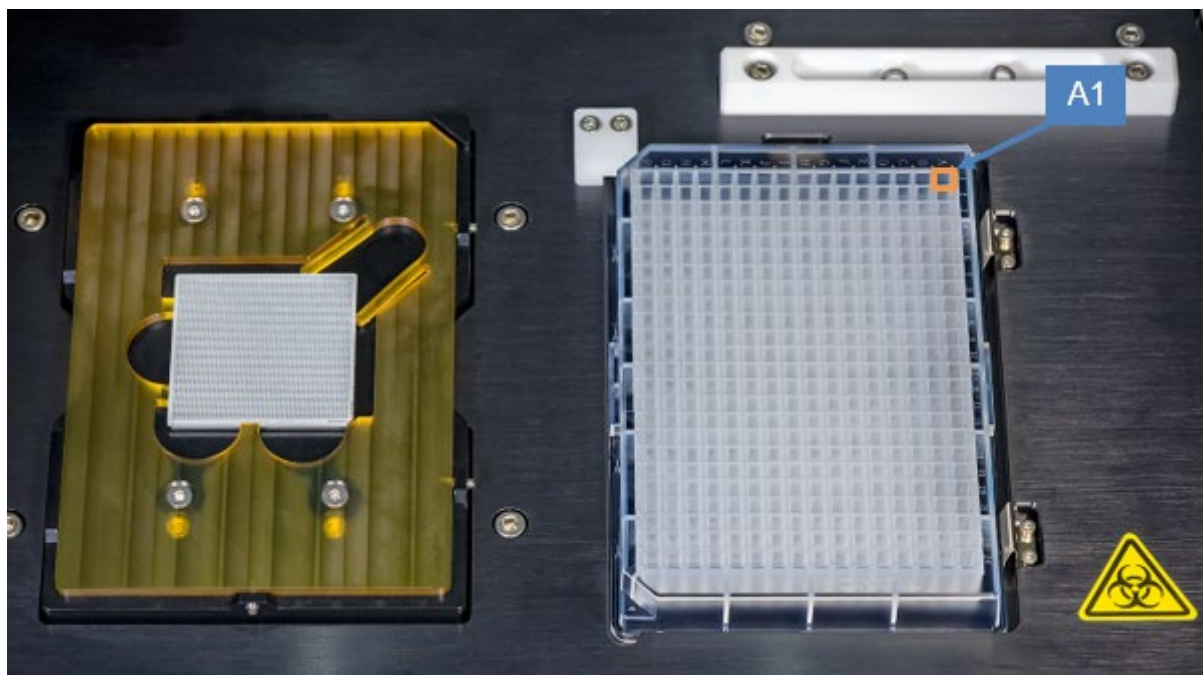


Figure 66. Sample source plate in the plate nest.

2. Close the front access panels of the ICELL8 cx module; both doors should remain closed during the dispensing process.



Figure 67. View of the ICELL8 cx module with the front doors open (left) and closed (right).

3. Let the system idle until the chamber has reached the relative humidity and dew point setpoints (this may take up to 2 min).

C. Dispense the Sample Cells and Experimental Controls into the Chip



IMPORTANT: Do not open the door of the stage module while the ICELL8 cx is dispensing. If the door is open, the chip can become contaminated. Additionally, when the door is open, evaporation from the nanowells can occur, resulting in changes to concentrations of the reagents in the chip.

1. (Optional) If your cells settle quickly, check the “Cell re-suspension” option on the *Advanced* tab (see [Section V.D](#)) to introduce pauses in the dispense process.
2. Click the first dispense button listed to initiate the dispense workflow.

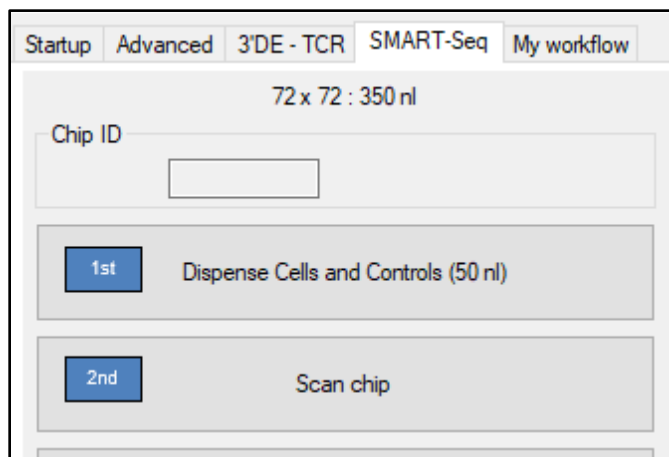


Figure 68. Identifying the first dispense button in the workflow. When working with cells, the cell dispensing step should be first. The [Scan chip] step is automatically added to the dispense list after the cell dispense step and is therefore second.

3. If using indexes, it will prompt you for which index set you will be using. Figure 69 shows the options for SMART-Seq application. Click [Done] once selected from the drop-down box.

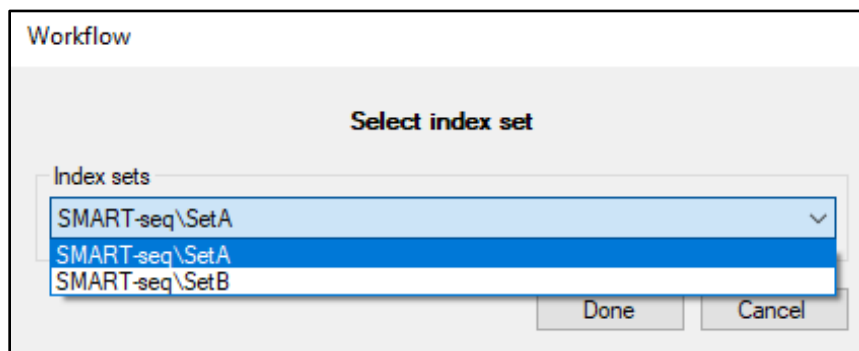


Figure 69. Example of multiple index set options for a primer dispense.

4. A message will pop up, reminding the user to make sure the source plate and chip are properly inserted. Click [Done].

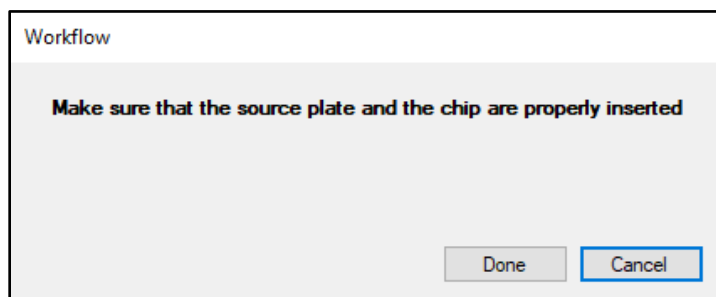


Figure 70. Prompt pop-up to confirm plate and chip loading.

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

5. The system will check to see if the minimum vacuum level has been reached.

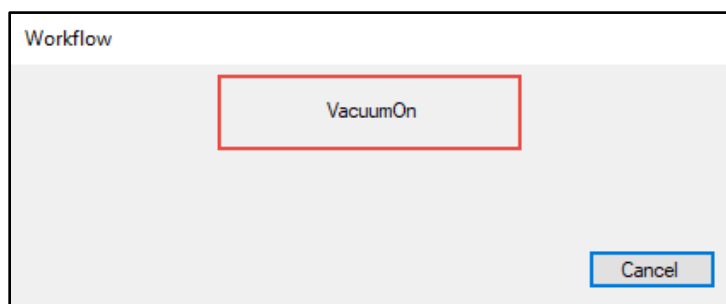


Figure 71. The dialog window when the system has initiated the chip nest vacuum. It will display this until the minimum threshold is reached.

If the minimum vacuum level is reached, the status button will show green and automatically continues to Step 6.



Figure 72. Vacuum status confirmation. (Left) The minimum vacuum level has been reached. (Right) Insufficient vacuum level indicator.

If the vacuum level is insufficient, the vacuum status will show a red dot, and an error message will display. Click [OK] to return to the main menu and run through the following steps:

- a. Click [Dispense cells] to restart the sequence. Follow the prompt by pushing the chip down and visually verifying that it is fully seated on the chuck. Then click [Done].
- b. If an error message indicates that vacuum levels are still not reached, but the chip is fully seated, click [Yes] to proceed to Step 6.

6. The software will remind the user to remove seals (on the chip and source place). Click [Done] in the *Workflow* window to confirm.

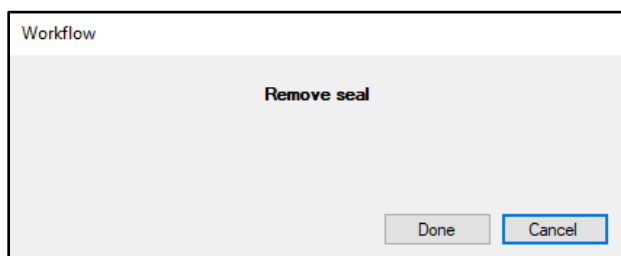


Figure 73. Confirm seal removal pop-up.

7. The system will perform autofocus and barcode read steps. The live image of chip scanning will display for user reference. The scanning process will take 1–2 minutes.

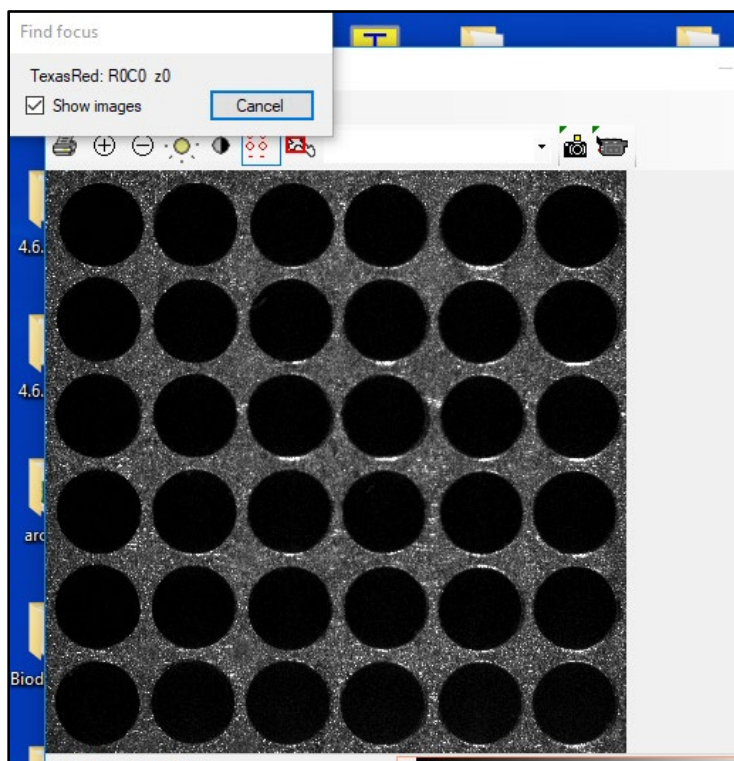


Figure 74. Live image of chip scanning.

- Occasionally, if the chip is not inserted correctly, the software will prompt the user to make sure that the chip is fully seated against the three alignment posts and oriented correctly (Figure 75).

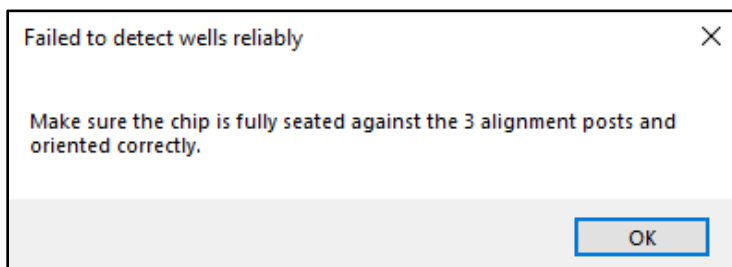


Figure 75. Pop-up message if a problem is detected during the chip scan.

If this happens:

- a. Click [OK].
 - b. Open the door to the ICELL8 cx module and visually check that the chip is placed correctly (refer above to Figure 65, Panel C).
 - c. Restart the workflow by clicking the [Dispense cells] button.
- If the chip ID is readable by eye (Figure 76), but the software is unable to read it, manually enter the ID into the “Chip ID” field.

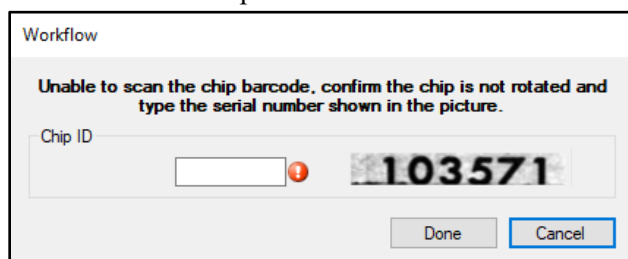


Figure 76. Input chip ID.

- If the scan completes, but there is no chip ID picture shown, as in Figure 76, that indicates the chip is likely in the incorrect orientation and needs to be rotated and resealed.
8. Once the Chip ID is input, click [Done] to proceed to the dispense.

If the chamber environment is not stabilized within the expected time (about 5 min), the following informational message will pop up. This message will automatically disappear when the required chamber humidity has been reached.

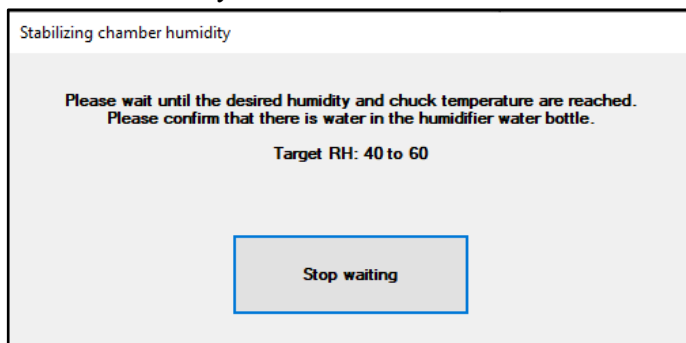


Figure 77. Stabilizing chamber humidity dialog message.

NOTE: [Stop waiting] should only be clicked in the rare instance of a system being unable to reach the desired humidity or chuck temperature after about 5 min. The system will then give the option to the user to continue or to abort the experiment.

Please contact Takara Bio technical support if the instrument is unable to reach the desired humidity and chuck temperature.

9. When the dispense is complete, the vacuum should turn off automatically. You can then remove the chip for blotting.

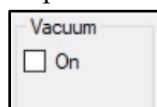


Figure 78. Vacuum status after dispense. Unchecked means the vacuum is off.

10. Perform the [Tip Clean] procedure 3–4 times (see [Section VIII.E](#)).

D. Blot and Centrifuge the Chip

After sample dispense is complete, promptly blot the chip for 2 seconds, as described below.

- Place the chip, wells facing up, on a clean lab wipe.
- 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.
- Pick up the Nanodispenser Blotter (Figure 79, 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 79. Blotting the chip with Blotting Paper and Nanodispenser Blotter. A blotter is provided with the ICELL8 cx Single-Cell System Accessory Kit.

- Let the blotter rest on top of the blotting paper for exactly 2 seconds without pressing down; the weight of the blotter is sufficient for adequate blotting.
- Remove the blotter, then gently remove the blotting paper. Check to see if there are any big droplets blotted, as shown in Figure 79. If big droplets are seen, refer to [Appendix A](#), Table 7 for troubleshooting tips.

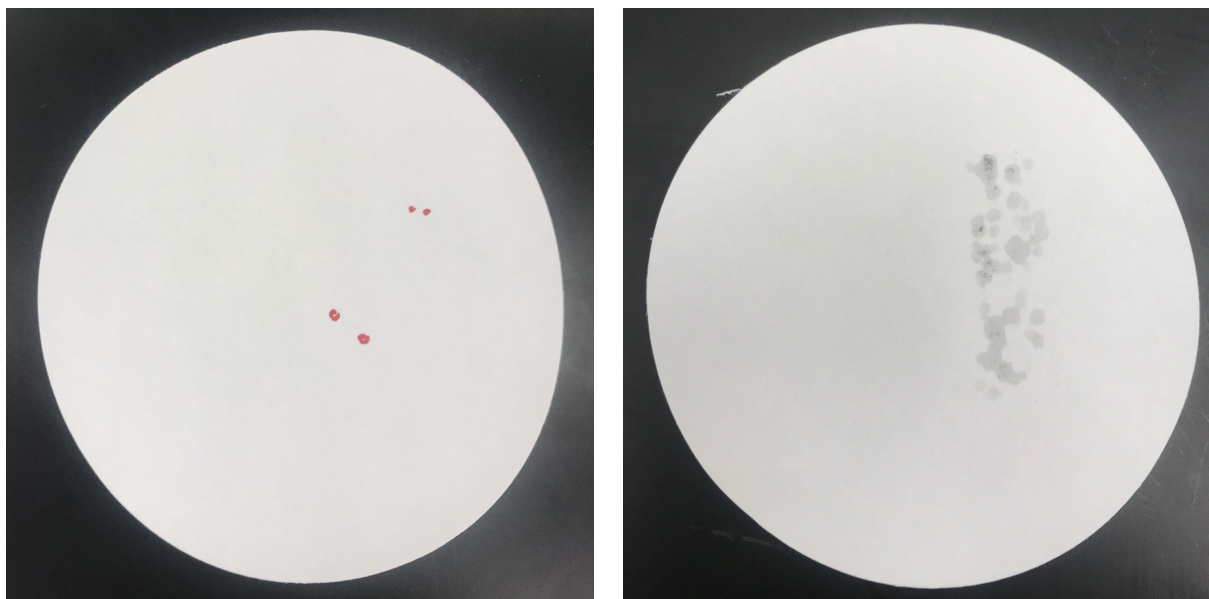


Figure 80. Examples of blotting results. (Left) Example of a good result; the red marks indicate spots on the paper where blots were observed prior to evaporation. (Right) Example of a poor blot; note the size and quantity of the damp spots on the paper.

- Dispose of the blotting paper in a biohazard container.

2. 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 81. Sealing the chip with film and a film-sealing roller.

3. Centrifuge as described below.
 - a. Place your chip on the centrifuge chip spinner.
 - b. Counterbalance with a second chip.
 - c. Centrifuge for 5 min at 22°C at 300g or the speed specified by your sample type or the protocol.

E. Scan Chip for Single Cells and Freeze the Chip (If Necessary)

NOTE: This section is only necessary when the sample source is single cells.

If starting with nucleic acids or your sample does not require imaging:

- The <chipID>_WellList.txt file that may be imported into Takara Bio bioinformatics software will be generated by CELLSTUDIO software. See [Appendix B.D](#) for more information.
- Skip the remainder of this section and go to the next ([Section X.F](#)).

1. After centrifugation of the chip with the samples is complete, remove the film on the chip and load the chip in the chip nest.
2. Press [Scan chip] to initiate the cell scan workflow.

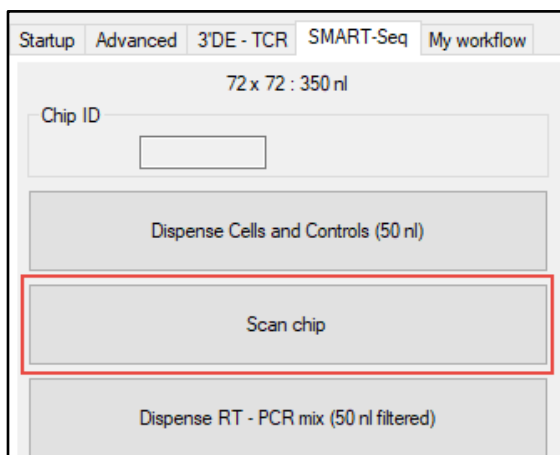


Figure 82. Initiate chip scanning.

3. Then select the Optical configuration that you would like to use for this scan from the drop-down menu (Figure 83). Custom optical configurations can be created following the guidelines in [Appendix E](#).

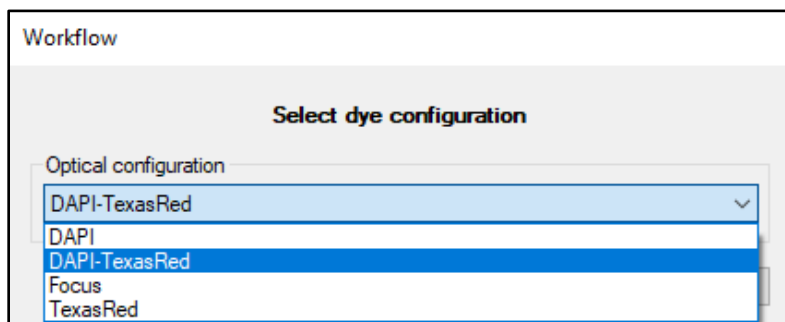


Figure 83. Initiate chip scanning. The default selection will match the previous user's choice.

4. Similar to the cell dispense workflow, the software will:
 - a. Prompt the user to verify that the chip is inserted properly.
 - b. Check that sufficient vacuum levels have been reached.
 - c. Remind the user to remove the seal on the chip.
 - d. Perform autofocus for chip barcode.
5. When the autofocus and barcode scanning steps are complete, if there is no chip ID picture shown, as in Figure 84, that indicates the chip is likely in the incorrect orientation and needs to be rotated and resealed.

If the chip ID is readable by eye, but the software is unable to read it, manually enter the ID into the "Chip ID" field.

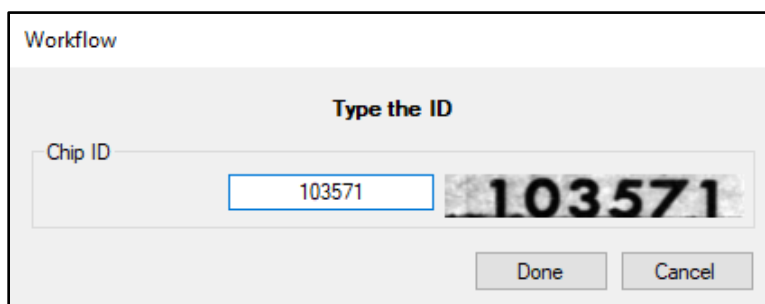


Figure 84. Enter the chip ID after autofocus and barcode scanning steps

Once the Chip ID is input, click [Done] to proceed to the dispense.

6. Select the proper analysis setting and, if necessary, the barcode .xml or .gal files. (Figure 85). Enter a description of the experiment in the “Chip comment” field, if helpful.

Figure 85. Selecting the analysis setting and barcode files to use during imaging. The “Barcodes” field is grayed out in this example screenshot because barcode files (SMART-seq application) were configured within the application, at the cell dispense step (first scenario, below).

- The “Barcodes” field will be grayed out in the *New static info* window if the barcode file to use was configured within the application properties ([Section VII.B](#)) or selected from a subfolder at the cell dispense step, such as with the SMART-Seq application.
 - If the application barcode folder is defined as ‘None/Predefined’ in the application properties, such as the case of the prevalidated 3’ DE or TCR applications, the “Barcodes” drop-down menu will be active. The assay map files stored in `C:\ProgramData\takara\CellSelect\AssayMaps` populates the menu. The appropriate assay file should be selected to match the known barcodes or preprinted chip type being used.
7. Press [OK] to confirm. At this point, CellSelect software will start to let you define the analysis settings.

8. Select the path and the name of the folder where data will be saved using the [...] button to the right of the “Base folder” path statement. Press [OK] to confirm.

NOTE: Make sure that the “Base folder” path is set to a location other than the C : \ drive. The scan folders are large and will quickly fill up the C : \ drive if set there. The red icon next to the path statement is a mouse-over tooltip if the current base folder is located on the C : \ drive.

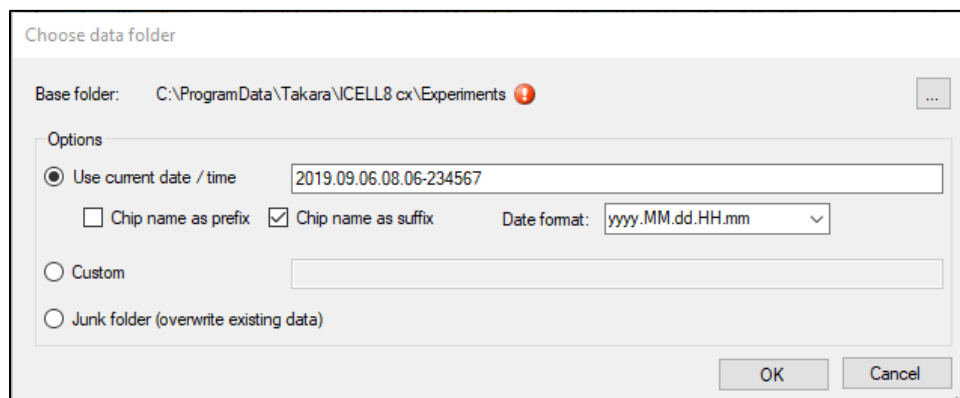


Figure 86. Selecting the path and file name for saving data on the *Choose data folder* window.

9. Chip scanning will begin. (The scanning channels are predefined in a configuration setting, see [Appendix E](#)).
10. When chip scanning and analysis is complete, the system will ask if you want to save the result file. Press [Save] to proceed.
11. Name the results file. The default file name is <chipID>.wcd. This folder will contain the filter file (with <the name given to the results file>.txt), which will be used in the next step to define which wells contain single cells and should be dispensed to.

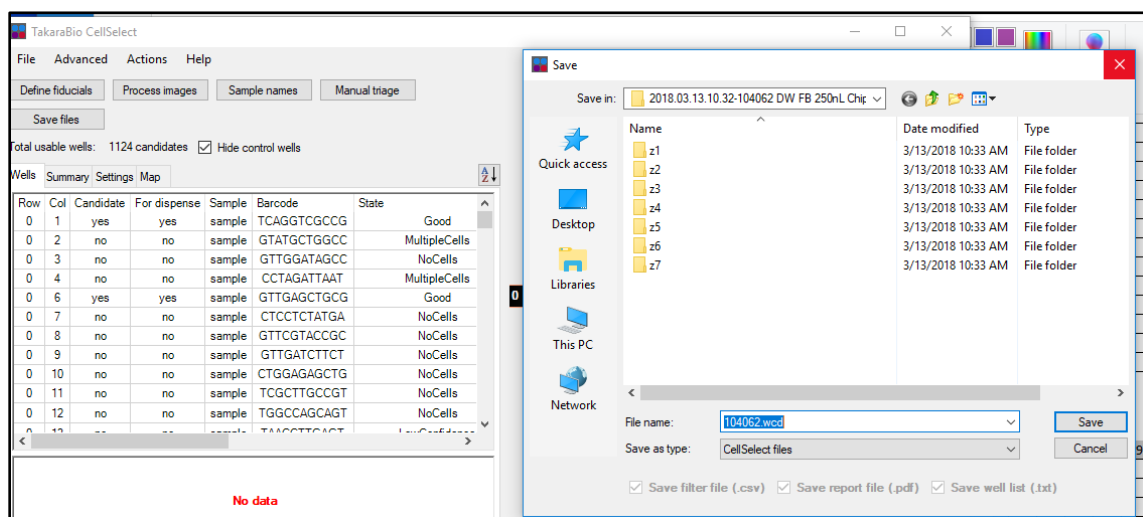


Figure 87. Naming and saving results file.

12. The vacuum should turn off automatically when scanning is complete.

13. Remove the chip from the chip nest, seal with sealing film, put it into a chip holder, and decide whether you want to freeze or store the chip at this time.
 - When working with cells, the chip should be frozen to lyse them. Freeze the chip at -70°C for 30 min or longer.
 - The chip should also be frozen at -70°C if it needs to be stored for a long time, if freezing is appropriate for your sample type.
 - 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.
14. (Optional) Manually inspect the selected nanowells in CellSelect software to exclude or include one or more candidate wells. Please refer to the “ICELL8 cx CellSelect v2.0 Software User Manual” for more information about this process.

During the inspection process, it is recommended that the chip be stored at -70°C or 4°C per the guidelines in Step 13 (above).
15. Close only the ICELL8 cx CellSelect v 2.0 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.

F. Dispense Reagents and/or Indexes into the Chip

The next steps are representative of the dispense of most downstream reagents or indexes. The example used is the first reagent dispense for the SMART-Seq application.

1. Check that the GUI is on the correct tab.

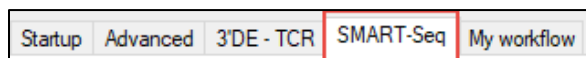


Figure 88. Selecting the application for dispensing reagents. The SMART-Seq application is used as an example here.

2. Pipette **only** the freshly prepared reagent or index being dispensed at this step into a source plate, according to the location(s) specified in the plate map for the application.

Cells	PosCtrl	NegCtrl	RT - PCR mix	Tagmentation mix	Index 1	Index 2																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	17	33		65	81	97	113	129	145	161		193	209	225	241	257	273						369
B	2	18	34		66	82	98	114	130	146	162		194	210	226	242	258	274						
C	3	19	35		67	83	99	115	131	147	163		195	211	227	243	259	275						
D	4	20	36		68	84	100	116	132	148	164		196	212	228	244	260	276						
E					69	85	101	117					197	213	229	245								
F					70	86	102	118					198	214	230	246								
G					71	87	103	119					199	215	231	247								
H					72	88	104	120					200	216	232	248								
I					73	89	105	121					201	217	233	249								
J					74	90	106	122					202	218	234	250								
K					75	91	107	123					203	219	235	251								
L					76	92	108	124					204	220	236	252								
M					77	93	109	125					205	221	237	253								
N					78	94	110	126					206	222	238	254								
O					79	95	111	127					207	223	239	255								
P					80	96	112	128					208	224	240	256								384

Figure 89. Source plate map for the SMART-Seq application. The highlighted wells contain the first reagent to be dispensed (Column 3, Rows A–D, wells #33–36). See [Appendix H](#) for additional plate map reference images.

3. Load the source plate into the plate nest.
4. Remove the ICELL8 cx chip from the chip holder, remove the chip sealing film, and place the chip securely flat on the chip nest.
5. Close the front door of the ICELL8 cx Instrument.

6. Click the [Dispense] button to initiate the reagent/index dispense.

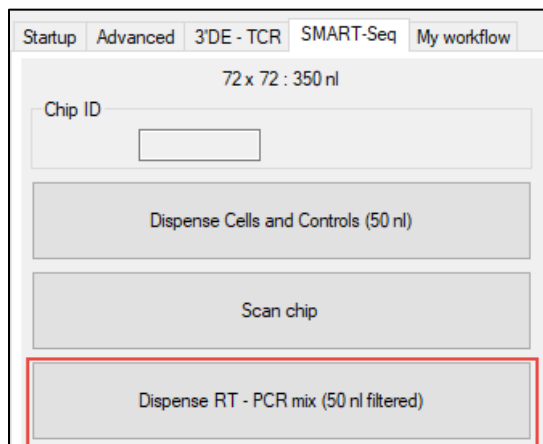


Figure 90. Click the [Dispense] button to initiate the first dispense. RT–PCR reagent dispense is shown as an example.

7. (Optional) If the dispense is configured with a constraint to follow a filter file:
 - a. Click the [...] button on the *Workflow* pop-up window to browse for the filter file.
 - b. Select the filter file generated from the cell scan step (previous step, [Section X.E](#)) **OR** one created manually ([Appendix D](#)) (Figure 91, top).
 - c. Once the file is highlighted, press [Open].
 - d. Back in the *Workflow* window, click [Done] to confirm.

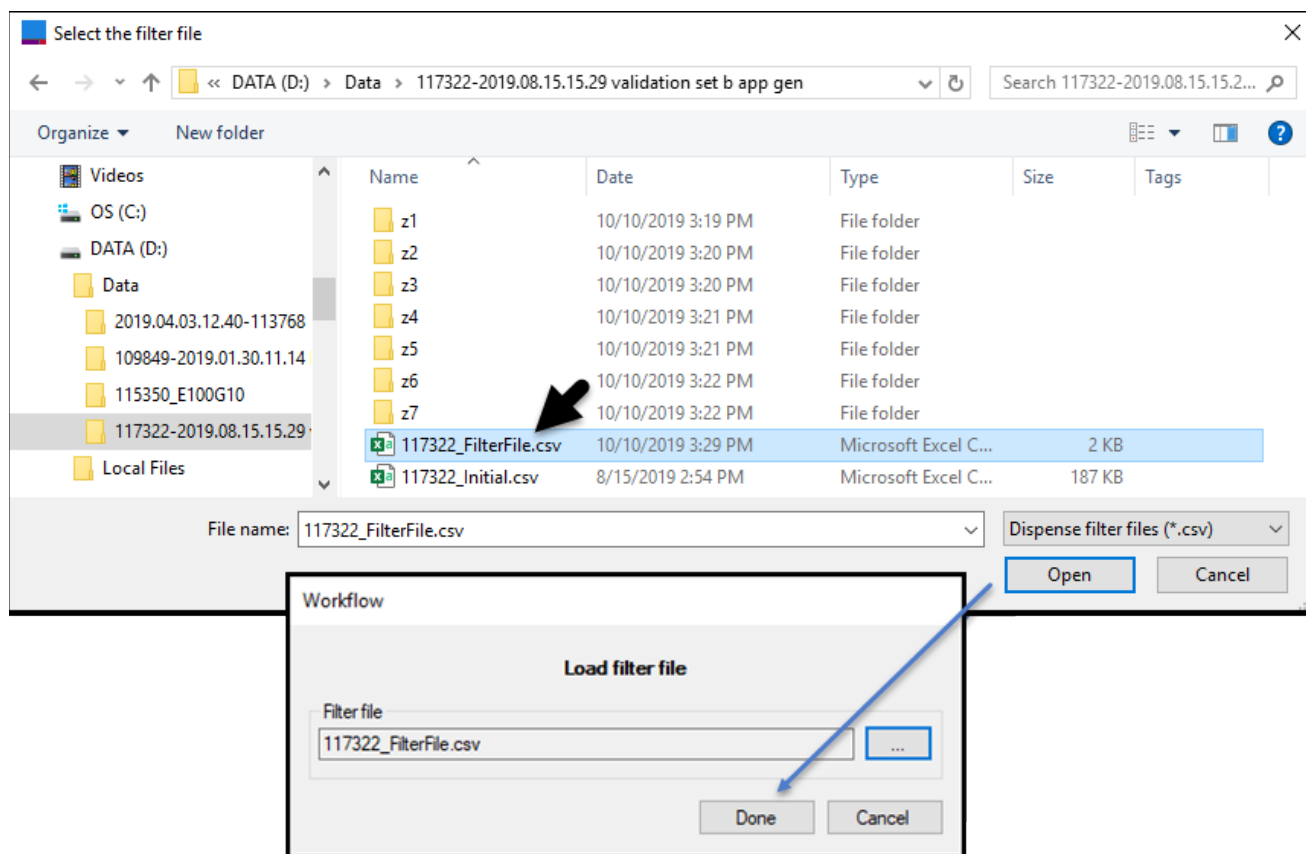


Figure 91. Selecting (top) and loading (bottom image) the filter file.

8. Similar to the cell-dispense workflow, the software will:
 - a. Prompt the user to verify that the chip is inserted properly.
 - b. Check that sufficient vacuum levels have been reached.
 - c. Remind the user to remove the seal on the chip.
 - d. Perform autofocus for the chip barcode.
9. When the autofocus and barcode scanning steps are complete, if there is no chip ID picture shown, as in Figure 92, that indicates the chip is likely in the incorrect orientation and needs to be rotated and repeated.

If the chip ID is readable by eye, but the software is unable to read it, manually enter the ID into the “Chip ID” field.

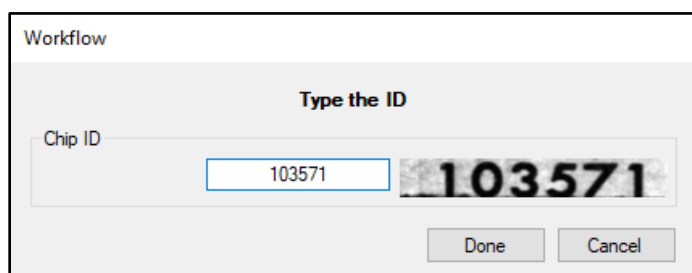


Figure 92. Enter the chip ID after autofocus and barcode scanning steps

Once the Chip ID is input, click [Done] to proceed to the dispense.

NOTE: If a filter file was selected in Step 7 and the chip ID does not match the chip ID prefix of the filter file, the software will give you the option to proceed or abort. Click [Yes] or [No], as appropriate.

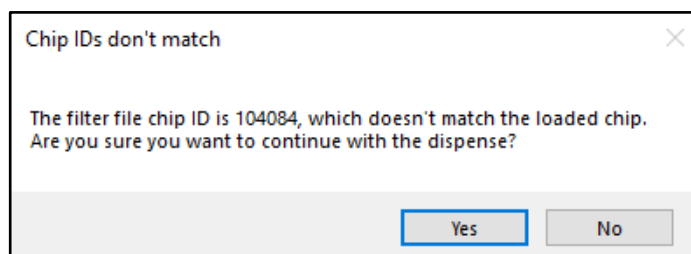


Figure 93. Confirm that the chip ID matches the filter file name.

10. After confirming the chip ID confirmation, the dispense will begin.
11. When the dispense step is complete, the vacuum should turn off automatically; you can then release the chip.
12. Carefully remove the chip from the chip nest.
13. Blot and seal the chip.
14. Centrifuge the chip at 3220g for 3 minutes at 4°C.
15. Perform the [Tip Clean] procedure 3–4 times ([Section VIII.E](#)).

NOTE: Repeat steps 1–14 as needed until all reagents and/or indexes are dispensed according to the application workflow. We recommend using a fresh source plate for each dispense.

G. Run your Reaction/Incubation on a 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 [Appendix F](#) for guidelines on designing a thermal cycler program.

If you are using the ICELL8 cx Single-Cell System (Cat. No. 640188), use the thermal cycler (Cat. No. 640002) included with the system. See the user manual shipped with the thermal cycler for complete instructions on thermal cycling the filled chip.

If you are using the ICELL8 cx Single-Cell System JPN (Cat. No. 640189), use a thermal cycler of your choice. See the manufacturer's user manual for further instructions on thermal cycling the filled chip.

H. Clean the ICELL8 cx System

1. After completing each dispense mapped for a source 384-well plate, remove the plate from the plate nest 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). **Do not** wipe the plastic side panels.



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 technical support (technical_support@takarabio.com).



WARNING: Ensure that no liquid drips below the platform, especially onto the temperature and humidity sensor, which is located just to the left of the chip nest.

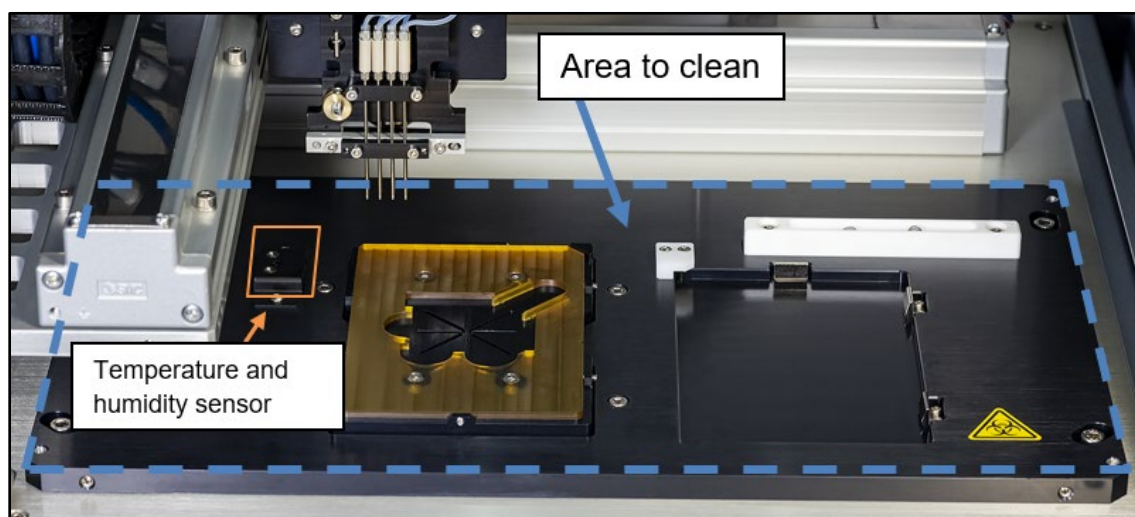


Figure 94. Area of the ICELL8 cx System to clean after a dispense run. The dispensing platform is outlined by the blue-dashed rectangle. Make sure to avoid liquid dripping onto the temperature and humidity sensor, outlined by the solid orange square.

3. After the final dispense of the day, perform the [Tip Clean] procedure ([Section VIII.E](#)).

NOTE: It is recommended to leave the instrument and software ON when not in use.

XI. Protocol: Extract library from the chip

This protocol extracts the resulting library from the chip.

A. Before You Start

- Set the centrifuge(s) used for spinning the chip to 4°C.
- This protocol requires components from the ICELL8 Collection Kit – L (Cat. No. 640212): Collection Fixture – L, Collection Tube, and Collection Film.

B. Procedure

1. Remove the chip from the thermal cycler. Centrifuge the chip at 3,220g for 3 min at 4°C.
2. Open the 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 95.

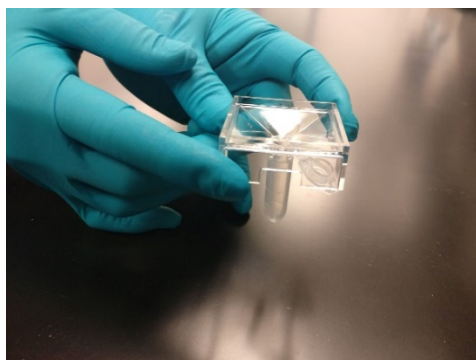


Figure 95. Assembling the collection module.

3. Carefully peel the film from the chip (Figure 96, below). 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.

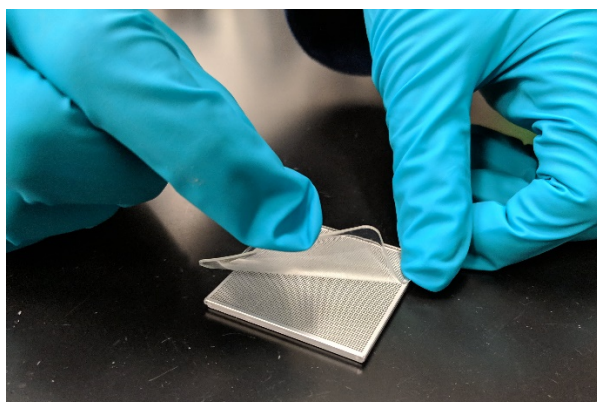


Figure 96. Removing the film from the chip.

4. With the nanowells facing down, place the chip into the assembled collection module (Collection Tube plus Collection Fixture; Figure 97, below). Surface tension will hold the liquid in the nanowells.



Figure 97. Placing the chip into the collection module.

5. Seal the chip and the top of the collection module with a supplied Collection Film (Figure 98, below).

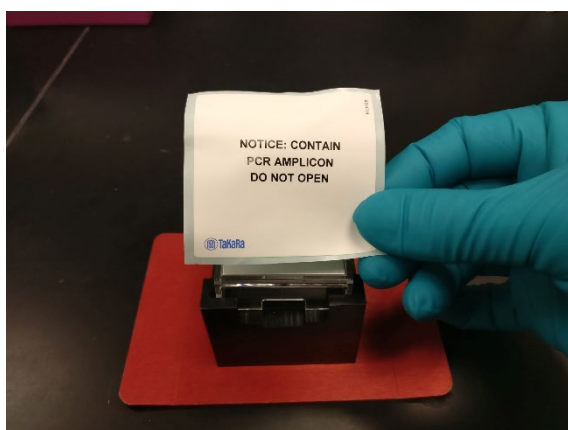


Figure 98. Securing the collection module with Collection Film.

6. Using a balance or blank chip, assemble another collection module. Centrifuge both collection modules at 3,220g (minimum 2,600g) for 10 min at 4°C.
7. Carefully remove the Collection Tube containing the extracted library (Figure 99, below).

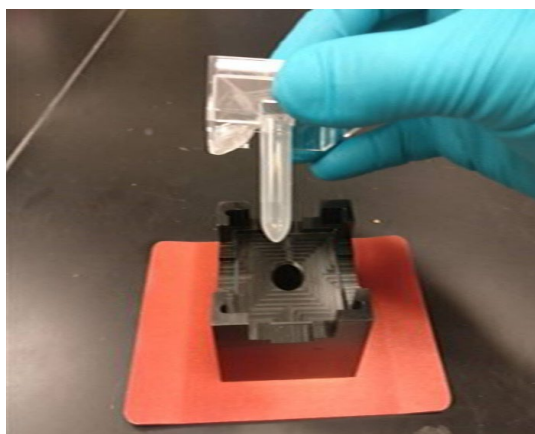


Figure 99. Removing the Collection Tube containing the eluate.

The volume of the eluate collected will be less than the calculated maximum volume (formula below). Evaporation, which occurs when the chip is not sealed, decreases the volume: having more dispense steps will increase the chances of evaporation, further decreasing the final volume.

To calculate the maximum potential volume (in µl) from the chip, use the formula:

$$\frac{(Total\ sample\ volume) + (Number\ of\ candidates \times (Well\ dispense\ volume))}{1,000\ nl/\mu l}$$

where:

$$Total\ sample\ volume = Number\ of\ wells \times Sample\ dispense\ volume\ per\ well$$

$$Well\ dispense\ volume = Total\ volume\ of\ reagent\ dispenses\ per\ well + Total\ volume\ of\ index\ dispenses\ per\ well$$

Example

When doing SMART-Seq full-length transcriptome analysis:

- The entire chip is used (72 x 72 dispense pattern), so *Number of wells* = 5,184
- *Sample dispense volume per well* is configured as: 50 nl/well
- There are two reagent dispenses (RT mix and Tagmentation Mix) of 50 nl each
(50 nl/well + 50 nl/well) = 100 nl/well
- There are two index dispenses (Index1 and Index2) of 50 nl each
(50 nl/well + 50 nl/well) = 100 nl/well

If 1,000 nanowells are selected as candidate wells (*Number of candidates*), the formula would be:

$$Maximum\ potential\ volume\ (\mu l) = \frac{(5,184 \times 50 \frac{nl}{well}) + (1,000 \times (100 \frac{nl}{well} + 100 \frac{nl}{well}))}{1,000\ nl/\mu l}$$

and the maximum potential value would be: 459 µl

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.

XII. 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. Daily Maintenance


Daily Maintenance procedures help ensure optimal instrument operation and prevent problems. They are described in “Protocol: Prepare the ICELL8 cx System” in [Section VIII](#).

B. System Shutdown Procedure

It is recommended to leave the instrument and software ON. If the ICELL8 cx Instrument needs to be shut down for long periods of time, the following procedure can be performed by qualified personnel.



WARNING: Moving the instrument may damage its enclosure if not done properly. Please contact Takara Bio technical support (technical_support@takarabio.com) for more details.

1. Follow the ICELL8 cx system cleaning procedure described above in [Section X.H](#).
2. Empty the contents of the water and humidifier reservoirs.
3. Press the [System Prime] button to empty the system tubing of water.
4. Dispose of the contents of the wash bottle.
5. On the *Advanced* tab, press [Wash Prime] until there is no new fluid coming up from the wash well.
6. Exit CELLSTUDIO software by clicking the [Close] button () at the top-right corner of the window.
7. Turn off the ICELL8 cx Instrument by toggling the power switch on back of the system.

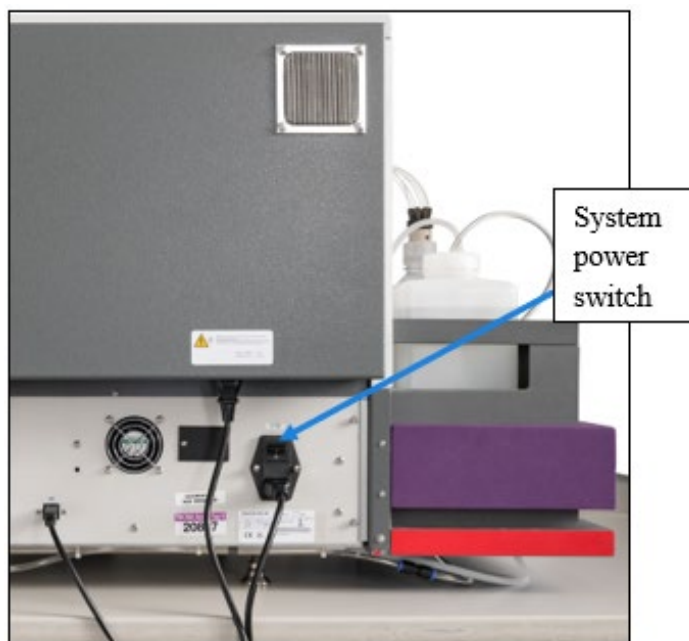


Figure 100. Location of the system power switch.

8. Check that all nanowell chips and source plates have been removed from the stage module.
9. Properly dispose of contents of waste container, rinse, and allow it to dry.
10. Remove the back cover, connect a tube to the drain valve on the humidifier tank, and drain the humidifier.
11. Power down the computer that controls the ICELL8 cx Instrument.

C. Annual Preventative Maintenance

The ICELL8 cx Instrument should be examined and calibrated every year by a Takara Bio service engineer.

Appendix A: Troubleshooting

If the ICELL8 cx system or CELLSTUDIO software does not respond as desired or a warning is displayed, please attempt to rectify the problem using the information below. If you cannot solve the problem, contact your authorized Takara Bio service technician or contact us directly at technical_support@takarabio.com.

Instrument issues

This section pertains specifically to the ICELL8 cx Instrument. Each of the following tables outlines potential root causes and suggested solutions for the observed problem symptoms described in the table header.

Table 7. Problem: Poor dispensing, excessive fluid on top of the chip

Possible cause	Solution
Trapped air in the system fluidics	Inspect the tubing between the tip and the 2 x 4 manifold (where not covered). Look for air or bubbles within the tube. If air is found within the tube, run the [Daily Warmup] 2–3 times (Section VIII.C). If the air bubble fails to be flushed out, perform a [System Prime] with isopropanol. Refer to an alcohol prime of the system (Section VIII.D).
Plugged tip	Run the [Tip Clean] procedure (Section VIII.E). If the tip is plugged with a soluble material, aspirating and dispensing a reagent capable of dissolving the material may clear the blockage. If this measure fails, contact Takara Bio technical support.
Leaking inlet valve	Let the system sit idle for 5 min and inspect the dispensing tip nozzles. If there are water droplets accumulating at any of the tips, it is possible that the solenoid is leaking. If the leaking continues after letting it idle, contact Takara Bio technical support.
Incompatible buffers and samples	Extremes of fluidic properties, such as viscosity, may result in poor performance. Instructions for testing reagents for dispensing quality are documented in a tech note at takarabio.com .
Crimped fluidic tubing	Inspect the tubing for crimps or bends. Straighten out crimped/bent sections if feasible. Contact Takara Bio technical support if the tubing needs to be replaced.

Table 8. Problem: Dispensing head does not home

Possible cause	Solution
No communication with the instrument	Check that the power cables and USB connection to the instrument are firmly plugged in on the ICELL8 cx unit (Figure 1, Section I.E). Close the CELLSTUDIO and CellSelect softwares, power cycle the instrument, and restart the CELLSTUDIO software.

Table 9. Problem: Low or partial dispenses

Possible cause	Solution
Microsolenoid valve failure	Contact Takara Bio technical support.
Crimped tubing	See Table 7.

Table 10. Problem: low number of visible cells in the Image Viewer

Possible cause	Solution
Syringe thumbscrew is loose	Tighten the syringe thumbscrew until finger tight. The thumbscrew is located at the bottom of the syringe in the syringe pump unit.
Tip is plugged	See “Plugged tip” (Table 7, above).
Air bubble in the syringe path	Prime the syringe path by performing the Daily Warmup.
Microsolenoid valve is leaky	See “Leaking inlet valve” (Table 7, above).
Syringe valve is blocked or leaky	See “Leaking inlet valve” (Table 7, above).
Low liquid level in the water container	Check the level of the system liquid in the water container. Make sure that the end of the tubing is submerged in the water. Add deionized filtered water as needed. Refer to “Check and Fill System Containers” in Section VIII.B .

Table 11. Problem: System stalls because the syringe does not move

Possible cause	Solution
No power	Check that the power cables to the instrument and the network switch are firmly plugged in on the ICELL8 cx unit. (See Figure 1 in Section I.E)
Syringe is not initialized	Close the CELLSTUDIO software, power cycle the instrument, and restart the software.
Obstruction	Verify that there is no object obstructing the movement of the syringe.

Appendix B: CELLSTUDIO Output Files

- A running debug log file for the CELLSTUDIO software itself can be found at:
C:\ProgramData\Takara\ICELL8 cx\Log\Debug.log
- A record of all dispenses can be found in the folder C:\ProgramData\Takara\ChipList\

For an ICCELL8 cx chip with a six-digit identification number represented by <chipID>, there are several files:

- <chipID>.xml
- <chipID>_initial.csv
- <chipID>_main.csv
- <chipID>_WellList.txt (situational, see description in [Appendix B.D.](#))

A description of what information each dispense file contains can be found below.

All files are human-readable. Files with the extensions *.csv and the WellList.txt should be opened in MS-Excel or another spreadsheet program. XML files (*.xml) and the debug log can be opened with a plain text editor like Notepad or a more advanced program like Notepad++, Internet Explorer, or a web browser.

A. <chipID>.xml

This file is a log file of all dispenses that have been performed on the chip. This file is updated after every dispense. For each dispense, the date and time, type of dispense, volume, and filter file name (if used) are written to this file.

B. <chipID>_initial.csv

This is a comma-separated file that is generated after the sample dispense. During the chip scan, this file provides information to CellSelect software about the locations of sample and control nanowells. It also provides barcode information for all nanowells, as there is no filter file information included in this file.

Only one of the columns “SampleSourceWell”, “PosCtrl”, “NegCtrl” will have text in it for a given row, as only one of these will be dispensed to any particular nanowell.

Table 12. Description of column headers in the <chipID>_initial.csv output file

Column header	Field description
Row	ICELL8 chip row, starting at 0
Col	ICELL8 chip column, starting at 0
SampleSourceWell	Position of sample well in source plate (alphanumeric, A1–P24)
PosCtrl	Position of positive control well in source plate
NegCtrl	Position of negative control well in source plate
Barcode1SourceWell	Position of barcode well in barcode Index1 source plate
Barcode1	Sequence of barcode Index1
Barcode2SourceWell	Position of barcode well in barcode Index2 source plate
Barcode2	Sequence of barcode Index2

Table 13. Example excerpt of a <chipID>_initial.csv output file

Row	Col	Sample Source Well	Pos Ctrl	Neg Ctrl	Barcode1 Source Well	Barcode1	Barcode2 Source Well	Barcode2
0	0	A1			A5	CGTTGGTT	A13	AACCGGTT
0	1	A1			A5	CGTTGGTT	B13	TCTAGGTT
0	2	A1			A5	CGTTGGTT	C13	CTGGTCTT
0	3	A1			A5	CGTTGGTT	D13	GTCGTTCT
1	71	A2			B5	CTATCGTT	D18	ATATGCAA
2	0	A1			C5	GTCTTCTT	A13	AACCGGTT
2	1	A1			C5	GTCTTCTT	B13	TCTAGGTT
2	2		P24		C5	GTCTTCTT	C13	CTGGTCTT
2	3	A1			C5	GTCTTCTT	D13	GTCGTTCT
2	4	A1			C5	GTCTTCTT	E13	AGAGTTCT
2	5	A1			C5	GTCTTCTT	F13	AGCTGAAT
2	6	A1			C5	GTCTTCTT	G13	GACGTATG
2	7	A1			C5	GTCTTCTT	H13	CATAATGG
2	8		P24		C5	GTCTTCTT	A17	TATACGGA
2	9	A2			C5	GTCTTCTT	A14	GGAGAAGC
4	66	A2			E5	TTGACTAT	L16	CGTAGAAC
4	67	A2			E5	TTGACTAT	M16	AGATACTA
4	68	A2			E5	TTGACTAT	N16	CCTAACTA
4	69			A24	E5	TTGACTAT	O16	GCGCAATA
4	70	A2			E5	TTGACTAT	P16	TTAACTGA
4	71	A2			E5	TTGACTAT	D18	ATATGCAA

C. <chipID>_main.csv

A comma-separated file that includes information about all reagent and index dispenses that were performed on that chip. The Barcode1 and Barcode2 columns will have text whether that index was dispensed or not. The file is updated after every dispense and only contains information about dispenses that have been performed; the exception to this is that the Barcode1 and Barcode2 columns will contain text about the primer indexes whether that index was dispensed or not.

An empty field indicates that the particular item has not been dispensed. For example, only one of the columns “SampleSourceWell”, “PosCtrl”, “NegCtrl” will have text in it.

If a dispense uses a filter file, fields for wells that were not dispensed into are left empty.

The columns for the reagent dispenses are only present in the file after the associated dispense has been carried out (e.g., The Reagent4WellId column will only be written to the file after the dispense for the reagent defined in the Reagent4 step of the application.) Note that the names of the Reagent columns do not change, even if the reagent names have been customized in the application.

Similarly, the Barcode1SourceWell and Barcode2SourceWell columns remain empty until the index dispenses have been performed; the Barcode1 and Barcode2 columns will have text whether that index was dispensed or not.

Table 14. Description of column headers in the <chipID>_main.csv output file

Column header	Field description
Row	ICELL8 chip row, starting at 0
Col	ICELL8 chip column, starting at 0
SampleSourceWell	Position of sample well in source plate
PosCtrl	Position of positive control well in source plate
NegCtrl	Position of negate control well in source plate
Barcode1SourceWell	Position of barcode well of barcode Index1 source plate
Barcode1	Sequence of barcode Index1
Barcode2SourceWell	Position of barcode well of barcode Index2 source plate
Barcode2	Sequence of barcode Index2
Reagent1WellId	Position of Reagent 1 well in source plate
Reagent2WellId	Position of Reagent 2 well in source plate
[...]	
Reagent6WellId	Position of Reagent 6 well in source plate

Example:

The following examples of an in-progress <chipID>_main.csv file were taken from a SMART-Seq full-length analysis run with Chip ID '99999'. The filter file has wells with the following (Row, Col) coordinates as (0,2), (0,4), and (0,6) as candidates.



Figure 101. Source well map for dispenses in an example SMART-seq full-length analysis run.

1. The file 99999_main.csv is created after the first reagent dispense: [Dispense RT – PCR mix], NOT after the cell dispense. The “Reagent1WellId” column has information added to it, but only for the wells that reagent is dispensed to (i.e., the wells designated by the filter file: (0,2), (0,4), and (0,6))

Table 15. Example <chipID>_main.csv file contents after first reagent dispense (Dispense #1)

Row	Col	Sample Source Well	PosCtrl	NegCtrl	Barcode1 Source Well	Barcode1	Barcode2 Source Well	Barcode2	Reagent1 WellId	
0	0	A1				CGTTGGTT		AACCGGTT		
0	1	A1				CGTTGGTT		TCTAGGTT		
0	2	A1				CGTTGGTT		CTGGTCTT	A3	
0	3	A1				CGTTGGTT		GTCGTTCT		
0	4	A1				CGTTGGTT		AGAGTTCT	A3	
0	5	A1				CGTTGGTT		AGCTGAAT		
0	6	A1				CGTTGGTT		GACGTATG	A3	
0	7	A1				CGTTGGTT		CATAATGG		
...										
2	2		P24			GTCTTCTT		CTGGTCTT		
...										

2. The second dispense is [Dispense Index 1]; information is added to the “Barcode1SourceWell” column in the file, again, for the wells specified by the filter file.

Table 16. Example <chipID>_main.csv file contents after first index dispense (Dispense #2)

Row	Col	Sample Source Well	PosCtrl	NegCtrl	Barcode1 Source Well	Barcode1	Barcode2 Source Well	Barcode2	Reagent1 WellId	
0	0	A1				CGTTGGTT		AACCGGTT		
0	1	A1				CGTTGGTT		TCTAGGTT		
0	2	A1			A5	CGTTGGTT		CTGGTCTT	A3	
0	3	A1				CGTTGGTT		GTCGTTCT		
0	4	A1			A5	CGTTGGTT		AGAGTTCT	A3	
0	5	A1				CGTTGGTT		AGCTGAAT		
0	6	A1			A5	CGTTGGTT		GACGTATG	A3	
0	7	A1				CGTTGGTT		CATAATGG		
...										
2	2		P24			GTCTTCTT		CTGGTCTT		
...										

- The third dispense is [Dispense Tagmentation mix]. Information is added to the “Reagent2WellId” column in the file; this column didn’t exist until the dispense occurred.

Table 17. Example <chipID>_main.csv file contents after second reagent dispense (Dispense #3)

Row	Col	Sample Source Well	PosCtrl	NegCtrl	Barcode1 Source Well	Barcode1	Barcode2 Source Well	Barcode2	Reagent1 WellId	Reagent2 WellId
0	0	A1				CGTTGGTT		AACCGGTT		
0	1	A1				CGTTGGTT		TCTAGGTT		
0	2	A1			A5	CGTTGGTT		CTGGTCTT	A3	A11
0	3	A1				CGTTGGTT		GTCGTTCT		
0	4	A1			A5	CGTTGGTT		AGAGTTCT	A3	A11
0	5	A1				CGTTGGTT		AGCTGAAT		
0	6	A1			A5	CGTTGGTT		GACGTATG	A3	A11
0	7	A1				CGTTGGTT		CATAATGG		
...										
2	2		P24			GTCTTCTT		CTGGTCTT		
...										

- The fourth dispense is [Dispense Index 2]; information is added to the “Barcode2SourceWell” column in the file.

Table 18. Example <chipID>_main.csv file contents after second index dispense (Dispense #4)

Row	Col	Sample Source Well	PosCtrl	NegCtrl	Barcode1 Source Well	Barcode1	Barcode2 Source Well	Barcode2	Reagent1 WellId	Reagent2 WellId
0	0	A1				CGTTGGTT		AACCGGTT		
0	1	A1				CGTTGGTT		TCTAGGTT		
0	2	A1			A5	CGTTGGTT	C13	CTGGTCTT	A3	A11
0	3	A1				CGTTGGTT		GTCGTTCT		
0	4	A1			A5	CGTTGGTT	E13	AGAGTTCT	A3	A11
0	5	A1				CGTTGGTT		AGCTGAAT		
0	6	A1			A5	CGTTGGTT	G13	GACGTATG	A3	A11
0	7	A1				CGTTGGTT		CATAATGG		
...										
2	2		P24			GTCTTCTT		CTGGTCTT		
...										

D. <chipID>_WellList.txt

<chipID>_WellList.txt is a comma-separated file containing the nanowell location of every sample and its unique barcode identifier. This file is generated by CELLSTUDIO software after a sample dispense and is equivalent, although not identical, to the <chipID>_<date>_WellList.txt file generated by CellSelect software after image processing (see “ICELL8 cx CellSelect v2.0 Software User Manual”, Section III.D).

After library generation and sequencing, either *_WellList.txt file may be used to demultiplex output data from the ICELL8 cx system using our bioinformatic software, mappa™ Analysis Pipeline. More information about the mappa software can be found at takarabio.com/icell8-software

The <chipID>_WellList.txt file generated by CELLSTUDIO software should be used as input to the mappa software in the case where imaging of the chip ([Section X.E](#)) is not performed. The structure of the file is described in Table 19, and an excerpt of an example file is shown in Table 20.

Table 19. Description of column headers in the <chipID>_WellList.txt output file

Column header	Field description
Row	ICELL8 chip row, starting at 0
Col	ICELL8 chip column, starting at 0
Sample	Position of sample well in source plate
Barcode	Combined barcode “Index2+Index1” (refer to Appendix C.B , Table 21, below) This column will be empty if no index primers are configured to dispense in the application

Table 20. Excerpt of an example <chipID>_WellList.txt generated by the CELLSTUDIO software. This file’s contents will differ from the <chipID>_WellList.txt generated by the CellSelect software.

Row	Col	Sample	Barcode
0	0	A1	TGACCGAT+TCAGATTG
0	1	A1	TCTAGGTT+TCAGATTG
0	2	A1	CTGGTCTT+TCAGATTG
0	3	A1	GTCGTTCT+TCAGATTG
0	4	A1	AGAGTTCT+TCAGATTG
0	5	A1	AGCTGAAT+TCAGATTG
0	6	A1	GACGTATG+TCAGATTG
0	7	A1	CATAATGG+TCAGATTG
0	8	A1	ATTCAAGG+TCAGATTG
0	9	A1	TGATTCCG+TCAGATTG
0	10	A1	GCAACTAG+TCAGATTG
0	11	A1	TACGCGAG+TCAGATTG
1	0	A1	TGACCGAT+CTATCGTT
1	1	A1	TCTAGGTT+CTATCGTT
1	2	A1	CTGGTCTT+CTATCGTT

Appendix C: Advanced Barcode File Configuration

Every sample on the chip should be able to be associated with its results after library generation and sequencing; this is done by having unique barcodes for each sample. With the Takara Bio prevalidated applications, barcoded primers may be preprinted on chips (3’ DE, TCR) or provided for dispense as part of the application (SMART-Seq ICELL8 Indexing Primer Set – A and/or Set B). The index files for these barcoded primers are available natively within the CELLSTUDIO software.

If you want to use different barcoded primers to generate a sequencing library for an application, custom index files detailing these custom primers will need to be created for use by CELLSTUDIO software that can be

associated with that application. CELLSTUDIO software supports the ability to create customized index files to accommodate the primer sequences you want to use in your experiment.

NOTE: For more information on applying index files to an application workflow, see [Section VII](#).

There are two concepts to understand when creating custom index files: the directory structure (as viewed in Windows Explorer) and the naming and configuration of the files which contain the barcode information.

A. Barcode Folder Directory Structure

- Barcode files are stored in subfolders of the barcode parent directory:
C:\ProgramData\takara\ICELL8 cx\Barcodes\
- The barcode folder is a subfolder of the barcode parent directory.
- Within the barcode folder are two files with these exact names:
 - Index1.csv
 - Index2.csv
- The name of the barcode folder will determine what name is displayed in the “Barcode folder” drop-down menu in the *Properties* window of the application editor.

Example:

If the user creates a subfolder with the path C:\ProgramData\takara\ICELL8 cx\Barcodes\Custom, the *Properties* “Barcode folder” drop-down menu will resemble Figure 102:

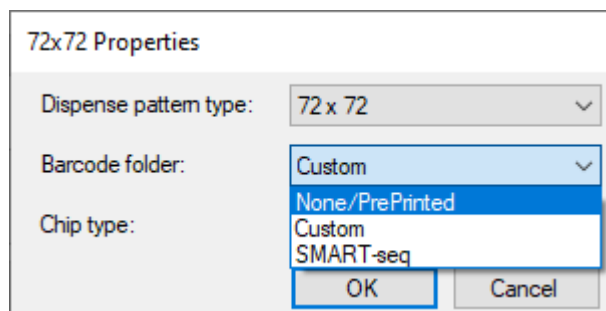


Figure 102. *Properties* window showing the “Barcode folder” with a custom barcode folder called ‘Custom’.

- Barcode folders can also contain subfolders, creating a hierarchy two levels deep. This additional layer of folders allows for greater organizational flexibility, such as multiple indexing set options for use with the same protocol.

An example of this two-tier barcode folder structure is the predefined SMART-seq indexes. The predefined SMART-seq app comes with two sets of barcodes to choose from: SetA, which corresponds to the indexes included in SMART-Seq ICELL8 Indexing Primer Set – A (Takara Bio, Cat. No. 640205) and SetB, which corresponds to the indexes in SMART-Seq ICELL8 Indexing Primer Set – B (Takara Bio, Cat. No. 640218). Using multiple barcode sets enables the multiplexing of chips in sequencing.

In the barcode parent directory, the SMART-seq index options are grouped under the SMART-seq subfolder; under this level subfolders for SetA and SetB also exist. This second set of subfolders (SetA and SetB) contain the index files.

Figure 103 shows how this hierarchy looks within Windows Explorer.

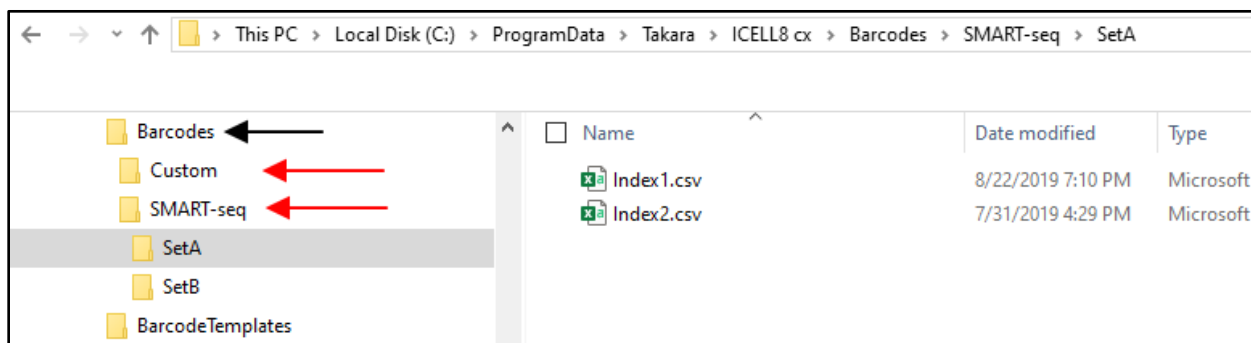


Figure 103. File hierarchy example for a two-tier barcode file organization shown in Windows Explorer. The parent directory Barcodes is indicated with a black arrow. First-level barcode folders are indicated by the red arrows. SetA and SetB are second-level barcode folders, under SMART-seq subfolder. The barcode files Index1.csv and Index2.csv located under the SetA/ directory are on the right-hand side of the display window.

B. Creating a Custom Barcode File

Users can begin to define their own barcode files by creating a new barcode folder in the barcode parent directory, C:\ProgramData\Takara\ICELL8 cx\Barcodes. The barcodes in this folder are used for two potential activities in the workflow:

1. Within CELLSTUDIO software, barcodes are used to generate the source well and sequence information found in the <chipID>_initial.csv and <chipID>_main.csv files, which in turn generate the <chipID>_WellList.txt file. These files are described in [Appendix B](#).
2. If the wells are imaged ([Section X.E](#)), the barcode file information is forwarded to CellSelect software in order to create the <chipID>_<date>_WellList.txt file during image processing (see the “ICELL8 cx CellSelect v2.0 Software User Manual”, Section III.D for more information).

Depending on the dispense pattern, different numbers of source plate locations and sequences need to be defined. The number of barcodes or barcode combinations should be equal to or greater than the number of wells in the dispense pattern.

Templates are included with the program for the dispense patterns available for use in an application. The files can be found in the C:\ProgramData\Takara\ICELL8 cx\BarcodeTemplates folder, with subfolders, named describing the grid pattern they’re based on. Figure 104 shows the template folder and the available dispense pattern templates.

NOTE: The source well locations for all patterns are subsets of the 72 x 72 pattern.

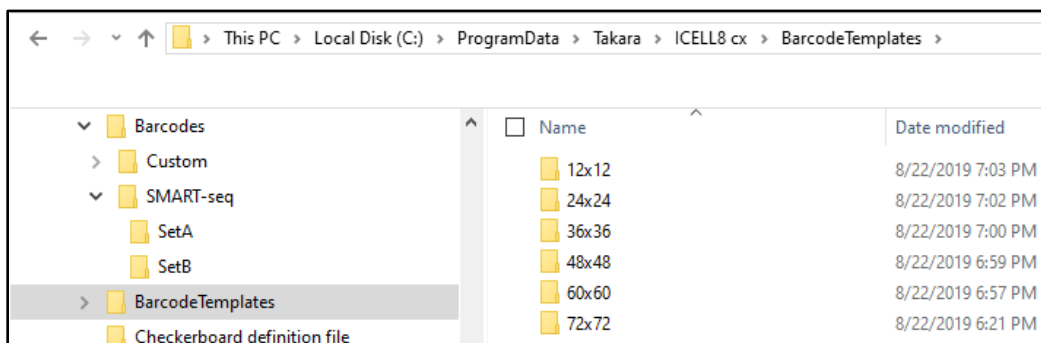


Figure 104. The BarcodeTemplates/ folder and available dispense pattern templates subfolders.

Procedure

1. In Windows Explorer, navigate into the barcodes parent folder:

C:\ProgramData\takara\ICELL8 cx\Barcodes

2. Right mouse-click on the folder content pane and select **New > Folder**.

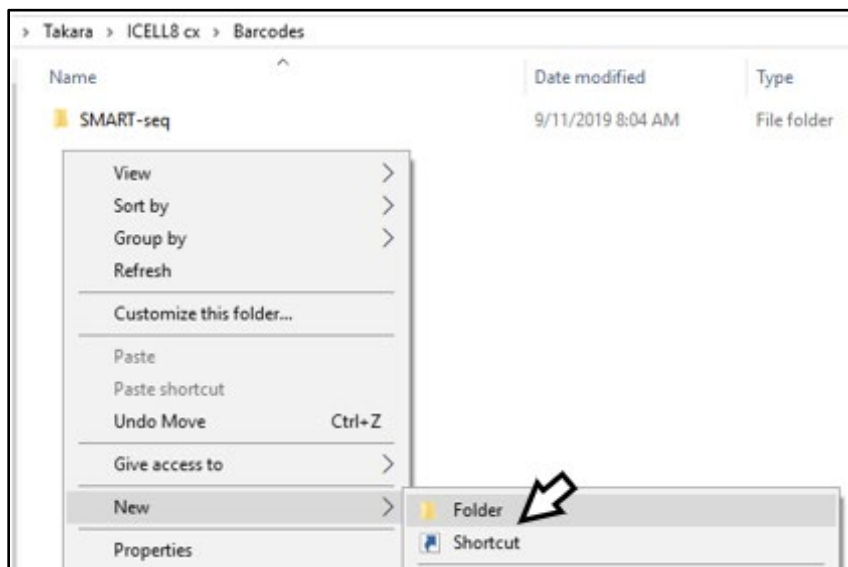


Figure 105. In the **Barcodes/** directory, create a new folder.

3. Name the folder something meaningful for the primer indexes or your experiment.
4. Determine what dispense pattern will be used for the application (e.g., 12 x 12).
5. In Windows Explorer, navigate into the barcodes templates folder for the determined dispense pattern.

Example:

C:\ProgramData\takara\ICELL8 cx\BarcodeTemplates\12x12\

6. Copy **Index1.csv** and **Index2.csv** files from the template folder and paste the copies into the folder you created in step 3.



Figure 106. Copies of the barcode template index CSV files in a new, custom barcode folder (**Barcodes\CustomIndexesSetC**).

7. Edit `Index1.csv` using a spreadsheet program like MS-Excel or a text editor such as MS-Notepad. The placeholder “Sequence” string, `NNNNNNNN`, (Column B in Figure 107, left) should be replaced with your custom barcode sequence. (See Example, below)

NOTE: The way the barcodes are output in `*.bcl` or `*.fastq` files from Illumina® sequencers can be dependent on the sequencer type.

8. Once all placeholder sequence strings are updated with the proper primer identification, save the file.
9. Repeat Steps 6–8 for `Index2.csv`.

	A	B
1	WellId	Sequence
2	A5	NNNNNNNN
3	B5	NNNNNNNN
4	C5	NNNNNNNN
5	D5	NNNNNNNN

Index1.csv - Notepad
File Edit Format View
WellId,Sequence
A5,NNNNNNNN
B5,NNNNNNNN
C5,NNNNNNNN
D5,NNNNNNNN

Figure 107. Template `Index1.csv` contents shown in (Left) MS-Excel and (Right) MS-Notepad.



IMPORTANT: The only change made to the files when creating custom barcodes should be the sequence value. The source well locations (`WellID`) defined in the template `Index#.csv` files (Figure 107, Column A example) should **NOT** be edited. The file names (`Index1.csv` and `Index2.csv`) also must **NOT** be altered.

Example

The following example uses only five forward and reverse indexes to keep the walkthrough for Step 7 simple. The same process would be expanded to cover more index sets.

Let’s say you are starting with the following forward and reverse primer indexes:

Table 21. Example forward (i5) and reverse (i7) primers for illustrating custom barcode file creation

Forward indexing (i5) primers			Reverse indexing (i7) primers		
Index number	Well position	Index sequence in primers	Index number	Well position	Index sequence in primers
Forward Index 1	A5	AACCAACG	Reverse Index 1	A13	AACCGGTT
Forward Index 2	B5	AACGATAG	Reverse Index 2	B13	AACCTAGA
Forward Index 3	C5	AAGAAGAC	Reverse Index 3	C13	AAGACCAG
Forward Index 4	D5	AGAGCCTA	Reverse Index 4	D13	AGAACGAC
Forward Index 5	E5	ATAGTCAA	Reverse Index 5	E13	AGAACTCT

The sequences become the field values input into Index1.csv and Index2.csv, respectively.

	A	B		A	B
1	WellId	Sequence	1	WellId	Sequence
2	A5	AACCAACG	2	A13	AACCGGTT
3	B5	AACGATAG	3	B13	AACCTAGA
4	C5	AAGAAGAC	4	C13	AAGACCAG
5	D5	AGAGCCTA	5	D13	AGAACGAC
6	E5	ATAGTCAA	6	E13	AGAACTCT
7			7		

Figure 108. Barcode files for example indexes from Table 21. (Left) Index1.csv would be populated with the forward indexing primers information. **(Right)** Reverse indexing primers are entered into Index2.csv

After the *_WellList.txt file is generated by:

- CELLSTUDIO software, if samples are not scanned ([Appendix B.D](#))
- CellSelect software after cell imaging (“ICELL8 cx CellSelect v2.0 Software User Manual”, Section III.D)

the sequences shown in the “Barcode” column are the combination of the reverse plus the forward index sequence, i.e., the Index2.csv value for the reverse index plus the Index1.csv value for the forward index.

A subset of the combinations from the example indexes in Table 21 is shown below:

Table 22. The barcodes displayed in the *_WellList.txt files “Barcode” column based on Table 21

Index numbers	Barcodes shown in *_WellList.txt (Index2+Index1)
Reverse Index 1+Forward Index 1	AACCGGTT+AACCAACG
Reverse Index 2+Forward Index 2	AACCTAGA+AACGATAG
Reverse Index 3+Forward Index 3	AAGACCAG+AAGAAGAC
Reverse Index 4+Forward Index 4	AGAACGAC+AGAGCCTA
Reverse Index 5+Forward Index 5	AGAACTCT+ATAGTCAA

Appendix D: 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 ICELL8 system to dispense to the corresponding well on the ICELL8 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 (e.g., 12 x 12, 24 x 24, etc.).
 - If the filter file dimensions exceed that of the dispense pattern (i.e., a 48 x 48 filter file applied to a 24 x 24 dispense pattern), values in the filter file outside the range of the dispense pattern are ignored.
 - If the filter file dimensions are smaller than the dispense pattern (i.e., a 12 x 12 filter file applied to a 24 x 24 dispense pattern), an error will display. E.g.:

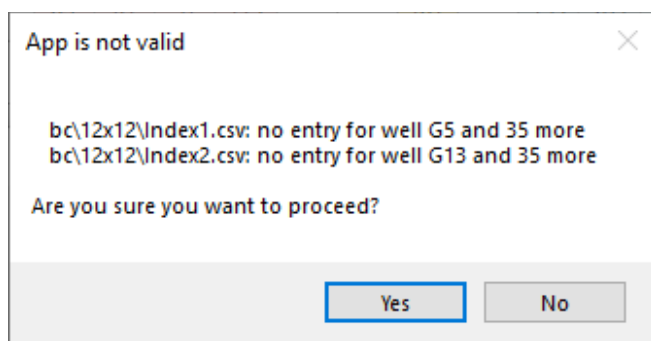


Figure 109. Example warning message if filter file dispense pattern dimensions are smaller than dispense pattern size.

- 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 $N \times N$ rows and columns with ones and zeroes (1 and 0), where N is the grid dispense pattern to use. Everything else in the spreadsheet should be empty.

Examples:

- If $N = 12$ for a 12 x 12 grid, then cells in the range would be A1:L12 (Figure 110)
- For a 72 x 72 filter file 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 110. Example of a manually created filter file in MS-Excel designed for a 12 x 12 sample dispense pattern. 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.

Under normal circumstances, you are statistically unlikely to get more than 37% of the wells that are dispensed containing a single cell. Based on this implication, the filter file should not contain more than 1,728 candidate wells for dispensing.

Appendix E: Imaging: Filter Configuration Editor with Live Images

The filter configuration editor allows you to create a new option for scanning a chip. The new configuration is chosen by adjusting the camera gain and exposure time for the filters that are best suited for a particular sample/dye combination.

For this procedure, you need a chip that has been dispensed with the appropriate cells; you may want to load cells at a higher concentration than usual to ensure that your cells of interest are represented. For each of the filters you intend to use, there should be cells that can be imaged with the filter set for that color. The system supports imaging with up to two filter sets.

1. Place the chip into the chip nest.
2. In the CELLSTUDIO main interface, navigate to **Utilities > Exposure Settings....** The *Start focus* window will pop up.

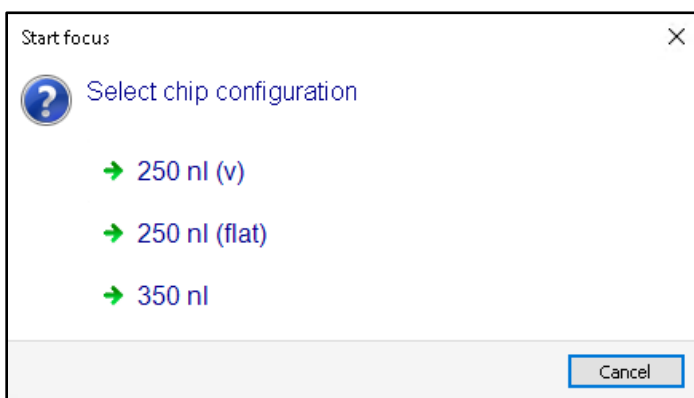


Figure 111. The *Start focus* window in Utilities > Exposure Settings.

3. Select what type of chip is in use. Refer to Table 5 in [Section VII.B](#) if additional information is needed to select the correct option based on the chip name.

The *Microscope* window will pop up, and focusing will start.

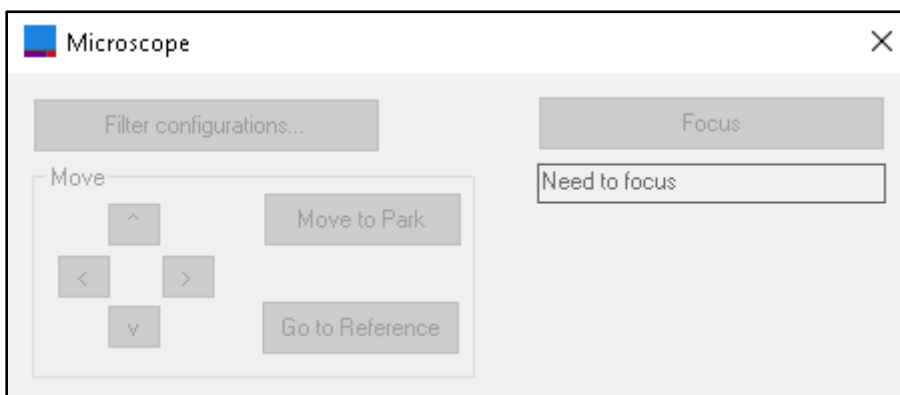


Figure 112. The *Microscope* window pop-up when accessing the filter configuration editor.

4. When focusing is complete, the message pop-up “Focus completed successfully” will display.

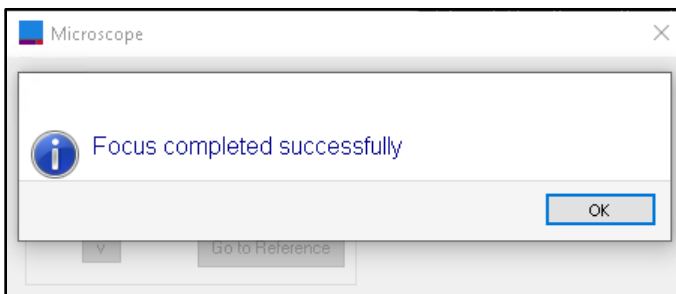


Figure 113. Success message accessing the focus configuration editor.

At this point, the camera is positioned over the top-left corner of the chip.

If successful, the *Filter configuration editor* window (Figure 114, below) will then display.

A. Interface

The editor allows you to select any existing filter configurations via the drop-down box in the top left. It also provides the option to generate new filter configurations for cells of interest.

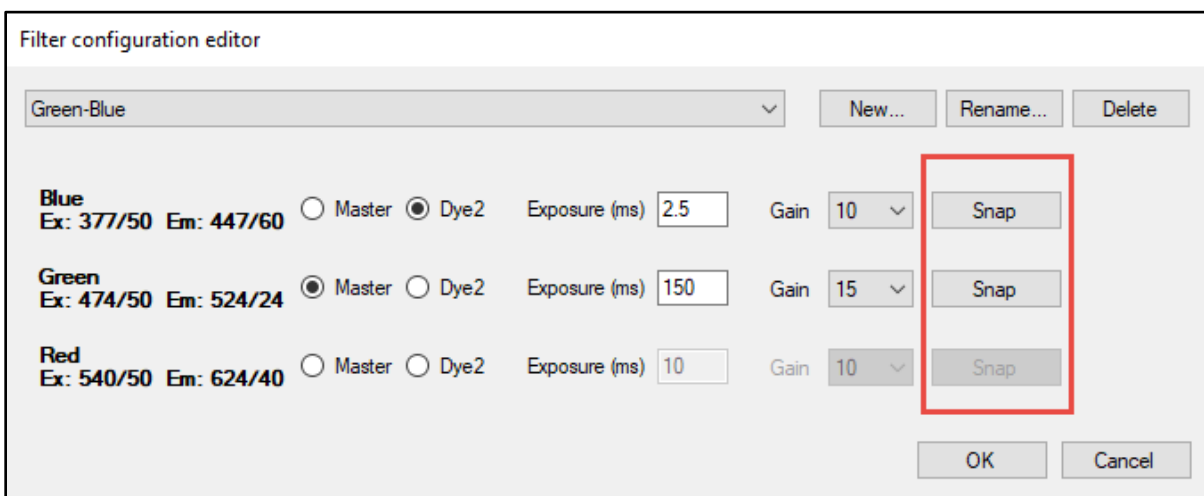


Figure 114. The *Filter configuration editor* interface window. The example shown is a user-generated filter configuration file where Green is the Master dye, and Blue is Dye2. Use the [Snap] buttons to confirm the configuration or optimize the settings against an object of interest.

[New...]

The [New...] button is clicked to create a new filter configuration. You will be prompted to choose the number of waves:

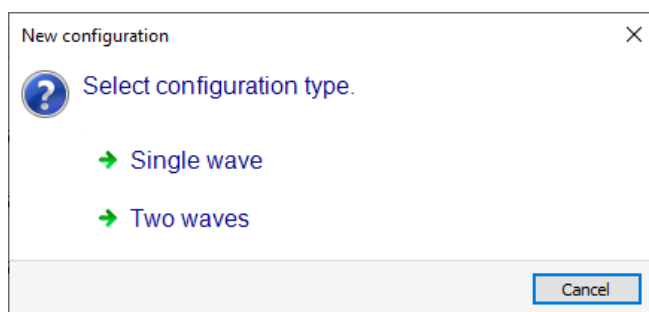


Figure 115. The *New configuration* dialogue window.

and a name:

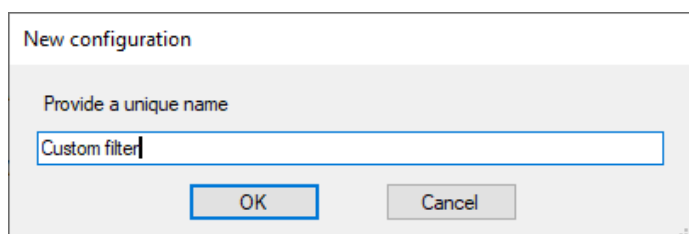


Figure 116. The *New configuration* naming window.

[Rename...]

Renames the currently selected filter configuration.

[Delete]

Deletes the currently selected Filter configuration.

[Exposure]

Ideally, the exposure times should be in the range of 2–500 ms. Values up to 2,000 ms are possible.

[Gain]

“Gain” values can be either ‘5’, ‘10’, or ‘15’. Higher values produce brighter images, but they also introduce more noise. We recommend that you start with a gain of 10.

[Snap]

Pressing the [Snap] button causes the system to acquire an image with the settings for that filter. You can use this to confirm whether an existing configuration will apply to your current cells of interest or to optimize a new configuration.

[OK]

Clicking this button saves the changes for the currently displayed filter configuration and closes the dialog window.

[Cancel]

Reverts all changes and close the dialog window.

B. Usage Guidelines

Decide whether you will modify an existing filter configuration or generate a new one.

Be sure that the dye selections reflect the candidate logic you wish to use in the CellSelect software. The Master dye is required to identify the presence of cells in the nanowell in the CellSelect software. Dye2 may be used for additional candidate selection. Refer to the “ICELL8 cx CellSelect v2.0 Software User Manual” for more information about candidate logic.

If the existing settings are good, select [OK] and proceed with this filter configuration. If the settings are not good, you have two options.

1. (Preferred) Select [New...] as described above to create a new filter configuration for these cells.
2. Change the Exposure or Gain values as described above. Click [Snap] to check. If the new settings look good to you, select [OK] and proceed. This option overwrites the existing filter configuration.

The image viewer displays 36 wells (6 x 6 well grid) and is configured to apply the auto-contrast procedure, once the image has been snapped. This means that the overall image appearance doesn't seem to change much when you snap images with quite different gains and/or exposure times. You need to watch the number in the auto-contrast bar (Figure 117) to quantify the difference in overall brightness, or you can open the *Image contrast* window (☀ icon in the *Image Viewer* window menu bar).

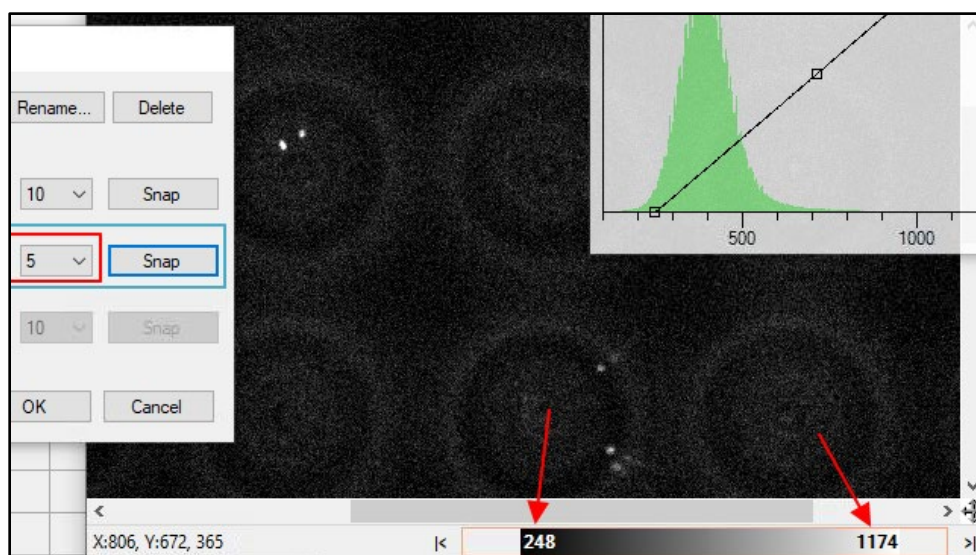


Figure 117. The auto-contrast bar at the bottom of the *Image Viewer* window displays two intensity values. The pixels with intensity equal to lower intensity value will be displayed black. The pixels with the higher value will be displayed white. These two values are automatically calculated based on the intensity histogram of the image.

What will change, however, are the signal-to-noise characteristics. Higher gains produce brighter but also noisier images. Additionally, if the exposure time is too high, brighter pixels on the camera may become more visible. Another consideration is that longer exposure times also mean longer scan times.

In general, you want to adjust the “Gain” and “Exposure” time so that all cells are clearly visible above the background, i.e., a high signal-to-noise ratio—but be careful to not overexpose the image. You should also be cognizant of having enough signal; the recommended minimum intensity is about 500 counts. One

can obtain the intensity of a cell by hovering the mouse over the cell in the *Image Viewer* window: the pixel value under the cursor is displayed in the status bar.

Two examples of this type of adjustments are documented in [Appendix E.C](#), below.

If you snap images too many times, you may bleach the cells, the pixel values will start to drop, and they won't be representative of the cells of the rest of the chip anymore. If that occurs, use the navigation buttons in the *Microscope* dialog window to move the camera by one field (6 wells). After you perform a move, press the [Snap] button to confirm that the camera is focused on a good place.

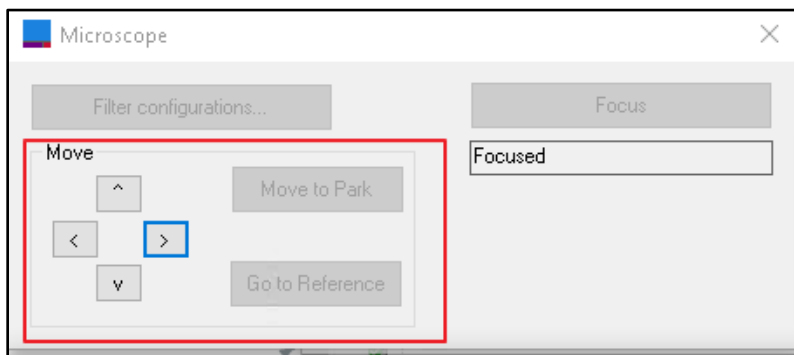


Figure 118. The Move area of the *Microscope* dialogue window. The arrow buttons can be used to move the focus of the camera to a different area of the chip. If the exposure settings are over-adjusted and the image bleaches out, move the camera six wells away and use [Snap] to recalibrate. [Move to Park] moves the dispense head to the Park position. [Move to Reference] moves the camera to the top-left corner of the chip.

If you cannot observe any cells, even at high gain and long exposures, this may be because of one of two reasons:

1. There simply are no cells present in the 36 visible wells. Try moving the camera to a different part of the chip and/or dispense a higher concentration of cells.
2. The cells are too dim. Reconsider your staining/labeling protocol.

C. Examples

Example 1: cells are quite bright

1. At a Gain of '5' and an "Exposure" time of '10 ms', the image is grainy.

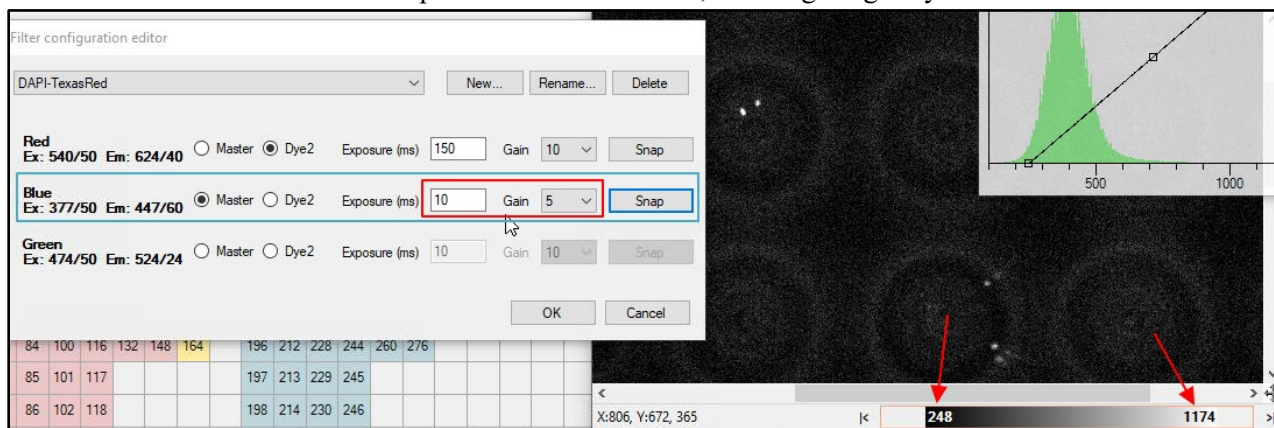


Figure 119. Example 1: Editing the filter configuration, Exposure time: '10 ms'.

- At a Gain of '5' and an Exposure time of '50 ms', the background is less grainy.

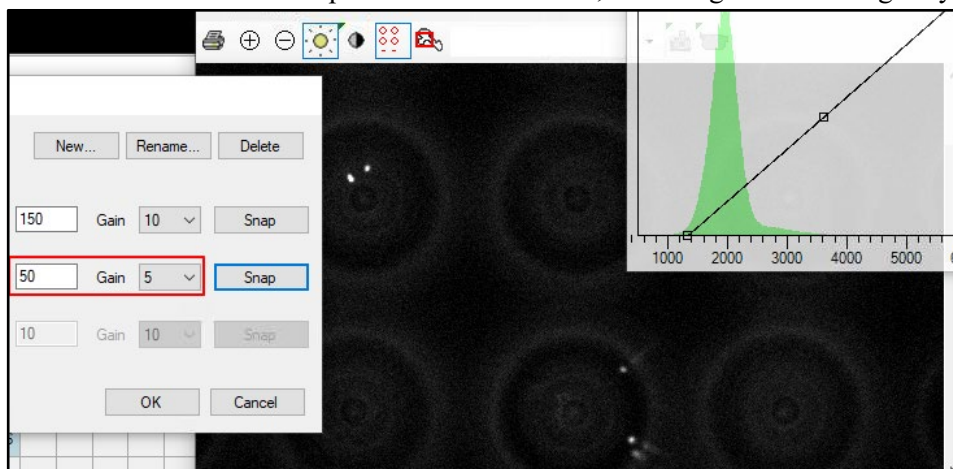


Figure 120. Example 1: Editing the filter configuration, Exposure time: '50 ms'.

- A Gain of '5' and an Exposure time of '500 ms' is too long; the brightest pixels are overexposed.

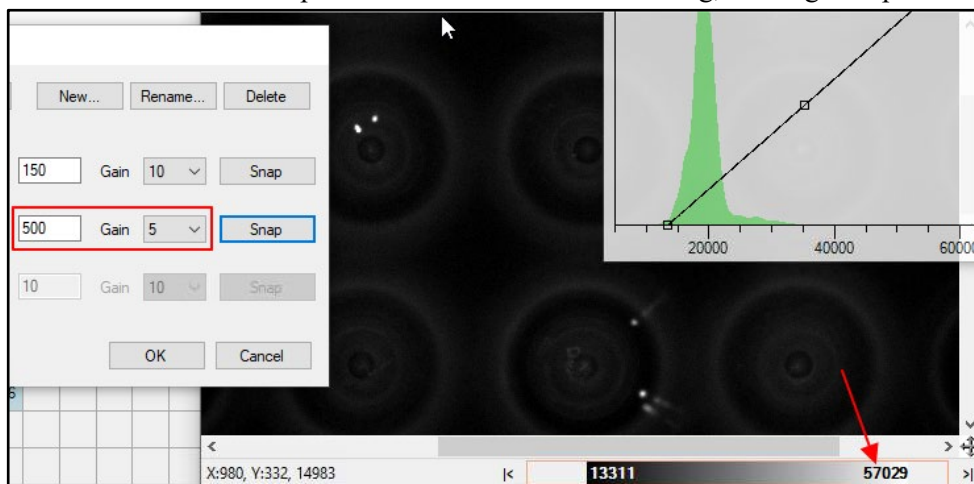


Figure 121. Example 1: Editing the filter configuration, Exposure time: '500 ms'. This image is overexposed; even the background is over 10,000.

Example 2: cells are very dim

- At a Gain of '5' and an Exposure time of '100 ms', the cell is barely visible.

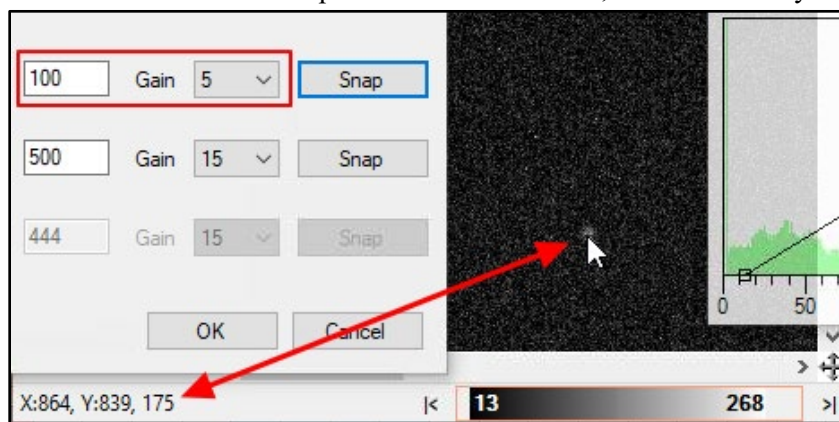


Figure 122. Example 2: Editing the filter configuration, Gain: '5', Exposure time: '100 ms'.

- At a Gain of '5' and an Exposure time of '100 ms', the cell is still dim.

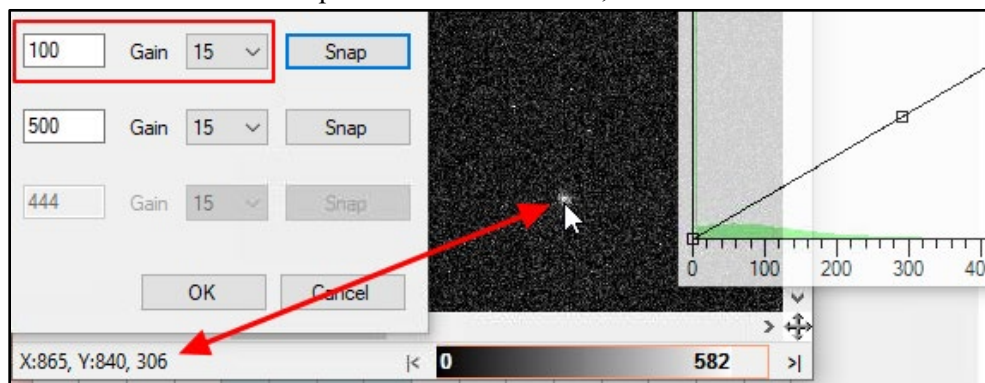


Figure 123. Example 2: Editing the filter configuration, Gain: '15', Exposure time: '100 ms'.

- At a Gain of '5' and an Exposure time of '300 ms', the cell looks okay.

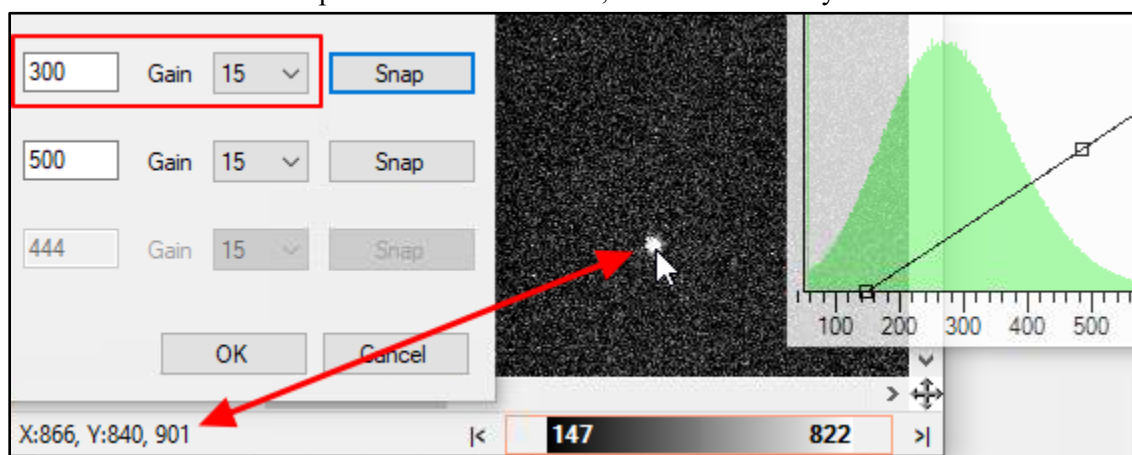


Figure 124. Example 2: Editing the filter configuration, Gain: '15', Exposure time: '300 ms'.

- At Gain: 15 and an Exposure time of 500 ms, the cell is nicely visible against the background.

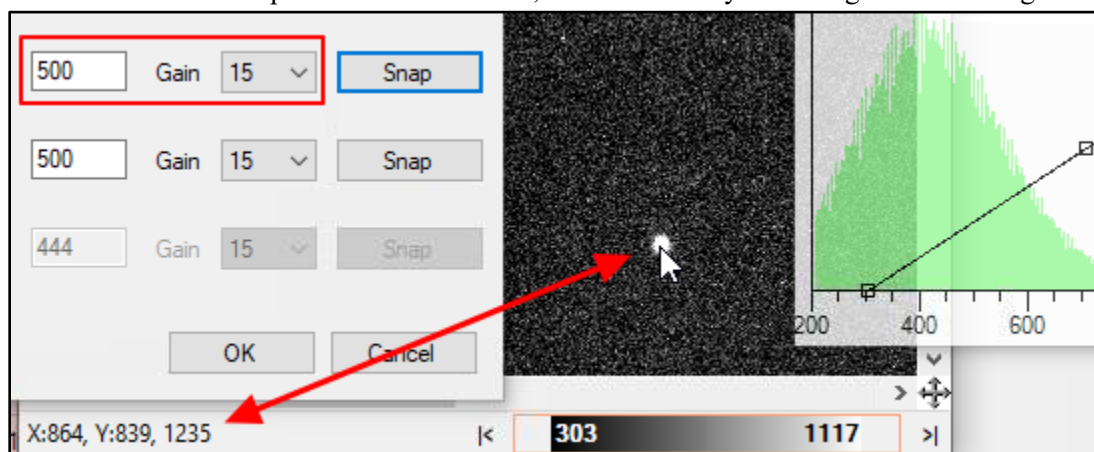


Figure 125. Example 2: Editing the filter configuration, Gain: '15', Exposure time: '500 ms'.

Appendix F: Designing Thermal Cycler Programs

In order to achieve the temperatures in the ICELL8 chip that are required by your protocol (as determined for in-tube reactions) the T-100 thermal cycler needs to be programmed appropriately. Table 23 is provided to aid in the selection of the temperature setpoints for the T-100.

- The first column is the desired protocol set point.
- The second column is the actual set point of the cycler.

To reach a desired temperature, the T-100 cycler must be programmed with a temporary over (or under) temperature value for the duration indicated in the table.

Example:

If the cycler is currently set at 72°C and you wish to go down to 40°C, the next 2 steps in your protocol would be:

- 34.5°C for 6 seconds
- 39.2°C for the duration required by your protocol.

If the cycler is at 20°C and you wish to go up to 40°C, the next 2 steps in your protocol would be:

- 45.2°C for 5 seconds
- 39.2°C for the duration required by your protocol

NOTES:

- The Initial Setpoint and its duration depend on the chip target temperature.
- Table 23 is only valid if the lid temperature is set to 72°C.

Table 23. Thermal cycler lookup table

Chip target (°C)	T-100 set point (°C)	Starting temperature lower than target		Starting temperature higher than target	
		Initial set point (°C)	Duration (seconds)	Initial setpoint (°C)	Duration (seconds)
16	13.3	NA	NA	12.9	8
17	14.5	NA	NA	13.7	8
18	15.7	NA	NA	14.6	8
19	16.8	NA	NA	15.5	8
20	17.9	NA	NA	16.3	8
21	19.1	NA	NA	17.2	8
22	20.2	NA	NA	18.1	8
23	21.3	NA	NA	19.0	10
24	22.4	NA	NA	19.8	10
25	23.5	NA	NA	20.7	10
26	24.6	NA	NA	21.6	10
27	25.7	NA	NA	22.5	10
28	26.7	NA	NA	23.4	10

Chip target (°C)	T-100 set point (°C)	Starting temperature lower than target		Starting temperature higher than target	
		Initial set point (°C)	Duration (seconds)	Initial setpoint (°C)	Duration (seconds)
29	27.8	NA	NA	24.3	10
30	28.8	NA	NA	25.2	10
31	29.9	36.6	5	26.2	10
32	30.9	37.5	5	27.1	10
33	32.0	38.5	5	28.0	10
34	33.0	39.4	5	28.9	10
35	34.1	40.4	5	29.8	6
36	35.1	41.4	5	30.8	6
37	36.1	42.3	5	31.7	6
38	37.2	43.3	5	32.7	6
39	38.2	44.3	5	33.6	6
40	39.2	45.2	5	34.5	6
41	40.2	46.2	5	35.5	6
42	41.2	47.2	5	36.5	6
43	42.3	48.1	5	37.4	6
44	43.3	49.1	5	38.4	6
45	44.3	50.0	5	39.4	6
46	45.3	51.0	5	40.3	6
47	46.3	52.0	5	41.3	6
48	47.3	52.9	5	42.3	6
49	48.4	53.9	5	43.3	6
50	49.4	54.9	5	44.3	6
51	50.4	55.8	5	45.3	5
52	51.4	56.8	5	46.2	5
53	52.4	57.8	5	47.2	5
54	53.5	58.7	5	48.3	5
55	54.5	59.7	5	49.3	5
56	55.5	60.6	5	50.3	5
57	56.5	61.6	5	51.3	5
58	57.5	62.6	6	52.3	5
59	58.6	63.5	6	53.3	5
60	59.6	64.5	6	54.4	5
61	60.6	65.5	7	55.4	5
62	61.7	66.4	7	56.4	5
63	62.7	67.4	7	57.5	5
64	63.7	68.4	8	58.5	5

Chip target (°C)	T-100 set point (°C)	Starting temperature lower than target		Starting temperature higher than target	
		Initial set point (°C)	Duration (seconds)	Initial setpoint (°C)	Duration (seconds)
65	64.8	69.3	8	59.6	5
66	65.8	70.3	8	60.6	5
67	66.9	71.2	9	61.7	5
68	67.9	72.2	9	62.7	5
69	69.0	73.2	9	63.8	5
70	70.0	74.1	10	64.9	5
71	71.0	75.1	10	66.0	5
72	72.1	76.1	10	67.0	5
73	73.2	77.0	11	68.1	5
74	74.2	78.0	11	69.2	5
75	75.3	79.0	11	70.3	5
76	76.3	79.9	12	71.4	5
77	77.4	80.9	12	72.5	5
78	78.4	81.8	12	73.6	5
79	79.5	82.8	12	74.7	5
80	80.6	83.8	13	75.8	5
81	81.6	84.7	13	76.9	5
82	82.7	85.7	13	78.0	5
83	83.8	86.7	14	79.1	5
84	84.8	87.6	14	80.3	5
85	85.9	88.6	14	81.4	5
86	86.9	89.6	15	82.5	5
87	88.0	90.5	15	83.7	5
88	89.1	91.5	15	84.8	5
89	90.1	92.4	16	NA	NA
90	91.2	93.4	16	NA	NA
91	92.2	94.4	16	NA	NA
92	93.3	95.3	17	NA	NA
93	94.4	96.3	17	NA	NA
94	95.4	97.3	17	NA	NA
95	96.5	98.2	18	NA	NA
96	97.5	99.2	18	NA	NA
97	98.5	100.0	18	NA	NA
98	99.6	100.0	18	NA	NA
99	100.0	100.0	18	NA	NA
100	100.0	100.0	18	NA	NA

Appendix G: Designing an Application: Advice from Our Scientists

A. How Should I Begin?

You can think of transitioning to the ICCELL8 cx system as miniaturization of an existing protocol that has already been tested at larger volumes. A protocol that is a good candidate for an application on the ICCELL8 system is one in which consecutive reactions are additive (i.e., only addition of reagents, no purification required between steps).

It may be a good idea to perform in-tube experiments to figure out the initial concentrations and volumes for each reagent and reaction conditions for each step, then scale down to apply them to the first experiment in chip. However, conditions that work in tube do not necessarily work in chip without some further optimization.

B. How Do I Choose What Chip to Use?

Chips may be unprinted (“blank”) or preprinted. Preprinted chips from Takara Bio for the ICCELL8 cx system are only available with a volume of 250 nl. The maximum dispensing volume allowed per well for these chips is 235 nl.

- The ICCELL8 cx 3' DE Chip (Cat. No. 640199) has 5,184 nanowells, each of which contains a preprinted oligo(dT) primer with a nanowell-specific barcode, so it can be used for capturing polyadenylated molecules. It can be used to perform in-chip sample processing reactions such as cDNA synthesis.
- The ICCELL8 cx TCR Chip (Cat No. 640200) contains 1,728 uniquely barcoded template switching oligos. Each barcode is printed three times on the chip to account for all 5,184 nanowells. (In this case, CellSelect software will downselect to generate a filter file using only uniquely represented barcodes, see the “ICCELL8 cx CellSelect User Manual”, Section III.H) It can be used for the ICCELL8 Human TCR a/b Profiling workflow and 5' DE assay or in applications that leverage the template-switching ability of SMARTScribe reverse transcriptase.

Currently, two blank ICCELL8 chips with volumes of 250 nl (Cat. No. 640183) and 350 nl (Cat. No. 640019), are available from Takara Bio for the ICCELL8 cx system. The maximum dispensing volume allowed per well for these chips are 235 nl and 300 nl respectively. Choose the correct chip according to the total volume being dispensed per well.

Unprinted chips are blank chips without any barcoding in the wells. As all samples will be extracted and pooled at the end of the application, if an unprinted chip is used, then each well needs to be identifiable by dispensing unique barcodes (or combinatorial index pairs) as part of the application.

C. What Volume Should I Dispense?

Calculate and choose the right dispense volume according to the number of steps and the concentration of reagents and indexes.

- The predefined volume options for cell, RNA, and DNA dispenses are 35 nl or 50 nl per well.
- The predefined volume options for reagents and indexes dispense are 35 nl, 50 nl, or 100 nl per well.
- The 100 -l dispense option without a filter file is only allowed for up to a 48 x 48 dispense pattern or smaller due to the volume limitation per source well.

We have guidelines for testing dispense quality of reagents acceptable concentrations of some common buffer components; see the tech note at takarabio.com. In cases where a larger volume of a less concentrated component is needed, the dispense volume for cells or other reagents may need to be reduced to accommodate the maximum allowable volume in the nanowell.

The number of steps available for your application depends on the dispense volume of each step and the ICELL8 chip type. Table 24 shows the maximum steps allowed for a 250 nl and a 350 nl chip at equivalent volumes for each step.

Table 24. Recommended volume fill of sample, control, reagent, or indexes added to the sample source plate

Dispense volume per step	250 nl chip (Max. vol.: 235 nl)	350 nl chip (Max. vol.: 300 nl)
35 nl	6	8
50 nl	4	6
100 nl	2	3

The dispense volume for each step can be different. An example is shown in Table 25.

Table 25. Example application workflow for a 350 nl chip with 7 steps and differing dispense volumes in the steps

Application workflow	350 nl chip
Step 1: Cell dispense	35 nl
Step 2: RT-PCR mix dispense	50 nl
Step 3: Master mix 1 dispense	50 nl
Step 4: Master mix 2 dispense	35 nl
Step 5: Index 1 dispense	35 nl
Step 6: Index 2 dispense	35 nl
Step 7: PCR mix dispense	50 nl
TOTAL: 7 steps	290 nl

D. Tips for Developing a New Protocol for the ICELL8 cx System

- If your application allows it, you should consider using nucleic acids to mimic single cells (or nuclei) as your starting material. For example, you could use purified total RNA (at an estimated 2–15 pg of total RNA per cell) or genomic DNA (at an estimated 6 pg of genomic DNA per cell).
- Nucleic acids should be diluted in whatever the cells will be dispensed in (i.e., PBS). We suggest confirming that the reagent used to dispense the sample dispenses without issue.
- We have observed that Second Diluent improves dispense quality of cells, thereby increasing the candidate count, and suggest adding ICELL8 Second Diluent 100X (Cat. No. 640016) to cells, nucleic acids, and all controls. During initial application development, test that the addition of Second Diluent will not interfere with your reaction, so this recommendation can be implemented. If you cannot use Second Diluent, you may see more doublets than expected from a standard Poisson distribution.
- Recombinant RNase Inhibitor (Takara Bio, Cat. No. 2313A or 2313B) should be included for RNA dispenses, and it should also be included with a cell dispense for any application that will use RNA.

- During the development of an application, you should also monitor your application at various steps. For example, if RT-PCR is the first step, you may want to extract the product off the chip after this stage to look at the profile on a fragment analyzer (e.g., Agilent2100 Bioanalyzer used with the Agilent High Sensitivity DNA Kit (Agilent, Cat. No. 5067-4626), the Fragment Analyzer and High Sensitivity Large Fragment Analysis Kit (Advanced Analytical Technologies, Inc., Cat. No. DNF-493), or an equivalent microfluidic device/kit to see if it looks as expected.
- You can also use an intermediate product to mimic being partway through protocol. To continue with the example above, if you use RT-PCR upstream, you could dispense cDNA you have generated into the tube to the chip, and then test downstream steps, like PCR or ligation.

E. Considerations for Special Types of Cells

If your cells settle quickly, such as cardiomyocytes, you should consider taking advantage of the CELLSTUDIO software “Pause” function ([Section V.D](#)).

If “Pause” is selected, load the source wells as usual. The ICELL8 cx Instrument will dispense only a portion of the chip and then pause and alert you that the instrument is paused. At this point, you can choose to:

1. Resuspend the cells in each source well,
2. Resuspend the cells in the original stock and add to the source wells, or
3. Remove what is in each source well and then use freshly resuspended cells.

For options 2 and 3, you may use 35 µl per source well per dispense rather than 80 µl.

F. Positive and Negative Control Recommendations

CELLSTUDIO software designates specific wells in the source plate for positive and negative controls. At early stages of application development, we recommend including a negative control (PBS or the dispense reagent your cells will be in are good choices). Water does not dispense well and is therefore not a good negative control.

Once you move to testing your protocol with cells ([Appendix G.K](#)), we recommend including a positive control in addition to the negative control. The purified nucleic acid that you used to develop your application is a good choice for a positive control. Positive control wells can be left empty if there is no proper positive control for an application.

G. Use Small Dispense Patterns at Early Stages of Application Development

When first moving the application from a tube to an ICELL8 chip, we recommend starting with small subsections of the chip, such as a 12 x 12 or 24 x 24 dispense pattern. Small subsections require less reagents and less time for dispensing and are suitable for testing the concentration of indexes and reagents as well as optimization of reaction conditions, such as thermal cycler parameters. Furthermore, if using an unprinted chip, small subsections require only 12 or 24 forward and reverse indexes, decreasing the need to invest in a large number of primers.

H. Indexing

We recommend combinatorial dual indexing, as CELLSTUDIO software allows for up to 72 forward and reverse indexes. Single indexing would require 5,184 unique indexes, each to be dispensed to the correct location, which is beyond the capabilities of the software. However, preprinted chips ([Appendix G.B](#)) enable single indexing and may be used if appropriate for your application.

I. Testing Different Conditions

- When using nucleic acids prior to using cells, you will most likely be dispensing reagents to every nanowell of the dispense pattern. For dispense patterns greater than a 48 x 48 wells, 100 nl dispenses to every nanowell (i.e., unfiltered) are not allowed by default.
- For unfiltered dispenses into a 48 x 48 dispense pattern and larger, up to eight different reagent compositions for each reagent can be added to their corresponding source wells for reagent dispensing (see source plate maps in [Appendix H.B](#)).
- For filtered dispenses into any pattern, up to four different reagent compositions for each reagent can be added to their corresponding source wells for reagent dispensing. This can be used to simultaneously test more than one experimental condition in a chip: different reagents or reagent compositions can be added to their corresponding source well for dispensing.

Example:

If you wanted to test four different magnesium concentrations in a PCR master mix in a 36 x 36 dispense pattern, and the master mix is Reagent 4, then each test concentration can be added to wells M11, N11, O11, and P11 in the source well plate (see Figure 129 in [Appendix H.A](#)). The `<chipID>_main.csv` output file will track which source well was dispensed to each chip nanowell so you can later review the effect of different magnesium concentrations on the results.

- If there is more than one reagent source well, CELLSTUDIO software has been designed to dispense approximately equal numbers of nanowells from each of the source wells.

Example:

If there were 1,000 wells being dispensed to the chip in the example above, each of the four master mix concentration source wells would be dispersed into approximately 250 nanowells.

- Different filter files can be manually created ([Appendix D](#)) to guide the instrument to dispense different reagent compositions into specific wells. This approach also allows selection of which positive and negative controls receive which reagent composition. Make sure to add reagent to the same location of a new source plate for each filter file using the same step.

Example:

One magnesium concentration can be used in each of the source wells, but a filter file can be used to define exactly which nanowells receive that reagent. Reagent4 would be selected for each of the dispenses, but a different filter file selected.

- After library generation and sequencing, the `*_WellList.txt` file ([Appendix B.D](#)) may be used as the `WellList.txt` for input into mappa Analysis Pipeline, our bioinformatic software designed to demultiplex output data from the ICELL8 cx system. More information about the mappa software can be found at takarabio.com/icell8-software.
- After the data are analyzed by mappa software, you can go back to the `<ChipID>_main.csv` file ([Appendix B.C](#)) to determine which nanowells (identified by their barcodes) received which reagent.

J. Using Cells: Improving Cell Reactivity

Including molecular-biology grade BSA in the cell dispense may improve cell viability. For RNA-based assays, we recommend Recombinant RNase Inhibitor (Takara Bio, Cat. No. 2313A or 2313B).

Once you move to cells, you will have to be sure that your cells are sufficiently lysed prior to moving to the next step. For many cells, a simple freeze/thaw of the chip—freezing at -80°C for at least 30 minutes—is sufficient.

K. Filtering Dispenses with Nucleic Acid Samples or Samples that are not Imaged

A filter file is automatically generated by CellSelect software after image processing (“ICELL8 cx CellSelect v2.0 Software User Manual”, Section III.D). The filter file is commonly used to dispense reagents into the specific wells, although you may also choose not to use one.

There is no filter file generated after dispensing nucleic acids or in cases where no cell scan (and therefore no image processing) is performed. In these situations, if you want to dispense reagents or indexes to only a subset of the nanowells, you would need to manually create your own filter file to target specific wells in the chip and choose to have those dispense steps require your custom filter file when you set up the application ([Section VII.C](#)).

For more information about creating or editing to customize an existing filter file, see [Appendix D.B](#).

L. Cell Labeling Options

CELLSTUDIO software allows any two combinations of the three filters (blue, red, and green) for taking cell images. Cells may be stained with dyes such as Hoechst and propidium iodide for the ICELL8 imager to identify live and dead cells. Cells may also be labeled with fluorescent antibodies or by expressing a fluorescent protein.

CellSelect software identifies wells containing signal and generates a filter file based on rules the user chooses to guide the ICELL8 cx Instrument to dispense reagents into those wells. For example, you may only want cells positive in Hoechst but negative for propidium iodide, or you may use a FITC-labelled CD4 antibody, an RFP-labeled CD8 antibody, use CellSelect software to identify your cell populations, and then select the cells you wish to process.

See [Appendix D.A](#) for more details about the structure of filter files, or the “ICELL8 cx CellSelect User Manual” (Section III.D and III.I) for more information about how the CellSelect software creates the files.

M. Reagent Parameters

In general, the ICELL8 cx Instrument is able to dispense a wide variety of reagents. Takara Bio has established guidelines for common reagent components, including those that are viscous or have low surface tension (see the tech note at takarabio.com), and we will continue to expand testing.

For reagents or combinations not in the tech note, we provide guidelines for testing the dispense quality. One simple test is to dispense a reagent and then use blotting paper on the chip. If the blotting paper has a large damp area (see Figure 126, below), that could indicate the dispense was not successful. If necessary, 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.

Pure water does not dispense well on the instrument. If you wish to dilute your reactions, we recommend dispensing 10 mM Tris or PBS into the chip. Additionally, you can try adding 100X Second Diluent (Takara Bio, Cat. No. 640016) or a low percentage of PEG8000 to a reagent that does not pass the blot test.

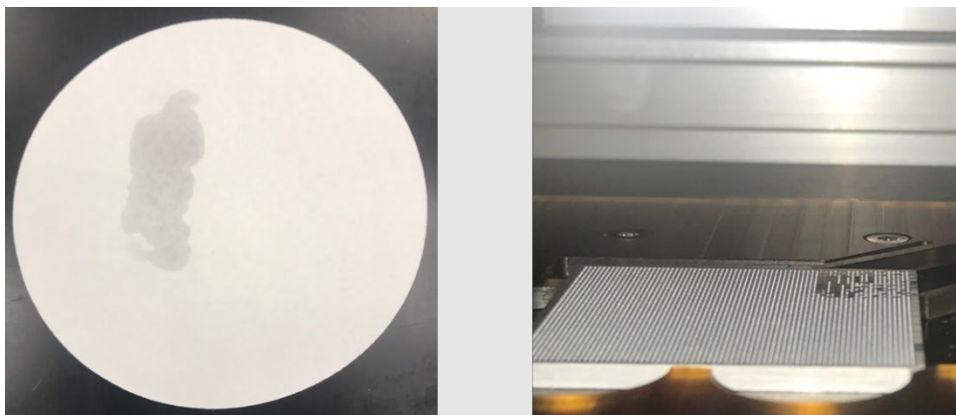


Figure 126. Images from failed dispenses. (Left) Blotting paper shows large damp area. **(Right)** Chip shows liquid on top of chip.

N. References

There have been a number of applications developed on the ICELL8 platform that have taken advantage of its flexibility. Many of these customer-created applications are described in articles at the takarabio.com website in the [ICELL8 System technical notes](#).

Appendix H: Source Well Layout Map Images

The images in this section represent the source plate maps for the six possible dispense patterns (12 x 12 through 72 x 72), using filter files (A) or unfiltered (B). They are included in this document so they can be printed out as a reference away from the ICELL8 cx system or at another workstation, such as at a bench when dispensing to the source plate.

A. Source Well Layout for Filtered Disperses



Figure 127. Source plate map: filtered 12 x 12 dispense pattern.

Cells	PosCtrl	NegCtrl	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 5	Reagent 6	Index 1	Index 2	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
A	1		33	65	81				161	193	209						289																	369
B	2		34	66	82				162	194	210						290																	
C				67	83					195	211																							
D				68	84					196	212																							
E				69	85					197	213																							
F				70	86					198	214																							
G				71	87					199	215																							
H				72	88					200	216																							
I				73	89					201	217																							
J				74	90					202	218																							
K				75	91					203	219																							
L				76	92					204	220																							
M			45						173								301																	
N			46						174								302																	
O																																		
P																																		384

Figure 128. Source plate map: filtered 24 x 24 dispense pattern.

Cells	PosCtrl	NegCtrl	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 5	Reagent 6	Index 1	Index 2															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1		33		65	81	97				161		193	209	225				289						369
B	2		34		66	82	98				162		194	210	226				290						
C	3		35		67	83	99				163		195	211	227				291						
D	4		36		68	84	100				164		196	212	228				292						
E					69	85							197	213											
F					70	86							198	214											
G					71	87							199	215											
H					72	88							200	216											
I					73	89							201	217											
J					74	90							202	218											
K					75	91							203	219											
L					76	92							204	220											
M			45		77	93					173		205	221					301						
N			46		78	94					174		206	222					302						
O			47		79	95					175		207	223					303						
P			48		80	96					176		208	224					304						384

Figure 129. Source plate map: filtered 36 x 36 dispense pattern.

Cells	PosCtrl	NegCtrl	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 5	Reagent 6	Index 1	Index 2														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	17	33		65	81	97	113			161		193	209	225	241			289					369
B	2	18	34		66	82	98	114			162		194	210	226	242			290					
C	3	19	35		67	83	99	115			163		195	211	227	243			291					
D	4	20	36		68	84	100	116			164		196	212	228	244			292					
E					69	85	101	117					197	213	229	245								
F					70	86	102	118					198	214	230	246								
G					71	87	103	119					199	215	231	247								
H					72	88	104	120					200	216	232	248								
I					73	89							201	217										
J					74	90							202	218										
K					75	91							203	219										
L					76	92							204	220										
M			45		77	93					173		205	221					301					
N			46		78	94					174		206	222					302					
O			47		79	95					175		207	223					303					
P			48		80	96					176		208	224					304					384

Figure 130. Source plate map: filtered 48 x 48 dispense pattern.

Cells	PosCtrl	NegCtrl	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 5	Reagent 6	Index 1	Index 2																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24			
A	1	17	33		65	81	97	113			161		193	209	225	241			289					369			
B	2	18	34		66	82	98	114			162		194	210	226	242			290								
C	3	19	35		67	83	99	115			163		195	211	227	243			291								
D	4	20	36		68	84	100	116			164		196	212	228	244			292								
E					69	85	101	117					197	213	229	245											
F					70	86	102	118					198	214	230	246											
G					71	87	103	119					199	215	231	247											
H					72	88	104	120					200	216	232	248											
I					73	89	105	121					201	217	233	249											
J					74	90	106	122					202	218	234	250											
K					75	91	107	123					203	219	235	251											
L					76	92	108	124					204	220	236	252											
M			45		77	93	109	125			173		205	221	237	253			301								
N			46		78	94	110	126			174		206	222	238	254			302								
O			47		79	95					175		207	223					303								
P			48		80	96					176		208	224					304					384			

Figure 131. Source plate map: filtered 60 x 60 dispense pattern.

Cells	PosCtrl	NegCtrl	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 5	Reagent 6	Index 1	Index 2	Index 3	Index 4	Index 5	Index 6	Index 7	Index 8	Index 9	Index 10	Index 11	Index 12	Index 13	Index 14	Index 15	Index 16	Index 17	Index 18	Index 19	Index 20	Index 21	Index 22	Index 23	Index 24	
A	1	17	33		65	81	97	113	129	145	161		193	209	225	241	257	273	289														369
B	2	18	34		66	82	98	114	130	146	162		194	210	226	242	258	274	290														
C	3	19	35		67	83	99	115	131	147	163		195	211	227	243	259	275	291														
D	4	20	36		68	84	100	116	132	148	164		196	212	228	244	260	276	292														
E					69	85	101	117					197	213	229	245																	
F					70	86	102	118					198	214	230	246																	
G					71	87	103	119					199	215	231	247																	
H					72	88	104	120					200	216	232	248																	
I					73	89	105	121					201	217	233	249																	
J					74	90	106	122					202	218	234	250																	
K					75	91	107	123					203	219	235	251																	
L					76	92	108	124					204	220	236	252																	
M			45		77	93	109	125			173		205	221	237	253			301														
N			46		78	94	110	126			174		206	222	238	254			302														
O			47		79	95	111	127			175		207	223	239	255			303														
P			48		80	96	112	128			176		208	224	240	256			304														384

Figure 132. Source plate map: filtered 72 x 72 dispense pattern.



Cells		PosCtrl	NegCtrl	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 4	Reagent 5	Reagent 6	Index 1	Index 2													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1			33		65	81				161			193	209					289					369
B	2			34		66	82				162			194	210					290					
C						67	83							195	211										
D						68	84							196	212										
E						69	85							197	213										
F						70	86							198	214										
G						71	87							199	215										
H						72	88							200	216										
I						73	89							201	217										
J						74	90							202	218										
K						75	91							203	219										
L						76	92							204	220										
M												173								301					
N												174								302					
O																									
P																									384

Figure 134. Source plate map: unfiltered 24 x 24 dispense pattern.

Cells	PosCtrl	NegCtrl	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 5	Reagent 6	Index 1	Index 2														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1		33		65	81	97				161		193	209	225				289					369
B	2		34		66	82	98				162		194	210	226				290					
C	3		35		67	83	99				163		195	211	227				291					
D	4		36		68	84	100				164		196	212	228				292					
E					69	85							197	213										
F					70	86							198	214										
G					71	87							199	215										
H					72	88							200	216										
I					73	89							201	217										
J					74	90							202	218										
K					75	91							203	219										
L					76	92							204	220										
M			45		77	93					173		205	221					301					
N			46		78	94					174		206	222					302					
O			47		79	95					175		207	223					303					
P			48		80	96					176		208	224					304					384

Figure 135. Source plate map: unfiltered 36 x 36 dispense pattern.

Cells	PosCtrl	NegCtrl	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 5	Reagent 6	Index 1	Index 2														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	17	33	49	65	81	97	113			161	177	193	209	225	241			289	305				369
B	2	18	34	50	66	82	98	114			162	178	194	210	226	242			290	306				
C	3	19	35	51	67	83	99	115			163	179	195	211	227	243			291	307				
D	4	20	36	52	68	84	100	116			164	180	196	212	228	244			292	308				
E					69	85	101	117					197	213	229	245								
F					70	86	102	118					198	214	230	246								
G					71	87	103	119					199	215	231	247								
H					72	88	104	120					200	216	232	248								
I					73	89							201	217										
J					74	90							202	218										
K					75	91							203	219										
L					76	92							204	220										
M			45	61	77	93					173	189	205	221					301	317				
N			46	62	78	94					174	190	206	222					302	318				
O			47	63	79	95					175	191	207	223					303	319				
P			48	64	80	96					176	192	208	224					304	320				384

Figure 136. Source plate map: unfiltered 48 x 48 dispense pattern.

Cells	Index 2																								
	PosCtrl	NegCtrl	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 5	Reagent 6	Index 1	Index 2															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1	17	33	49	65	81	97	113			161	177	193	209	225	241			289	305					369
B	2	18	34	50	66	82	98	114			162	178	194	210	226	242			290	306					
C	3	19	35	51	67	83	99	115			163	179	195	211	227	243			291	307					
D	4	20	36	52	68	84	100	116			164	180	196	212	228	244			292	308					
E					69	85	101	117					197	213	229	245									
F					70	86	102	118					198	214	230	246									
G					71	87	103	119					199	215	231	247									
H					72	88	104	120					200	216	232	248									
I					73	89	105	121					201	217	233	249									
J					74	90	106	122					202	218	234	250									
K					75	91	107	123					203	219	235	251									
L					76	92	108	124					204	220	236	252									
M			45	61	77	93	109	125			173	189	205	221	237	253			301	317					
N			46	62	78	94	110	126			174	190	206	222	238	254			302	318					
O			47	63	79	95					175	191	207	223					303	319					
P			48	64	80	96					176	192	208	224					304	320					384

Figure 137. Source plate map: unfiltered 60 x 60 dispense pattern.

Cells	Index 2																								
	PosCtrl	NegCtrl	Reagent 1	Reagent 2	Reagent 3	Reagent 4	Reagent 5	Reagent 6	Index 1	Index 2															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	
A	1	17	33	49	65	81	97	113	129	145	161	177	193	209	225	241	257	273	289	305					369
B	2	18	34	50	66	82	98	114	130	146	162	178	194	210	226	242	258	274	290	306					
C	3	19	35	51	67	83	99	115	131	147	163	179	195	211	227	243	259	275	291	307					
D	4	20	36	52	68	84	100	116	132	148	164	180	196	212	228	244	260	276	292	308					
E					69	85	101	117					197	213	229	245									
F					70	86	102	118					198	214	230	246									
G					71	87	103	119					199	215	231	247									
H					72	88	104	120					200	216	232	248									
I					73	89	105	121					201	217	233	249									
J					74	90	106	122					202	218	234	250									
K					75	91	107	123					203	219	235	251									
L					76	92	108	124					204	220	236	252									
M			45	61	77	93	109	125			173	189	205	221	237	253			301	317					
N			46	62	78	94	110	126			174	190	206	222	238	254			302	318					
O			47	63	79	95	111	127			175	191	207	223	239	255			303	319					
P			48	64	80	96	112	128			176	192	208	224	240	256			304	320					384

Figure 138. Source plate map: unfiltered 72 x 72 dispense pattern.

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