

Takara Bio USA, Inc.

SmartChip ND® and qPCRStudio™ Software User Manual

Cat. No. 640290
(120925)

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I. Introduction

The **SmartChip ND Real-Time PCR System** (Cat. No. 640290, referred to as the SmartChip ND system in this manual) enables automated, high-throughput, high-density, real-time PCR for gene expression or single nucleotide polymorphism (SNP) genotyping analysis.



Figure 1. The SmartChip ND Real-Time PCR System. For more information about the hardware, see Section IV.

This manual provides instructions for the safe operation and maintenance of the SmartChip ND (referred to as the SmartChip ND dispenser), the nanodispenser module of the SmartChip ND system. This manual also includes instructions for using the SmartChip ND qPCRStudio Software (not sold separately). Operation of the SmartChip ND Real-Time PCR Cycler and qPCR Software is covered in the [SmartChip ND Real-Time PCR Cycler and Software User Manual](#).

Please review the information in this manual thoroughly before starting your experiments. If you require technical support, contact your authorized Takara Bio Service Technician or field_support@takarabio.com.

Symbols and conventions

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

Table 1. User manual symbols and conventions.

Symbol	Description
	WARNING: Indicates a potentially hazardous situation that could result in injury to the user or damage to or destruction of the system.
	CAUTION: Indicates a hazard that could result in loss of data or damage to the system.
	Indicates the presence of an electrical shock hazard. Proceed with caution. This symbol may appear next to either a WARNING or CAUTION 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.

II. Safety Information

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



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



Operating conditions:

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



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

Instrument use:



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



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



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



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

Moving the system:



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

III. List of Components

The complete SmartChip ND Real-Time PCR System (Cat. No. 640290) includes the components listed below. Please note that this manual only covers operation of the SmartChip ND dispenser and qPCRStudio Software.

Table 2. SmartChip ND Real-Time PCR System components.

	640290
SmartChip ND Real-Time PCR System	
SmartChip ND Crate Components (Cat. No. 640292)	1
Package 1 (Store at room temperature)	
SmartChip ND	1
Package 2 (Store at room temperature)	
Barcode Scanner	1
Mobile Scissor Lift	1
Regional Power Cord	2
Universal Power Strip	1
SmartChip ND Real-Time PCR System Accessory Kit	1
Nanodispenser Chip Centrifuge Spinner	2
Nanodispenser Chip Centrifuge Balance	1
Nanodispenser Chip Blotter	1
384-Well Plate Seal Applicator	1
SmartChip ND Real-Time PCR Cyclers (Cat. No. 640023)	1
Cycler Computer	1
Cycler Monitor	1
Cycler Monitor Mount	1
SmartChip ND 5KT Cycler Assembly	1
Region specific power cord and transformer set	1
SmartChip ND Real-Time PCR System Installation Kit (Cat. No. 640294)	1
Package 1 (Store at room temperature)	
SmartChip® MyDesign Chips	3
Blotting Paper (Pack of 10)	1
Nanodispenser Alignment Chip Film	1
Cycler Sealing and Pressure Film	3
384-Well Source Plate Seal	3
384-Well Source Plate	3
Nanodispenser Imitation Mastermix	1
Nanodispenser Chip Centrifuge Balance	2
Package 2 (Store at -20°C)	
Lightcycler 480 SYBR Green I Mastermix	3 x 1 ml
1X TE Buffer, pH 8	1 x 1 ml
DTT (134 mM)	2 x 50 µl
Positive Control Primers	2 x 200 µl
PCR-grade Water	2 x 1 ml
PC Binding Reagent	2 x 50 µl
Package 3 (Store at -70°C)	
Positive Control Template	3 x 5 µl

SmartChip Real-Time PCR System Training Kit – Genotyping (Cat. No. 640154)	1
Package 1 (Store at room temperature)	
Blotting Paper (10 per pack)	1
Nanodispenser Chip Intermediate Film (10 per pack)	1
384-Well Source Plate Seal (10 per pack)	1
Cycler Sealing and Pressure Film	3
SmartChip MyDesign Chips	3
384-Well Source Plate	6
96-well Axygen Plates	3
Package 2 (Store at –20°C)	
SmartChip Probe qPCR Mix	3
PCR grade water	1
ROX Reference Dye (50X)	1
GT Training Assay Plate (5X, 5 SNPs), 100 µl/well	1
Package 3 (Store at –70°C)	
GT Training Samples Plate (25 ng/µl, 8 Samples), 75 µl/well	1

IV. Overview: The SmartChip ND dispenser

A. Main Components

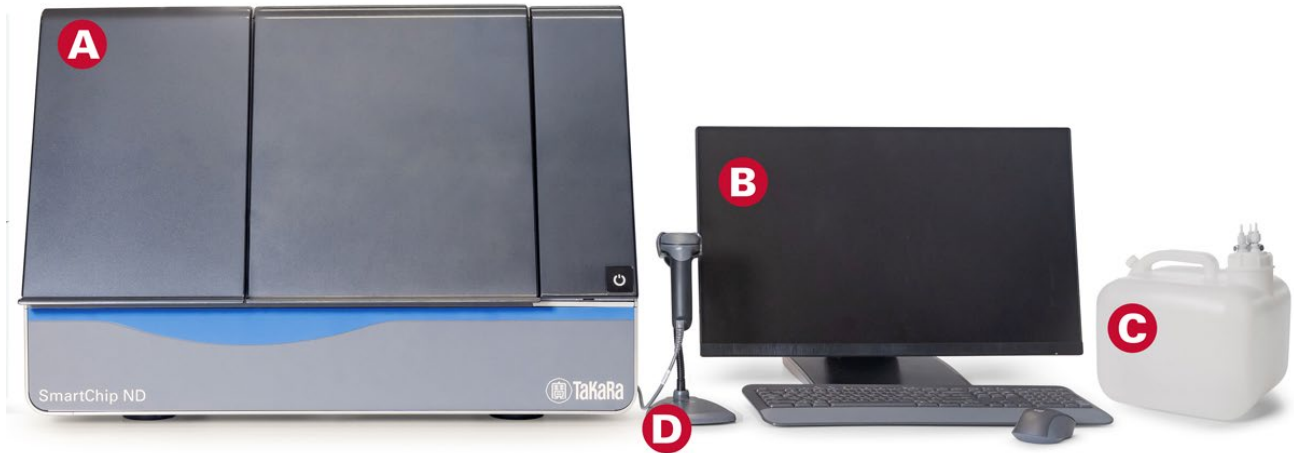


Figure 2. The SmartChip ND dispenser (external view).

The SmartChip ND dispenser includes the following main components:

- [A] SmartChip ND dispenser
- [B] Touch-screen monitor, wireless mouse, and keyboard (preloaded with qPCRStudio Software).
- [C] Waste container—the waste container collects the fluid that is discarded through the tip nozzles into the waste well of the tip wash trough. The container sits outside of the SmartChip ND.
- [D] Handheld barcode reader—using the barcode reader provided with the system is recommended for best results.

B. SmartChip ND Electrical Inputs and Outputs

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

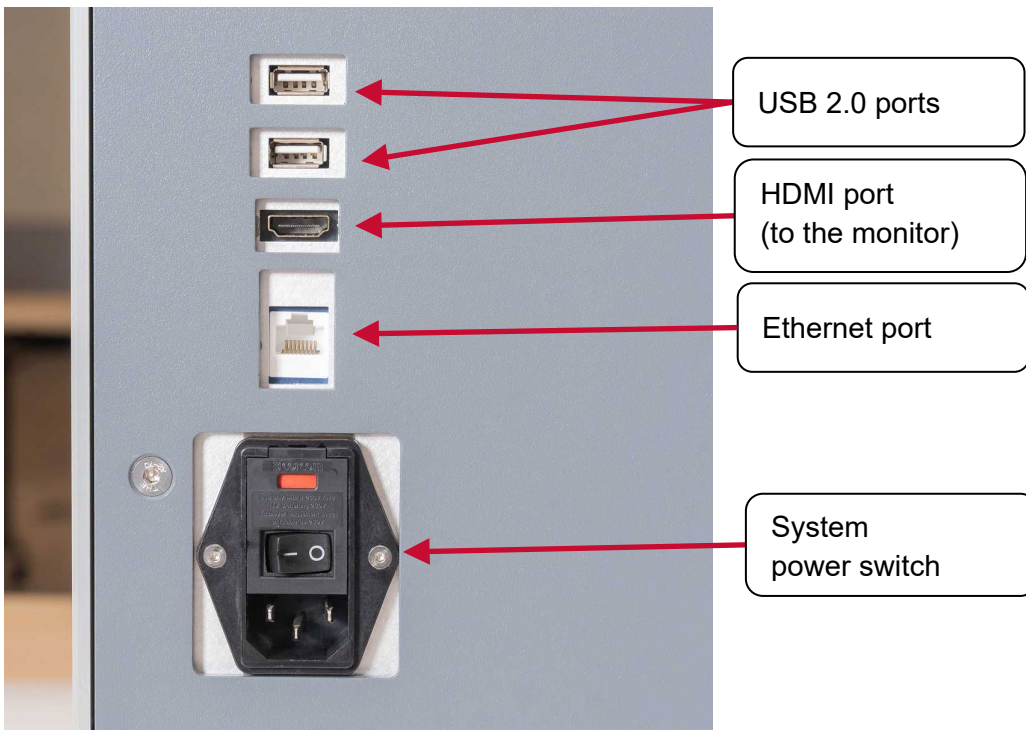


Figure 3. Identification of the electrical input and output on the back of the SmartChip ND dispenser.

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

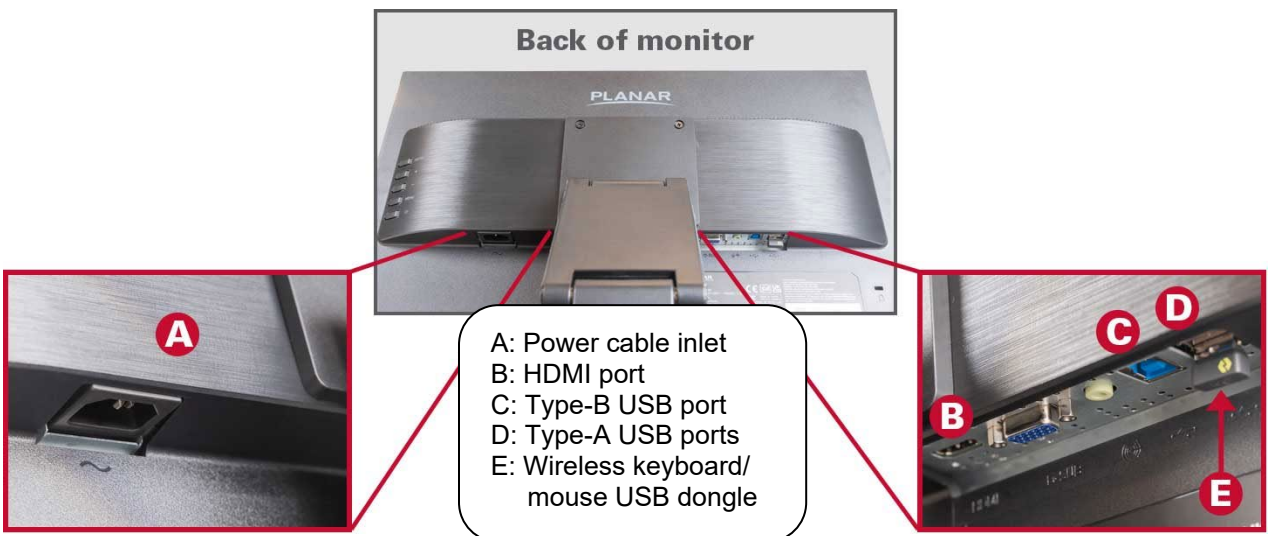


Figure 4. Identification of inputs and outputs on the back of the touchscreen monitor.

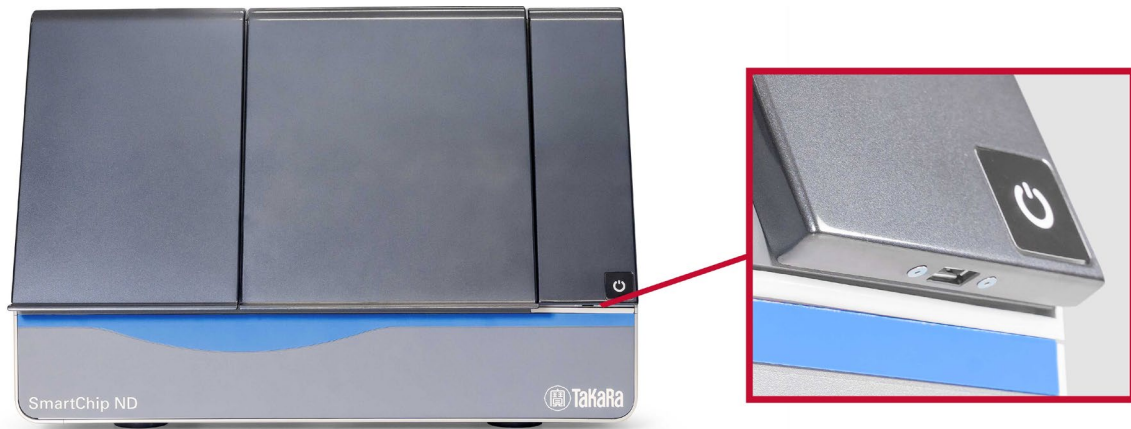


Figure 5. Location of the USB 2.0 port on the front of the SmartChip ND dispenser.

C. SmartChip ND Dispenser Internal Components

This section describes the internal components of the SmartChip ND dispenser. Opening the door on the left side of the dispenser reveals the fluidic source bottles (Figure 6; F, G, and H). There are three fluidic source bottles: the 2 L water source bottle, the 500 ml wash source bottle, and the 500 ml humidifier source bottle.



Figure 6. The SmartChip ND dispenser (internal view).

Opening the door on the right side of the dispenser reveals the inside of the environmental chamber. As shown in Figure 6, the inside of the environmental chamber consists of the dispensing Platform (I), the tip nozzle assembly (J), and the temperature/humidity sensor (K). The dispensing platform contains the chip nest in the center, 384-well plate nests to the left and right of the chip nest, and a tip wash trough. The environmental control hardware (humidifier, dehumidifier) is located on the internal right-hand wall (Figure 7; L).

The door sensor and door lock, LED status indicator, and power touch button are located on the bottom right of the SmartChip ND (Figure 7; M, N, O).



Figure 7. The SmartChip ND dispenser (internal view), with a focus on the right side.

Fluidic source bottles

Figure 8 shows the positions of the water source bottle, the bleach source bottle, and the humidifier source bottle under the left panel within the SmartChip ND dispenser.

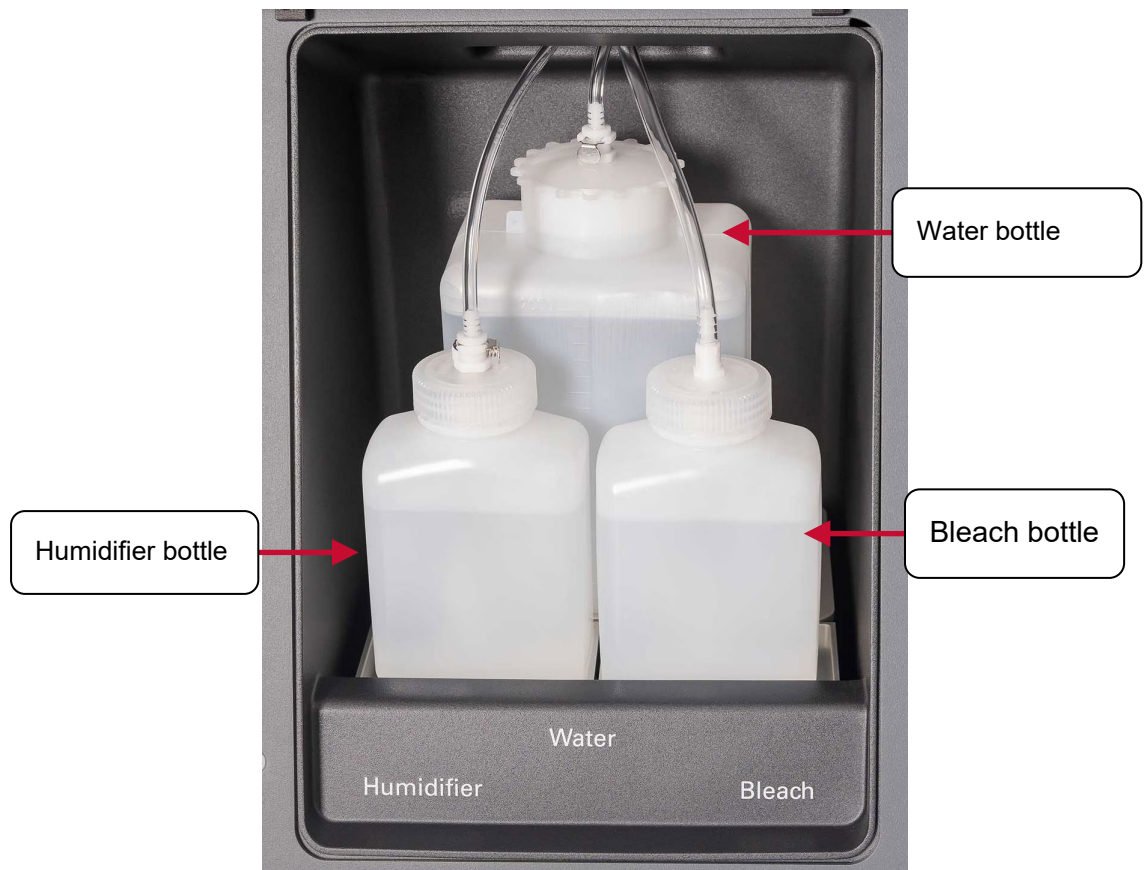


Figure 8. SmartChip ND dispenser (internal view) source bottle positions.

Water source bottle, 2 L

The water in the water source bottle is used for rinsing dispenser tips. The water source bottle should be filled with deionized filtered water (Milli-Q, Elga system or equivalent; 0.2 µm filtration). The

water source bottle sits on a scale that monitors the water level via weight. This allows the software to alert the user before starting a chip dispense if the bottle needs refilled.

Bleach source bottle, 500 ml

The bleach source bottle is the source for the wash well in the tip wash trough, and it sits to the front and right of the water source bottle. It should be filled with a 0.3% sodium hypochlorite solution, which is used for cleaning the tip nozzles between dispense rounds to prevent cross-contamination. Like the water source bottle, it sits on a scale so the system can alert users if the bottle needs refilled before dispensing.

Humidifier source bottle, 500 ml

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

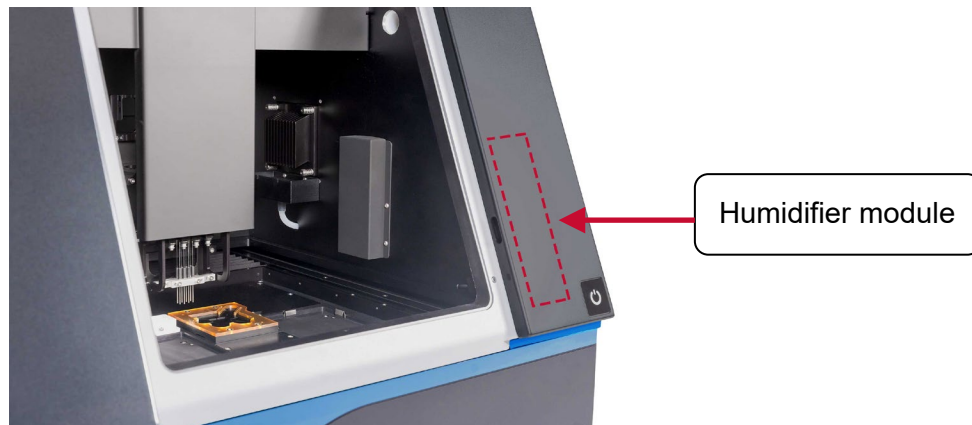


Figure 9. Position of the SmartChip ND dispenser humidifier module. The red outline on the front right of the instrument is where the humidifier module is located.

Dispensing platform

The dispensing platform consists of the cooled chip and 384-well plate nests, and a tip wash trough. The entire platform can move on the XY axes underneath the tip nozzles. The tip nozzles are raised and lowered independently of the platform on the Z axis.

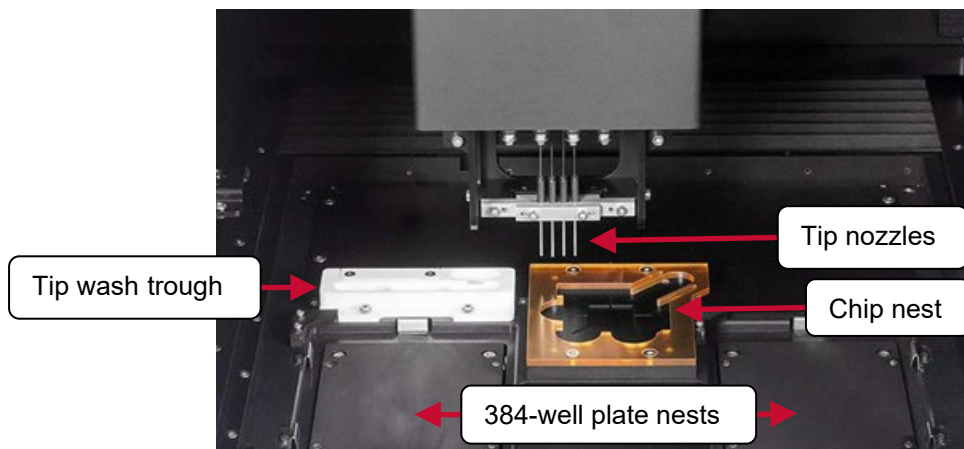


Figure 10. SmartChip ND dispensing platform.

Chip Nest and 384-well Plate Nests

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

Tip Wash Trough

The Tip Wash Trough consists of four wells: wash, waste, secondary rinse, and primary rinse (Figure 11). The rinse wells are used for rinsing the inside and outside of the tips with water and drain to the waste container. The waste well is where any excess system fluid, reagents, or samples are discarded. The wash well is connected to the wash bottle (which contains 0.3% sodium hypochlorite solution) and used for washing the inside and outside of the tips with the washing solution.

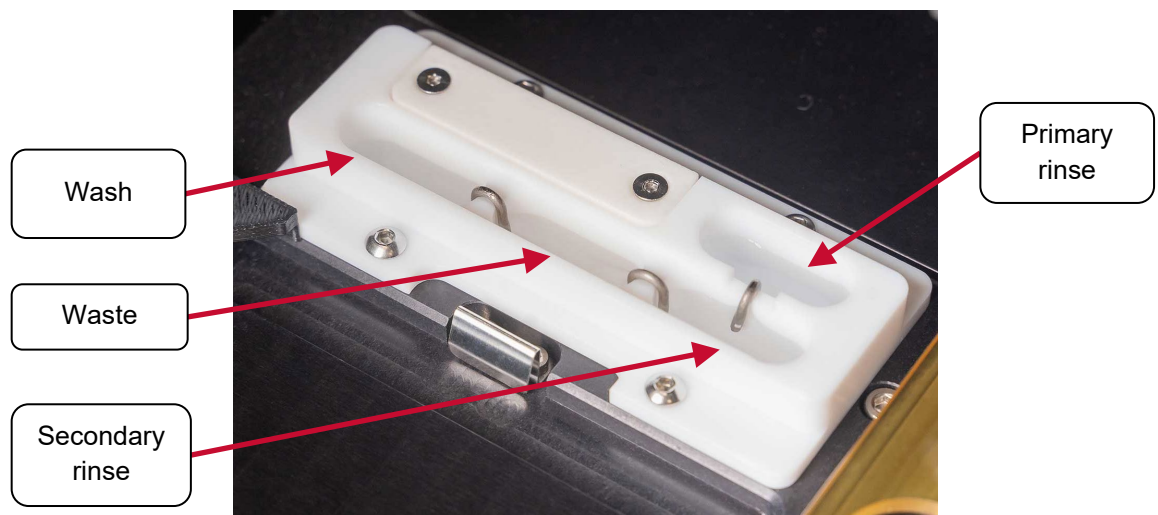


Figure 11. Tip wash trough well locations.

Tip nozzle

The tip nozzle is raised and lowered to the desired height for aspiration, dispense, and wash steps. When the door to the stage module is closed, an environmental chamber is created that allows the system to control the chamber to the optimal humidity levels during sample and reagent dispensing.

Assays or samples are aspirated from a 384-well plate that sits in the source plate nest and then dispensed into a chip that sits in the chip nest. The tips are brought to the trough to discard excess fluids to waste and to perform tip cleaning.

Environmental Control Module

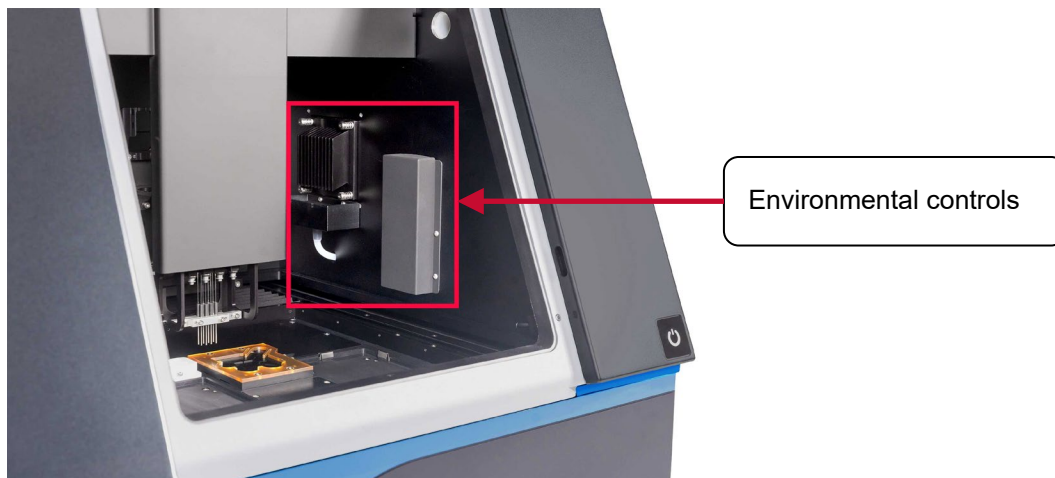


Figure 12. Location of the environmental control module in the SmartChip ND dispenser.

Temperature/humidity sensor

The temperature/humidity sensor (Figure 6; L and Figure 12) are mounted on the inside chamber on the left wall. They are used to monitor the chamber's environmental conditions. The sensor allows the environmental control system to minimize evaporation or condensation during dispensing.

Humidifier and dehumidifier modules

The two environmental control units are the humidifier and dehumidifier Modules (Figure 12). Based on the temperature and humidity sensor readings, the environmental control system increases or decreases the humidity in the chamber to maintain conditions that minimize reagent evaporation or condensation during the dispensing process. The control system also attempts to maintain the chip temperature of 10°C, but a deviation of a few degrees higher or lower, while the humidity conditions stabilize, may occur.

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

Additional Components

Door lock and door sensor

The door lock and door sensor (Figure 6; M) are used to ensure safe operation of the SmartChip ND dispenser. The software will not proceed if it does not sense that the door is closed. Once a dispense operation is started, the door lock prevents users from opening the door and accessing the machine while parts are in motion.

LED status indicator

The LED status indicator (Figure 6; N) is on the front-right side of the machine and provides a visual indicator of the status of the SmartChip ND dispenser. A blue light means that the system is idle. The light turns green during operation, and a yellow light indicates an error.

Software power touch-button

The software power touch-button is located on the front-left of the SmartChip ND dispenser. After the system is powered on by the switch in the back, it will still be in sleep mode. The software power

touch-button will slowly blink. To wake up the system and start the software, press and hold the button for 1 sec.



Figure 13. Software power touch-button location.

D. SmartChip ND Dispenser Specifications and Lab Requirements

The table below outlines the specifications and requirements for the SmartChip ND dispenser. For specifications and requirements of the SmartChip ND Real-Time PCR Cycler, please refer to the [SmartChip ND Real-Time PCR Cycler and Software User Manual](#).

Table 3. SmartChip ND dispenser specifications and lab requirements.

Category	Specification
Dispense volume	35–150 nL per nanowell, in 5 nL increments
Software	qPCRStudio Software (preinstalled)
Computer	Enterprise-level Windows 11 PC (included with system)
Power requirements (for different power supply types)	120 VAC/60 Hz mains: two 15 or 20A outlets 220–240 VAC/50 Hz mains: two 10A outlets 100 VAC/50–60 Hz mains: two 15A outlets
Fuses	T 10A H, 250V, 5x20mm type
Environmental conditions	Ambient temperature: 18–26°C Temperature variability <10°C per 10 min Relative humidity, noncondensing: 10–70% RH% variability <10 percentage points per 10 min Altitude: <2,000 m above sea level Dust level: ISO 9 Vibration level: (<12.5 µm/s VC-C)
Dimensions	32" W x 22" H x 24" D (81 cm x 54 cm x 60 cm)
Bench space	Includes clearance for SmartChip ND dispenser and monitor: 54" W x 26" H x 30" D (138 cm x 66 cm x 76 cm) <i>Note: Bench space must be capable of supporting 400 pounds (180 kg)</i>
Floor space	Waste container: 10" W x 10" H x 10" D (25 cm x 25 cm x 25 cm)
Weight	240 pounds (109 kg)
Minimum space to back wall (for ventilation)	4" (10 cm)

E. Setup and Installation

Your Takara Bio Service Engineer will unpack and install the SmartChip ND dispenser and explain the basic operation of the system. They will use material from the SmartChip ND Real-Time PCR System Installation Kit to qualify the instrument after installation and will leave any reusable and/or remaining materials at your site.

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

F. Required Equipment and Supplies from Your Lab or Other Suppliers

Reagents for the SmartChip ND Dispenser Fluidic Source Bottles

- Deionized filtered water (Milli-Q or Elga system or equivalent; 0.2 µm filtration)
- 0.3% sodium hypochlorite solution made from reagent-grade sodium hypochlorite in deionized filtered water

Other Reagents and Materials

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

General Lab Equipment

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

G. SmartChip Technology

SmartChip technology distinguishes Takara Bio's PCR platform from other systems. Each nanodispenser chip has a 72 x 72 array of nanowells and can accommodate up to 5,184 150 or 250 nl real-time PCRs in a single run. There are three types of chips:

- **SmartChip MyDesign Chip, 150 nl** (sold as part of Cat. No. 640032 & 640036): You add experimental samples, PCR reagents, and PCR assays to these chips using the SmartChip ND.
- **SmartChip MyDesign Chip, 250 nl** (sold as part of Cat. No. 640253): You add experimental samples, PCR reagents, and PCR assays to these chips using the SmartChip ND.
- **SmartChip Custom Panels 150 nl:** contain PCR assays dispensed into the chips at Takara Bio. You add your experimental samples and PCR reagents to these chips using the SmartChip ND dispenser.

Gene Expression Analysis

For mRNA expression analysis, the SmartChip ND Real-Time PCR System has been tested with cDNA from human brain (Cat. No. 637242), human lung (Cat. No. 637206), human placenta (Cat. No. 637208), and a pooled reference sample (Cat. No. 637260) with SmartChip TB Green Gene Expression Master Mix (Cat. No. 640210).

NOTE: Other master mixes have not been validated for performance and are not supported. Precipitate may be observed in the SmartChip TB Green Gene Expression Master Mix. This precipitate does not affect the performance of the kit. The precipitate can be dissolved easily by warming to room temperature and mixing for a few minutes. Ensure that the precipitate is fully dissolved before use.

- **SmartChip MyDesign Chips** are provided empty. Use the SmartChip ND to add both PCR assay(s) and experimental cDNA or DNA sample(s) in any of the 14 configurations supported for expression analysis.
- **SmartChip Custom Panels** are designed for targeted expression analysis; they are custom-manufactured to your specifications. Choose from commercially available assays or have your assay design of choice predispensed into a chip. We offer SmartChip Custom Panels for expression analysis in six different configurations, designed for analysis of 3–96 samples using 384–12 assays in quadruplicate.

SNP Genotyping

The SmartChip ND system can be used for SNP genotyping using TaqMan SNP Genotyping Assays (Thermo Fisher Scientific) with Minor Groove Binder (MGB), BHQ*plus* assays (LGC Biosearch), rhAmp assays (IDT), and, with minor modifications, KASP assays (LCG Genomics). Please contact [field support](#) if you have questions about implementing any of these assays.

- **SmartChip MyDesign Chips** are provided empty. Use the SmartChip ND dispenser to add SNP genotyping assays, reagent master mix, and experimental DNA samples in any of the 14 configurations supported for SNP genotyping analysis.
- **SmartChip SNP Genotyping Panels** contain TaqMan SNP Genotyping Assays that you have shipped to Takara Bio. They are available in eight configurations to analyze 12–384 samples using a single replicate with 384–12 TaqMan SNP Genotyping Assays. We dispense the assays and send the resulting SmartChip SNP Genotyping Panels with a CD containing files with assay locations, thermal profile, and data analysis parameters. Use the SmartChip ND dispenser to add your experimental DNA samples to the SmartChip Custom Panel.

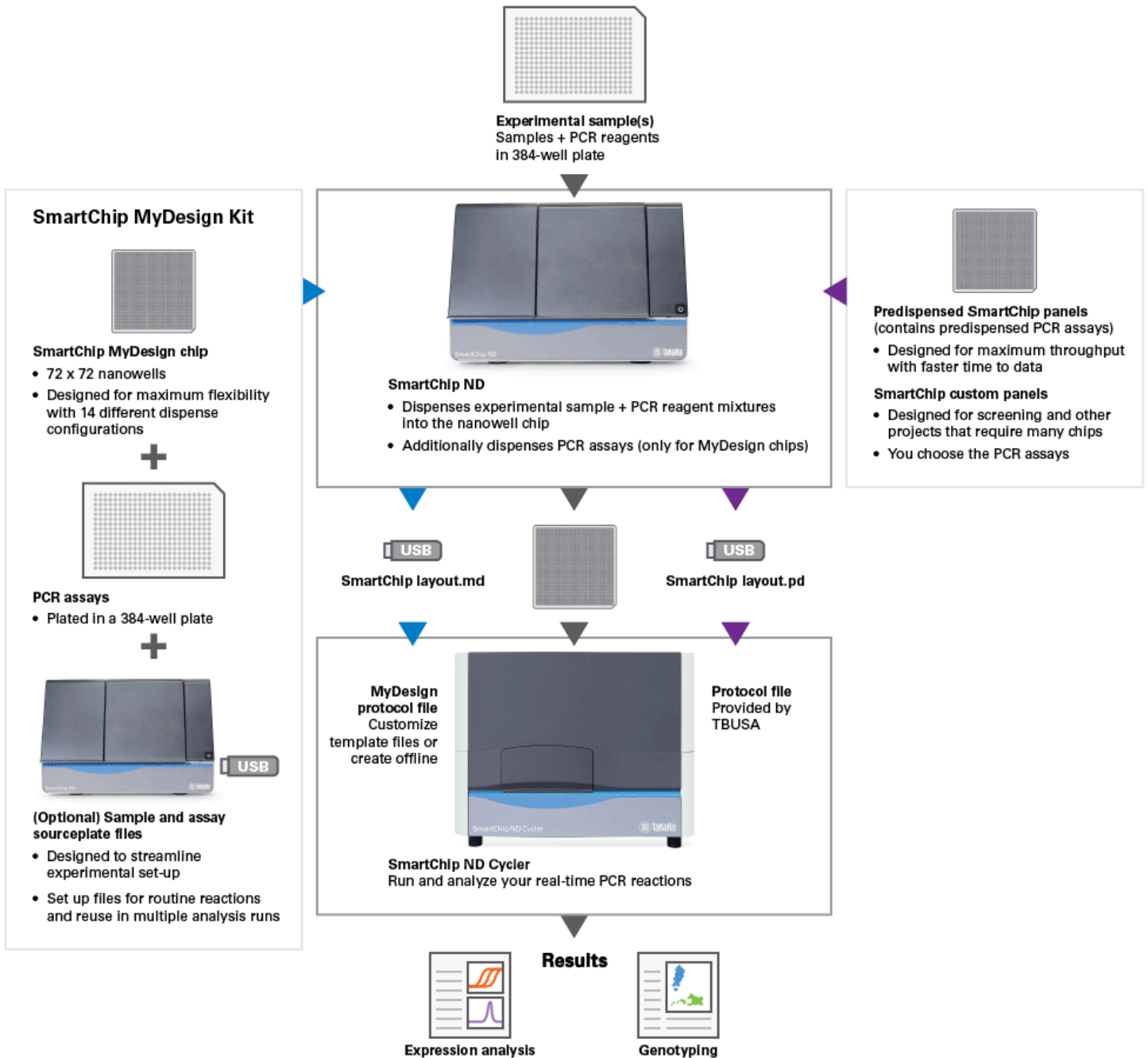


Figure 14. SmartChip ND Real-Time PCR System workflow.

V. Overview: qPCRStudio Software

qPCRStudio software is preinstalled on the computer within the SmartChip ND dispenser. This software controls the instrument, including chamber environmental control, assay/sample dispensing, and generating chip content outputs to the SmartChip cycler for analysis. qPCRStudio software provides a guided dispense workflow to execute jobs of different combinations of assays and samples with the option for replicates. All jobs are organized and saved as independent instances of a pre-planned application. Jobs can also be stopped and resumed from any step.

A. Starting qPCRStudio Software

1. To start qPCRStudio, first ensure that the SmartChip ND dispenser is powered by toggling the power switch on the back of the instrument (Figure 3)
2. **Press and hold the power button for 1 sec** until the LED status indicator light changes from blue to green, indicating the software is initializing. On software startup, the XY and Z axes and dispenser hardware will automatically initialize on the system. On the monitor, the initialization splash screen (Figure 15) will display.



Figure 15. Example qPCRStudio initialization screen.

3. Upon successful completion of the system initialization, the LED status indicator will change back to blue, and you will be presented with either *Action Center* view (Figure 16), if there are any system actions required, or the *Home* screen if there are not.

B. Getting the System Ready

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

For a comprehensive list of potential action items, please refer to [Appendix B, Section B](#), "Action Center Alerts".

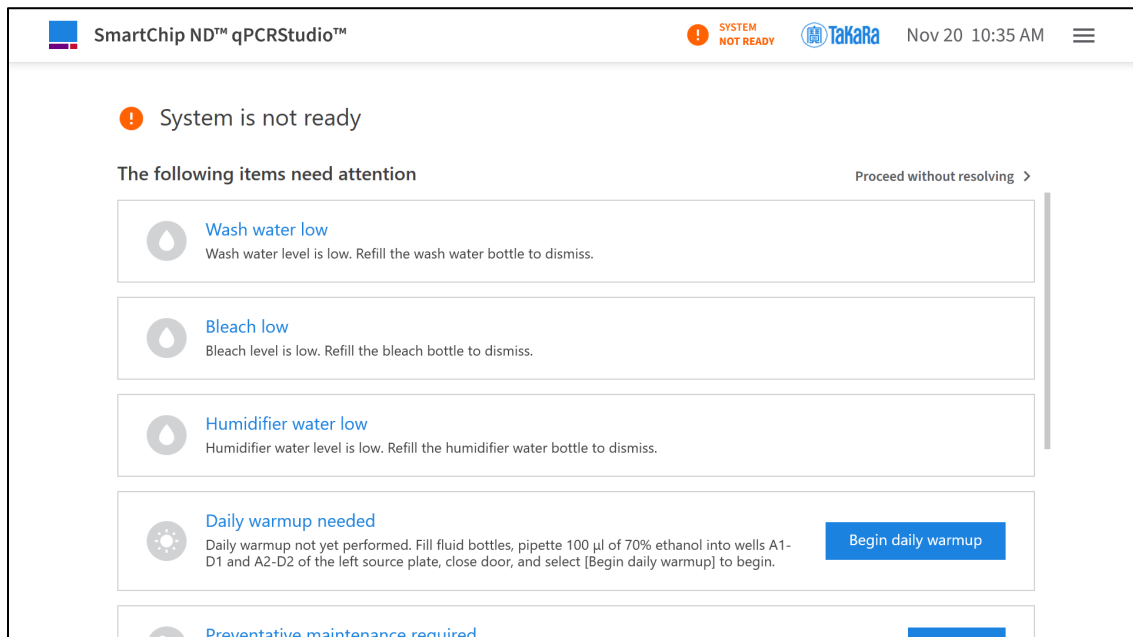


Figure 16. An example *Action Center* view.

Typically, an action item includes:

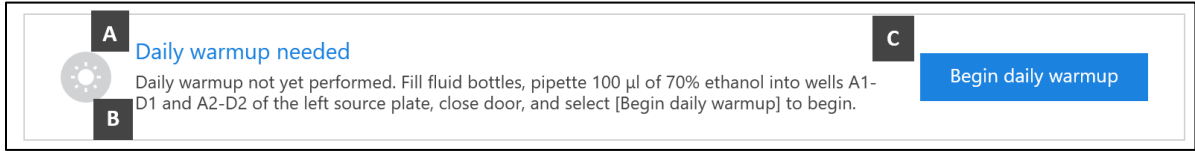


Figure 17. An example action item.

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

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

[C] Action button (optional): A button that, when pressed, performs the necessary action.

C. The Home Screen Title Bar

The title bar on the *Home* screen displays useful information such as system status, current date and time, and more options (Figure 18). For more information about potential messages displayed in the title bar, refer to [Appendix B, Section A](#), "Home Bar Alerts".



Figure 18. Home screen menu bar.

D. Guided Workflow

Execution through the steps is accompanied by easy-to-follow on-screen instructions. The guided dispense workflow at the bottom of the screen walks you through the experimental setup, layout, dispense, and export of files.

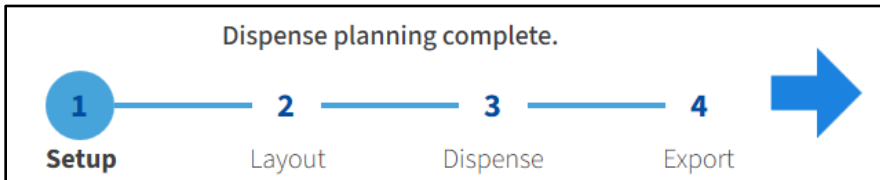


Figure 19. Example Guided Dispense workflow.

E. Exiting qPCRStudio Software

To shut down the software, click on the options button on the upper right side of the user interface and select **Exit SmartChip ND qPCRStudio**.

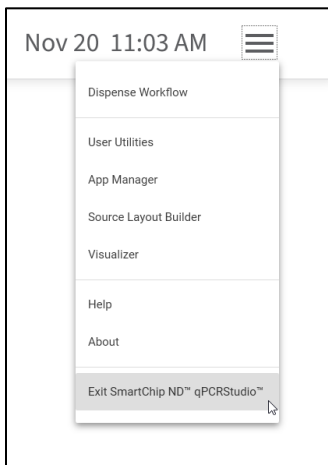


Figure 20. How to exit SmartChip ND qPCRStudio Software.

VI. Protocol: Prepare the SmartChip ND dispenser

A. Power on the System (If Needed)

If the instrument is powered down, turn it on by flipping the switch at the back-right of the SmartChip ND dispenser (Figure 3). The Software Power Touch-button (front, Figure 21) will slowly blink to indicate that the software is ready to be started.

B. Start Up the Software

To start the qPCRStudio software, press and hold the power touch-button for 1 sec. Refer to [Section VI.B](#) for more information on the software startup.



Figure 21. Software power touch-button located on the front right panel of the SmartChip ND dispenser.

When initialized, the qPCRStudio Software will automatically check for additional actions that may be needed for optimal instrument performance. If any such actions are found, the *Action Center* screen will automatically appear prior to starting a job and alert the user of the actions to take. Resolve the alerts to proceed. Note that the *Action Center* may also be displayed after startup if there is a new actionable item that comes up (e.g., notification to refill fluid in a source bottle).

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

VII. Protocol: Prepare the Source Plates

A source plate is a 384-well plate containing either the samples (i.e., a sample source plate) or the PCR assays (i.e., an assay source plate) that are to be dispensed using the SmartChip ND dispenser.



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

This section has instructions for preparing source plates for the following applications or SmartChip type:

- Expression or genotyping analysis using SmartChip MyDesign Chips
- Expression or genotyping analysis using predispensed SmartChip Custom Panels



IMPORTANT: Avoid introducing dust and debris to solutions that will be dispensed with the SmartChip ND dispenser. They can cause the tips to clog.

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

- Consider assembling source plates in a dead air box 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

A. Prepare Source Plates for SmartChip MyDesign Chips

1. Prepare your samples and assays using the [SmartChip MyDesign Kit User Manual](#) or the protocol provided with your SmartChip Custom Panel.
2. Pipette the following material into a 384-well plate based on the type of job you will be running:
 - For gene expression analysis: sample/PCR reagent mixtures containing cDNA or DNA
 - For genotyping: sample or sample/PCR reagent mixture containing DNA
 - Assays (on a separate source plate)

To make it easier to manually pipette into the 384-well source plates, we suggest implementing one of the following:

- Use the *Source Plate Layout Builder* under the **User Utilities** in the qPCRStudio software to view the relevant source plate map(s).
- Use the source plate map(s) on the "Source Plate Layout" step of the job you will be running.

Tables 4 and 5 below show recommended volumes per well and the number of wells in your sample source plate depending on the well volume of the chip.

Table 4. 384-well plate preparation for a 150 nl MyDesign chip: recommended volumes per well and number of wells for samples and assays.

SmartChip layout		Recommended volume and # of wells in source plate			
# of assays	# of samples	Assay source plate (volume/well)	# of wells per assay	Sample source plate (volume/well)	# of wells per sample
6	768	11.7 µl	8	10.0 µl	1
12	384	11.7 µl	4	10.0 µl	1
24	216	12.3 µl	2	10.0 µl	1
36	144	14.1 µl	1	10.0 µl	1
48	36	12.3 µl	1	14.1 µl	1
48	108	12.3 µl	1	10.0 µl	1
54	96	11.7 µl	1	10.0 µl	1
72	24	10.5 µl	1	30.6 µl	1
72	72	10.5 µl	1	10.5 µl	1
80	64	10.1 µl	1	10.9 µl	1

SmartChip layout		Recommended volume and # of wells in source plate			
# of assays	# of samples	Assay source plate (volume/well)	# of wells per assay	Sample source plate (volume/well)	# of wells per sample
96	54	10.0 µl	1	11.7 µl	1
120	42	10.0 µl	1	12.9 µl	1
144	36	10.0 µl	1	14.1 µl	1
172	30	10.0 µl	1	15.5 µl	1
216	24	10.0 µl	1	12.3 µl	2
248	20	10.0 µl	1	13.1 µl	2
296	16	10.0 µl	1	10.6 µl	4
384	12	10.0 µl	1	11.7 µl	4

Table 5. 384-well plate preparation for a 250 nl MyDesign chip: recommended volumes per well and number of wells for samples and assays.

SmartChip layout		Recommended volume and # of wells in source plate			
# of assays	# of samples	Assay source plate (volume/well)	# of wells per assay	Sample source plate (volume/well)	# of wells per sample
6	768	16.5 µl	8	10.0 µl	1
12	384	16.5 µl	4	10.0 µl	1
24	216	25.2 µl	2	10.0 µl	1
36	144	30.6 µl	1	10.5 µl	1
48	36	25.2 µl	1	46.7 µl	1
48	108	25.2 µl	1	11.7 µl	1
54	96	16.5 µl	1	12.3 µl	1
72	24	14.1 µl	1	61.2 µl	1
72	72	14.1 µl	1	14.1 µl	1
80	64	13.3 µl	1	14.9 µl	1
96	54	12.3 µl	1	16.5 µl	1
120	42	11.1 µl	1	42.3 µl	1
144	36	10.5 µl	1	30.6 µl	1
172	30	10.0 µl	1	31.1 µl	1
216	24	10.0 µl	1	25.2 µl	2
248	20	10.0 µl	1	30.4 µl	2
296	16	10.0 µl	1	14.3 µl	4
384	12	10.0 µl	1	16.5 µl	4

- Pipette your assay/PCR reagent mixtures into a 384-well source plate.

NOTE: For SmartChip MyDesign chips, the system is set up to include a single replicate of each reaction. To run replicates, use the same sample or assay/PCR reagent mixture for more than one sample or assay indicated in the source plate guides.

- After filling, seal the plate(s) with the adhesive film from the Nanodispenser 384-Well Source Plate and Seals. Sufficient pressure must be applied to activate the silicone adhesive

NOTES:

- The 384-Well Source Plate Seals included in Nanodispenser 384-Well Source Plate and Seals should be used for this. Please do not substitute with other sealing films.
- Sufficient pressure must be applied to activate the silicon adhesive. Multiple swipes of the sealing paddle in both directions are highly recommended.

- Centrifuge at 3,220g for 30 sec at room temperature.

B. Prepare Source Plates for Predispensed SmartChip Panels

- Prepare your samples and assays using the protocol provided with your SmartChip Custom Panels.
- Pipette the following mixture into a 384-well plate based on the type of experiment you will be running:
 - For gene expression analysis: SmartChip TB Green Gene Expression Master Mix/your cDNA
 - For genotyping: SmartChip Probe qPCR Master Mix/your DNA

To make it easier to manually pipette into the 384-well source plates, we suggest implementing one of the following:

- Use the *Source Plate Layout Builder* under the **User Utilities** in the qPCRStudio software to view the relevant source plate map(s).
- Use the source plate map(s) on the "Source Plate Layout" step of the job you will be running.

Tables 6 and 7 below show recommended volumes per well and number of wells for sample source plates targeting predispensed SmartChip Panel chips for each dispense volume supported for each layout.

Table 6. Sample source plates for gene expression analysis: predispensed SmartChip Panels.

SmartChip layout		Recommended volume and # of wells in source plate		
# of assays	# of samples	Dispense volume	Sample source plate (volume/well)	# of wells per sample
12	96	50 nl	10.0 µl	1
12	96	100 nl	11.7 µl	1
24	48	50 nl	11.7 µl	1
24	48	100 nl	16.5 µl	1
48	24	50 nl	11.7 µl	2
48	24	100 nl	16.5 µl	2
96	12	50 nl	11.7 µl	4
96	12	100 nl	16.5 µl	4
96	18	50 nl	30.2 µl	1
96	18	80 nl	47.5 µl	1
96	18	100 nl	49.5 µl	1

SmartChip layout		Recommended volume and # of wells in source plate		
# of assays	# of samples	Dispense volume	Sample source plate (volume/well)	# of wells per sample
96	54	50 nl	11.7 µl	1
96	54	80 nl	14.6 µl	1
96	54	100 nl	16.5 µl	1
192	6	50 nl	12.9 µl	7
192	6	100 nl	25.8 µl	7
384	3	50 nl	12.9 µl	14
384	3	100 nl	25.8 µl	14

Table 7. Sample source plates for SNP genotyping: predispensed SmartChip Panels.

SmartChip layout		Recommended volume and # of wells in source plate		
# of assays	# of samples	Dispense volume	Sample source plate (volume/well)	# of wells per sample
12	384	50 nl	10.0 µl	1
12	384	100 nl	10.0 µl	1
24	216	50 nl	10.0 µl	1
24	216	100 nl	10.0 µl	1
36	144	50 nl	10.0 µl	1
36	144	100 nl	10.5 µl	1
48	108	50 nl	10.0 µl	1
48	108	100 nl	11.7 µl	1
96	18	50 nl	30.2 µl	1
96	18	80 nl	47.5 µl	1
96	18	100 nl	49.5 µl	1
96	54	50 nl	11.7 µl	1
96	54	80 nl	14.6 µl	1
96	54	100 nl	16.5 µl	1
120	42	50 nl	12.9 µl	1
120	42	100 nl	29.4 µl	1
192	24	50 nl	11.7 µl	2
192	24	100 nl	16.5 µl	2
384	12	50 nl	11.7 µl	4
384	12	100 nl	16.5 µl	4

3. After filling, seal the plate(s) with the adhesive film from the Nanodispenser 384-Well Source Plate and Seals. Sufficient pressure must be applied to activate the silicone adhesive.
4. Centrifuge at 3,220g for 30 sec at room temperature.

C. Prepare Source Plates Using Automation

If you plan to dispense from a 384-well source plate containing assays and/or samples filled by an automated pipette, please refer to [Appendix E, Section B](#) for best practices and sample plate maps.

VIII. Protocol: Configure qPCRStudio Software For Dispense

A. Set Up Your Job

Before dispensing samples and/or assays, you must choose an application and set up the parameters for your "job" within the qPCRStudio software. This is Step 1 in the guided dispense workflow.

1. Click on the desired application under the "Start a new job" heading on the qPCRStudio home page. The *Job Setup* view will appear (Figure 22). More information about creating custom applications can be found in [Appendix A](#).

The screenshot shows the 'Job Setup' window in the qPCRStudio software. The window title is 'SmartChip ND™ qPCRStudio™'. The top right corner shows 'SYSTEM READY', the Takara logo, and the date/time 'Nov 20 11:04 AM'. The left sidebar contains 'Job information', 'App edit', 'App: GT Training Kit', 'SmartChip information', 'Dispense layout', and 'Job notes'. The main area is titled 'Job Setup' and contains the following fields:

- Customer name (with a grid icon) and Customer (optional)
- User name (with a person icon) and User (optional)
- Chip ID: Scan chip barcode (with a red asterisk indicating it is required)
- Job name: New job (with Job name below it)
- Source plate ID: Scan source barcode (with a 'Skip' checkbox)
- Reagents kit ID: Scan reagents barcode (with a 'Skip' checkbox)

At the bottom, a progress bar shows 'Dispense planning in progress...' with four steps: 1 Setup, 2 Layout, 3 Dispense, and 4 Export. A red asterisk at the bottom right indicates '* Required entries'.

Figure 22. The *Job Setup* window.

2. In the *Job Setup* window, enter your job information.
 - a. Enter the job name and the SmartChip Chip ID either using the barcode scanner, or manually entering the ID. When this information is entered under *Job Setup*, it will automatically fill the information under "Job Information" and "SmartChip Information" on the left hand side of the screen.
 - b. Enter relevant information in the "User" field.
 - c. (Optional) Enter relevant information in the "Customer" field.
 - d. (Optional) Enter the Source plate ID
 - e. (Optional) Enter the Reagents kit ID

- Determine if the information under "SmartChip Information" or "Dispense Layout" needs to be edited. To edit the information under "SmartChip Information" or "Dispense Layout", toggle the "App Edit" switch to "on" under "Job Information" (Figure 23).

NOTE: To be able to choose different options from the dropdown menus under "SmartChip Information" or "Dispense Layout" the "App Edit" button (on the left navigation bar) must be toggled to "on" as in Figure 23.

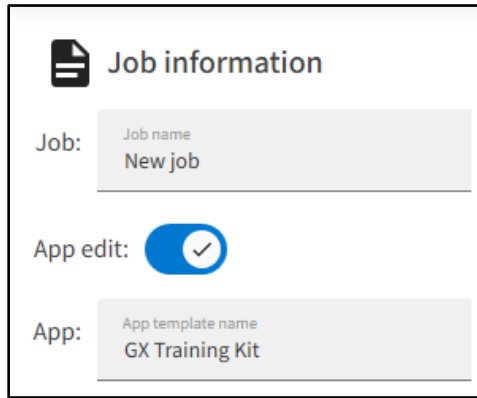


Figure 23. The "App Edit" switch is toggled "on" under the "Job Information" heading.

- When all required job information is entered, the guided dispense workflow at the bottom of the screen will display "Dispense planning complete". Click the large blue arrow to proceed (Figure 24).

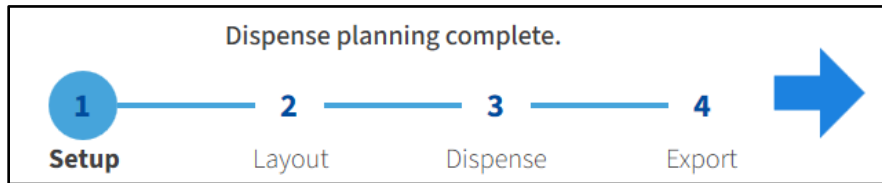


Figure 24. Guided dispense workflow at the bottom of the Job Setup window when dispense planning is complete.

B. Confirm the Sample and/or Assay Source Plate Layouts

Upon moving to Step 2 in the guided dispense workflow (Layout), you will have a chance to edit the sample and/or assay source plate, depending on the application.

- To change between the sample source plate layout and the assay source plate layout, use the "Source" dropdown menu in the upper left corner of the screen (Figure 25). If your application involves only loading a sample plate and not an assay plate, this dropdown menu will not be displayed.

ID	Sample	Assay	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	UHR1	UHR1	Lung3	Lung3																				
B	UHR1	UHR1	Lung3	Lung3																				
C	UHR2	UHR2	Placenta1	Placenta1																				
D	UHR2	UHR2	Placenta1	Placenta1																				
E	UHR3	UHR3	Placenta2	Placenta2																				
F	UHR3	UHR3	Placenta2	Placenta2																				
G	Brain1	Brain1	Placenta3	Placenta3																				
H	Brain1	Brain1	Placenta3	Placenta3																				
I	Brain2	Brain2	NTC	NTC																				
J	Brain2	Brain2	NTC	NTC																				
K	Brain3	Brain3	EMPTY	EMPTY																				
L	Brain3	Brain3																						
M	Lung1	Lung1																						
N	Lung1	Lung1																						
O	Lung2	Lung2																						
P	Lung2	Lung2																						

Figure 25. The Source dropdown menu.

- Values within the wells of the source plate can be edited by clicking on the desired well. Right-clicking on a well gives you the option to cut, copy, or paste contents of a previously copied well. By clicking and dragging the mouse, multiple wells can also be selected at once, right-clicked on, and cut, copied, or pasted from/to using Microsoft Excel. For faster operation, the keyboard shortcuts for copy (Ctrl + C) and paste (Ctrl + V) can also be used.
- Once the sample/assay source plate(s) are confirmed, click the large blue forward arrow (Figure 26) to move to the "Dispense" step.

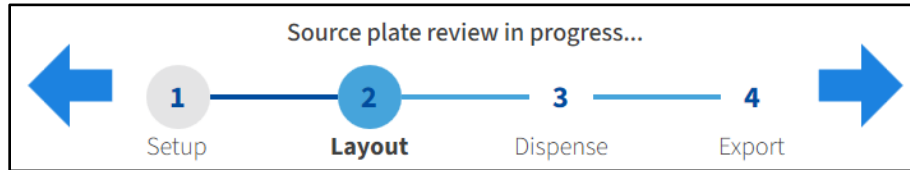


Figure 26. Guided dispense workflow at the bottom of the *Layout* window.

IX. Protocol: Dispensing Using the SmartChip ND Dispenser

A. Place the Chip in the SmartChip ND Dispenser

1. Once on the *Dispense Run* step of the job, wait for "Chip T" to reach "Dewpoint T", at which point the SmartChip ND dispenser door will unlock and allow you to load your consumables.
2. Visually inspect the chip nest and clean it if there is any debris (Figure 27).

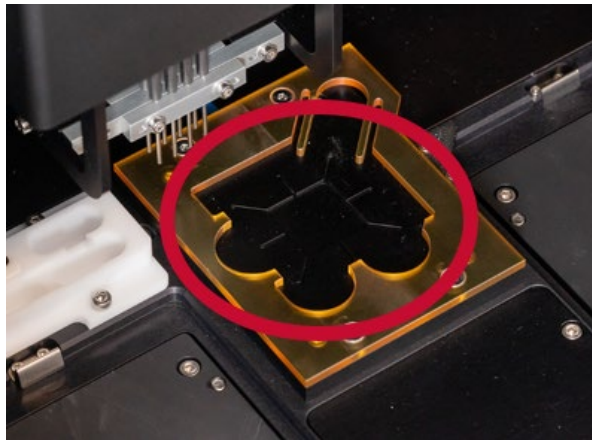


Figure 27. Area of the chip nest to inspect and clean prior to loading a chip.

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

The parts of the chip nest referenced in the procedure below are identified in Figure 28.

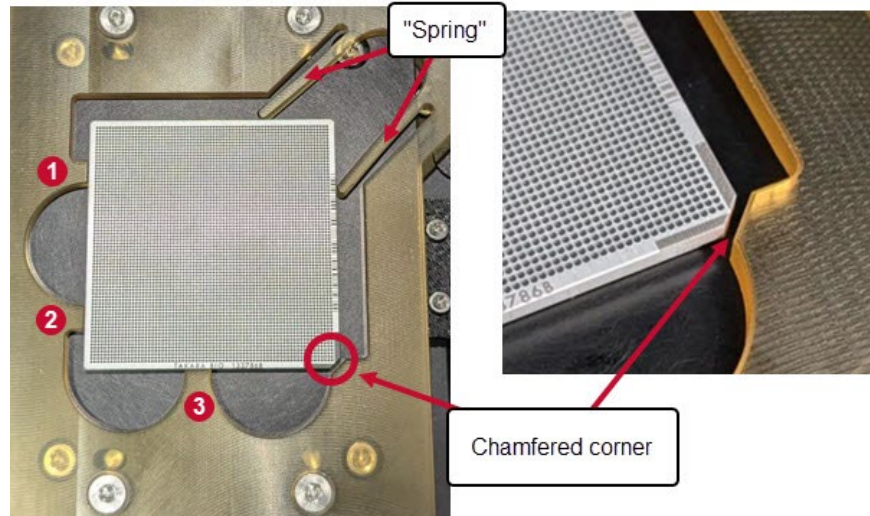


Figure 28. The parts of the chip nest identified. The "spring" is in the top right corner. The chamfered corner of the chip will be placed against the bottom right corner. On the bottom and left edge are three alignment posts protruding into the chip nest well. When inserted correctly, the spring, chamfered corner, and alignment posts ensure the chip is seated correctly in the nest.

- a. Grip the chip in such a way that the film does not become separated from the surface of the chip. The chamfered corner of the chip should be oriented on the lower right corner of the chip nest (Figure 29).
- b. Angle the chip so the top right corner of the chip nest (the "spring") is engaged first. To do this, the right index finger should press slightly on the top right corner as shown in Figure 29.

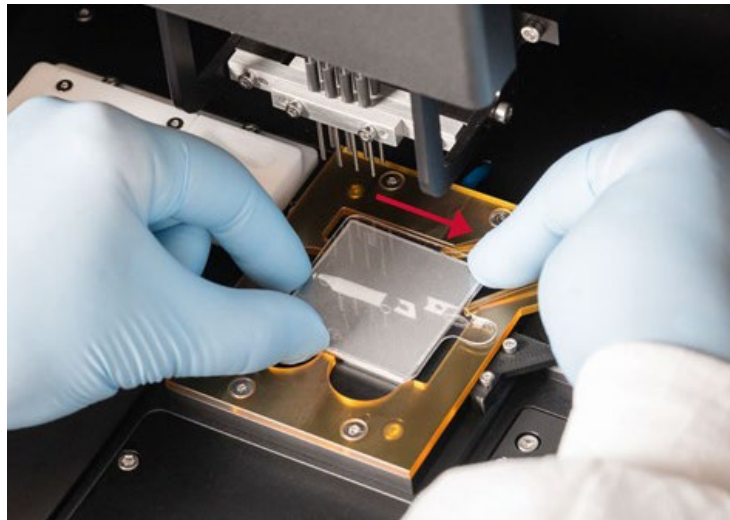


Figure 29. Engaging the spring on the chip nest. Place the right index finger between the two prongs to gently spread them apart so the chip can be fit between them.

- c. Guide the chip towards the spring so that it can be engaged (Figure 29, red arrow). The chip should be pushed far enough to the upper right that it can be placed so that the three alignment posts (Figure 29) will press into each side of the chip.
- d. Lower the chip into the nest. The chip can be pressed down on top of the film to ensure it is seated but be careful not to move the film when doing so.

- e. Visually check to make sure the bottom face of the chip is flat against the black anodized aluminum surface, and the top face of the chip is flush with the surface of the chip nest (Figure 30).

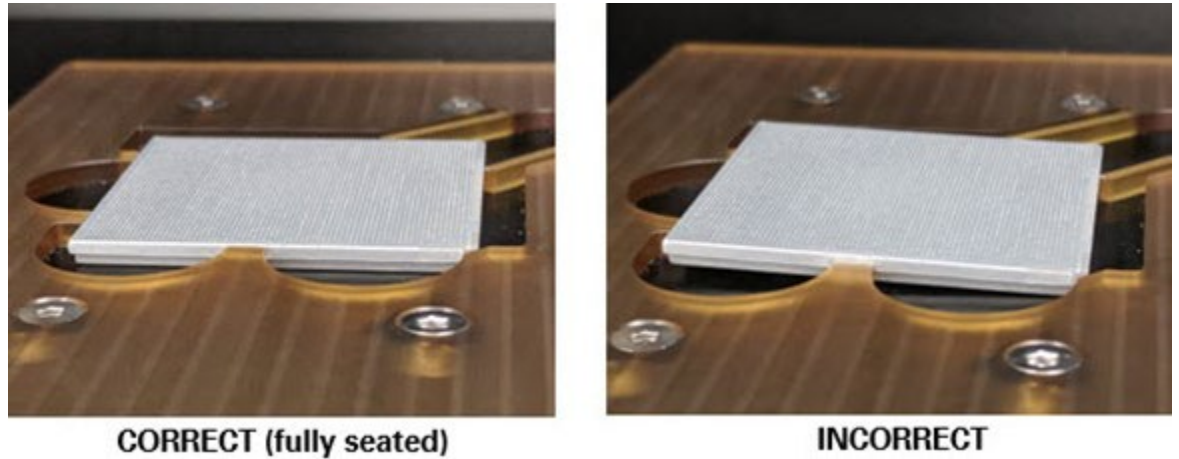


Figure 30. Examples of a fully-seated chip and a chip incorrectly inserted into the chip nest. It is important to check that the chip is fully seated before dispensation to avoid interference with the dispenser tips.

4. Remove the seal from the chip.
 - a. With one finger pressed in the middle of the film (Figure 31), grasp the tab of the film between two fingers of the other hand.
 - b. Peel the film from the chip. To avoid pulling the chip out of the chip nest, keep pressing down on the film with one finger until the film is almost fully removed before releasing the pressure.

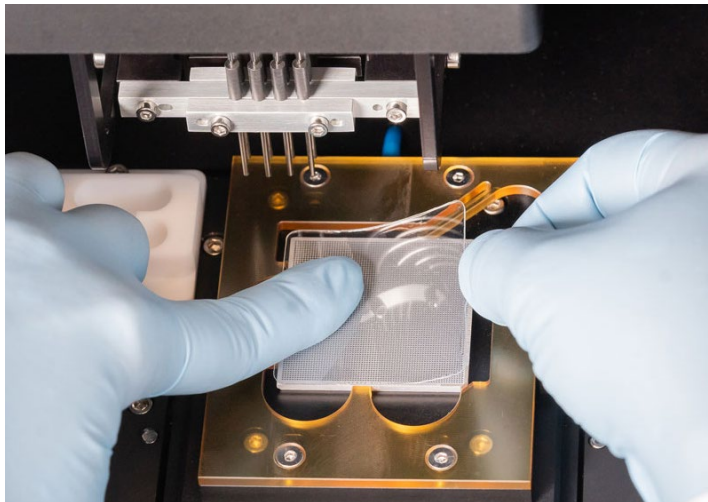


Figure 31. Demonstration of proper chip film removal. One finger (left) should remain pressing down on the film as long as possible while the film is being peeled (right).

5. Check the "SmartChip load confirmed" box in the *Dispense* window of the qPCRStudio software (Figure 32).

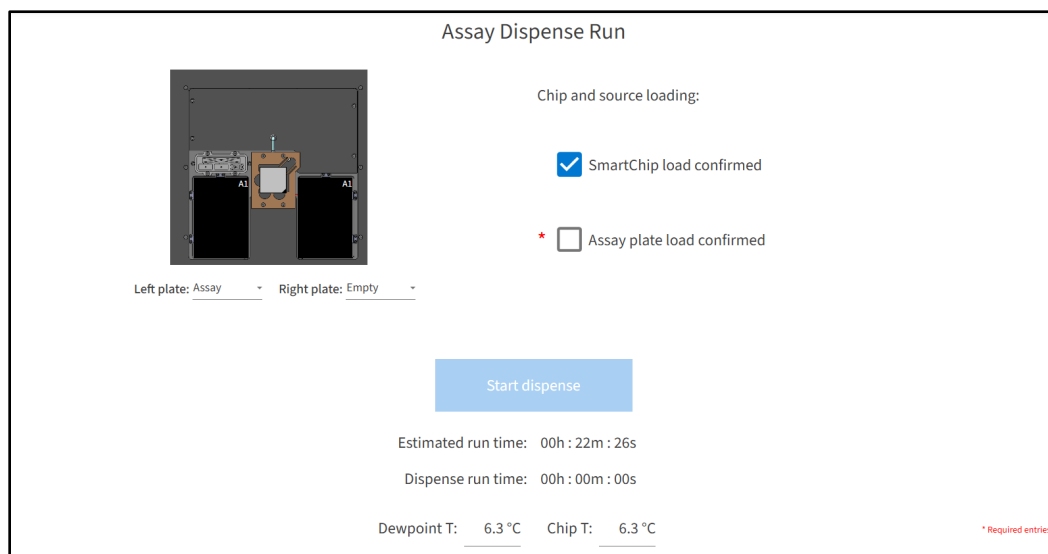


Figure 32. Checking the "SmartChip load confirmed" box in the qPCRStudio software.

B. Place the Source and/or Assay Plate(s) in the SmartChip ND Dispenser

1. If you haven't already done so, centrifuge the source plate at 3,220g for 30 sec at 20–25°C.
2. Remove the Nanodispenser 384-Well Source Plate and Seals adhesive film from the source plate.
3. Depending on the application, place the 384-well sample plate(s) and/or assay plate(s) on either left or right plate nest with the A1 well positioned in the upper left corner of the plate nest.

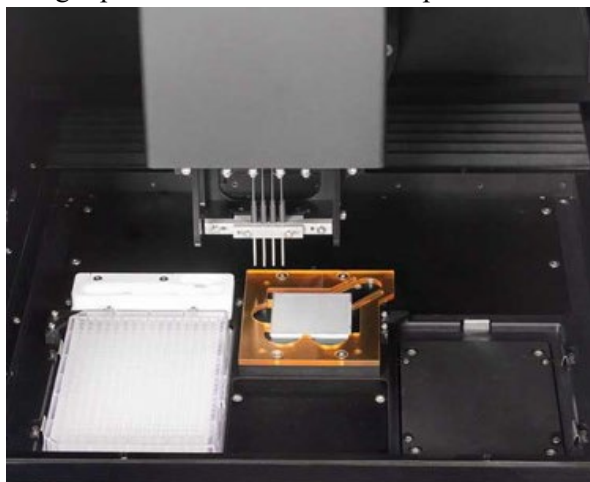


Figure 33. Sample source plate on left-side SmartChip ND dispenser plate nest.

4. Choose the correct source plate(s) and/or assay plate(s) positions in the "Left plate" and "Right plate" dropdown menus in the *Dispense* window (Figure 34).



IMPORTANT: Confirm that the source or assay plate position chosen in the software matches where the plate was loaded in the SmartChip ND dispenser.

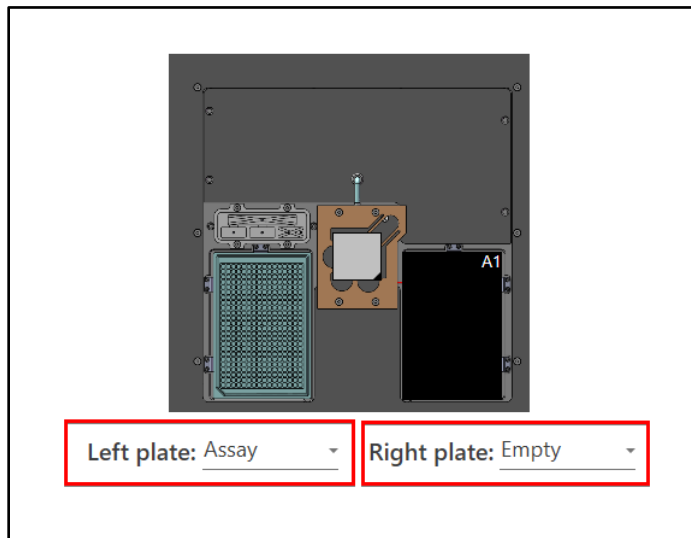


Figure 34. Dropdown menus for "Left plate" and "Right plate" in the *Dispense* window.

5. Gently close the front access panels of the SmartChip ND dispenser; both doors should remain closed during the dispensing process.
6. Check the "Assay plate load confirmed" or "Sample plate load confirmed" box in the *Dispense* window of the qPCRStudio software (Figure 35).

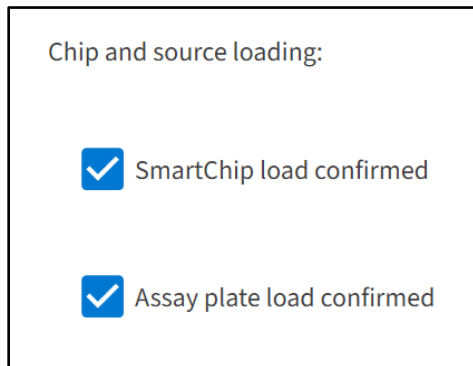


Figure 35. Checking the "Sample plate load confirmed"/"Assay plate load confirmed" box.

C. Dispense into the Nanowell Chip Using the SmartChip ND

1. Select [Start Dispense] (Figure 36) to begin the dispense process.

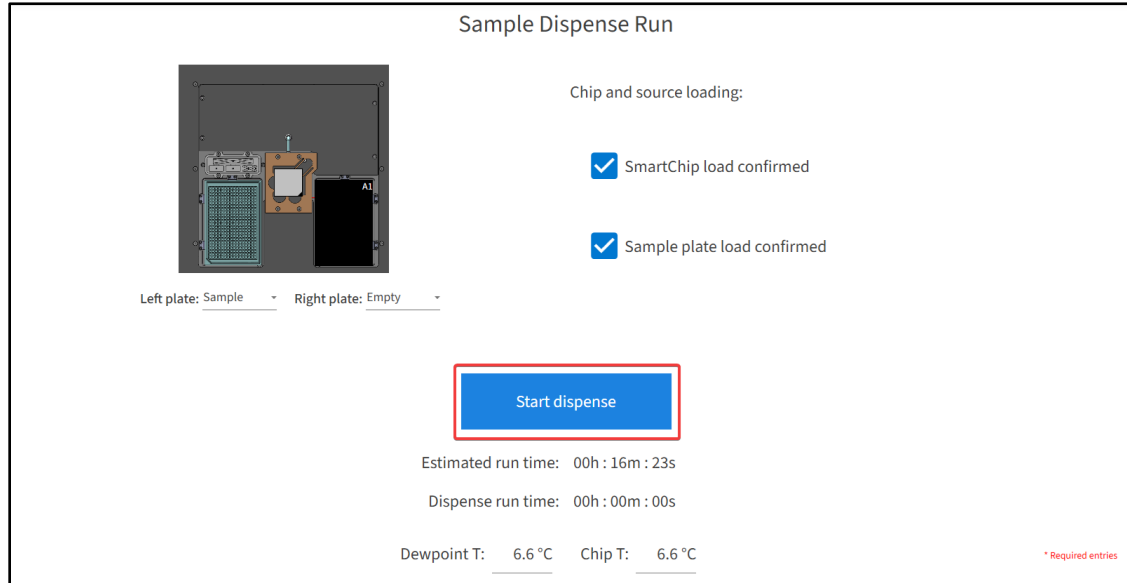


Figure 36. *Sample Dispense Run* page with [Start dispense] button indicated by the red box.

2. Once the dispense is initiated the software will automatically start the dispense run timer and complete all dispense steps (Figure 37).

NOTE: The "Estimated run time" indicates the total time expected for the dispense step to progress to completion. The actual elapsed time may vary slightly from the estimated time; this is normal.

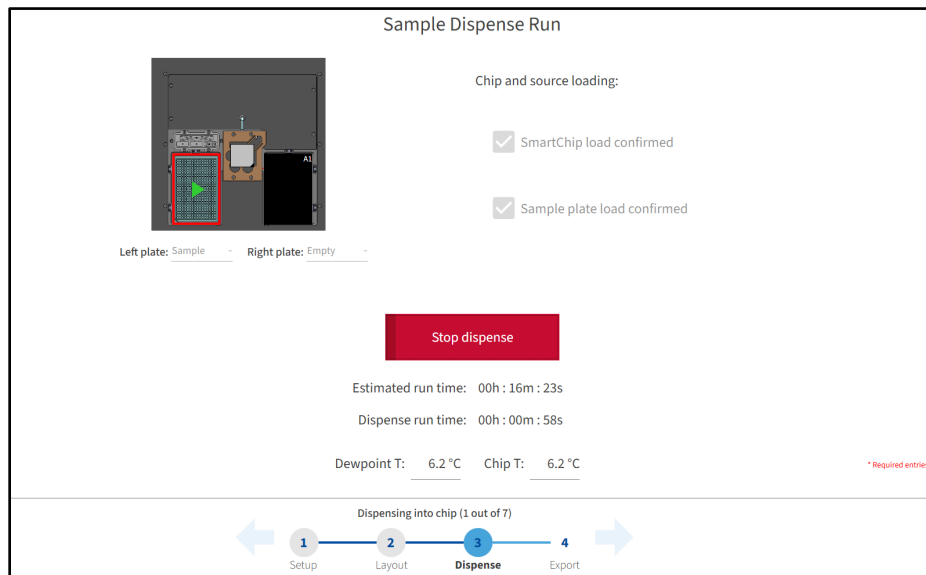


Figure 37. *Dispense in progress.*

The dispense can be aborted, if necessary, by clicking the [Stop dispense] button. You will be asked to confirm your choice to stop the dispense before the dispense is aborted. Once the abort is completed, the message shown in Figure 38 will display.



IMPORTANT: Once a dispense has been aborted, it cannot be resumed.

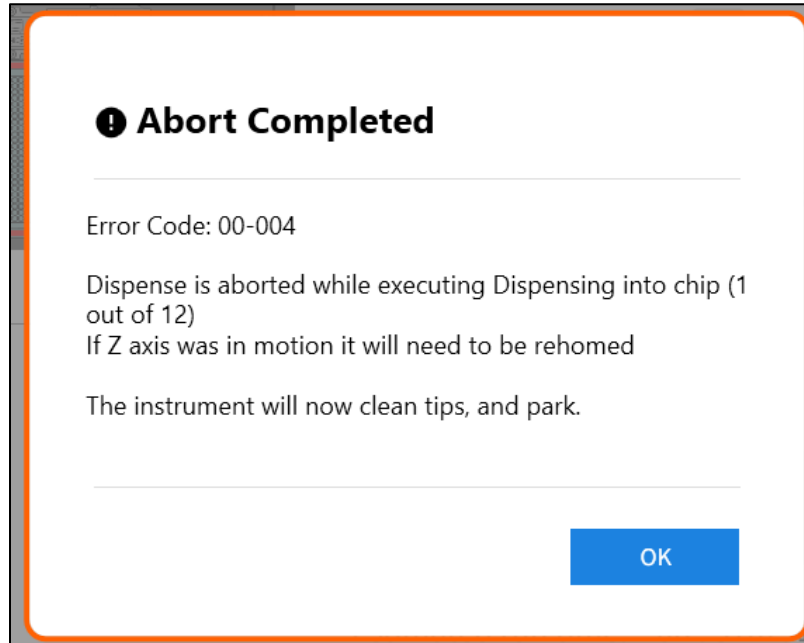


Figure 38. Dispense run aborted.

3. If the application involves more than one dispense, after the first dispense is complete the software will notify the user of its completion and prompt them to prepare for the next dispense step (Figure 39). Select [OK] to continue.

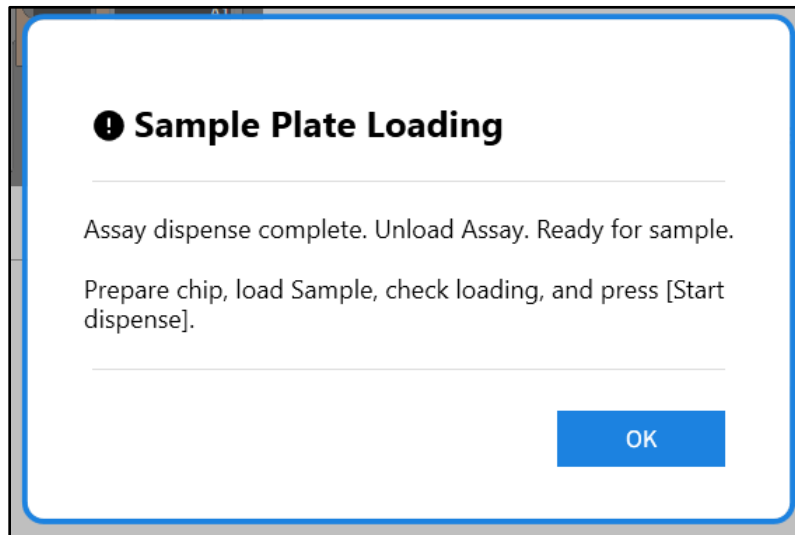


Figure 39. The user is notified when the first dispense is completed.

4. The software will then prompt the user to confirm chip and source or assay plate loading and positioning for the next dispense. Refer to [Section XI.B](#) to complete this part of the workflow for the next dispense step.
5. Once the next dispense step is completed, select the large blue right arrow in the bottom navigation bar (Figure 40) to proceed to the *Dispense Run Summary* window.

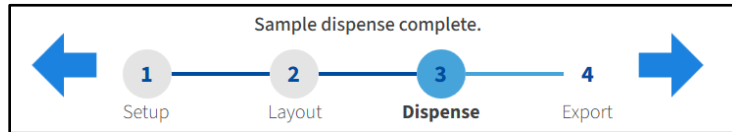


Figure 40. Guided dispense workflow when the dispense is complete

D. Generate and Save Your SmartChip ND Layout File

The SmartChip ND Layout file is needed by the SmartChip ND qPCR Cyclers Software to run your sample and assays on the SmartChip ND cycler. The extension on the SmartChip ND Layout file indicates the type of information it contains:

- `<filename>.pd` : contains only sample information. For use with a predisposed SmartChip Panel.
- `<filename>.md`: contains sample and PCR assay information. For use with a SmartChip MyDesign chip.

After completing all dispenses, the SmartChip Layout File is automatically generated and saved in the default `C:\ProgramData\takara\qPCRStudio\Jobs\<ChipID>\` directory. By default, the SmartChip Layout File name includes the job name and SmartChip ID.

Alternatively, the saved SmartChip Layout file can be exported and saved to another location by clicking the export button (outlined in red) on the *Dispense summary* window (Figure 41).

1. Choose a location to save the file.
2. Choose a file name.
3. Click [Save].

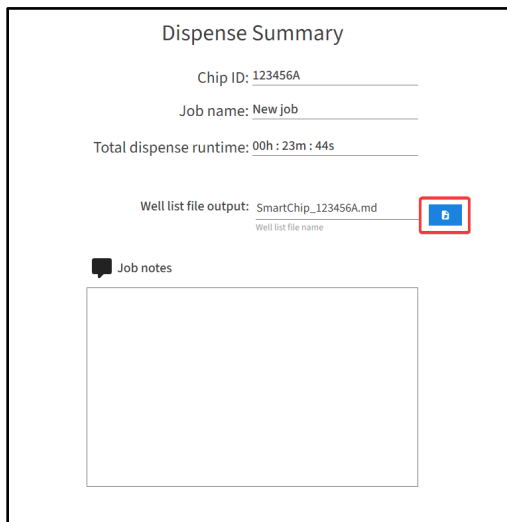


Figure 41. Exporting the .md or .pd file from the *Dispense summary* window.

E. Blot, Seal, and Centrifuge the Chip

1. After dispensing is complete, promptly blot the chip for 2 sec.
 - a. Place the chip with the wells facing up on a clean lab wipe.

- b. 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.
 - c. Pick up the Nanodispenser Chip Blotter 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.
 - d. Let the blotter rest on top of the blotting paper for exactly 2 sec without pressing down—the weight of the blotter is sufficient for adequate blotting.
 - e. Remove the blotter and then gently remove the Blotting Paper and dispose of it in a biohazard container.
2. Quickly seal the loaded chip with the appropriate film.

Table 8. Selecting the correct sealing film to use on the nanowell chip for each SmartChip type.

Chip type	Dispense type	Film type
Predispensed Custom Panel	Sample dispense	Cycler Sealing and Pressure Film
MyDesign chip	Sample dispense	Nanodispenser Chip Intermediate Film*
	Assay dispense	Cycler Sealing and Pressure Film*

*Film is included in the SmartChip MyDesign Kit.

The steps below illustrate the application of the Cycler Sealing and Pressure Film.

NOTE: Other than how the backing is peeled from the film, application of the intermediate film will be performed similarly.

- a. Remove the backing from the adhesive film.
Remove the backing only from the center of the film (Figure 42). Leave the blue backing around the periphery of the film in place.



Figure 42. Removing the backing from the Cycler Sealing and Pressure Film.

- b. Center the square adhesive portion of the film over the chip and press into place. The film must cover the chip entirely but does not need to align perfectly.
Place the film in the orientation shown below (Figure 43).

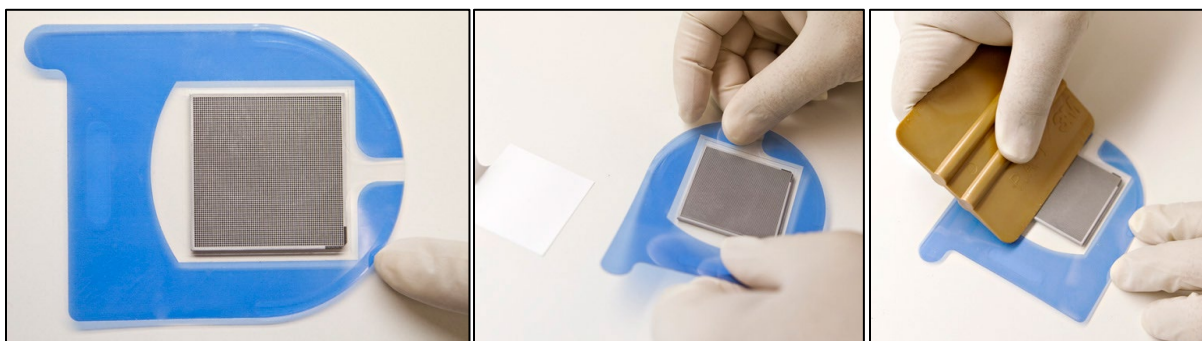


Figure 43. Placing the Cyclor Sealing and Pressure Film on the chip.



IMPORTANT: Be sure to place the Cyclor Sealing and Pressure Film on the chip in the orientation shown in the photos above. In Figure 43, the chamfered corner of the chip is at the bottom right in the left-most photo; our scientist is pointing to it.

- c. Use your fingers to press and smooth the seal, starting at the center of the chip and moving toward the edges.
- d. To make sure that a strong seal is achieved, apply pressure with the seal applicator from the center of the chip outward. Repeat eight times, rotating the chip 45 degrees each time to seal all edges and corners.

NOTE: Small bubbles on the periphery of the chip will not cause problems, but bubbles on top of the nanowells may interfere with gathering information on the wells under the bubbles. It is recommended to continue the procedure, as it will only affect a small area of the chip. Do NOT try to remove and reapply the film to correct it—this will likely cause cross-contamination between wells.

3. Using the Nanodispenser Chip Centrifuge Spinner, centrifuge the chip at 3,220g for 5 minutes at 4°C as described below.
 - a. Place your chip(s) in the Nanodispenser Chip Centrifuge Spinner(s).
 - b. Counterbalance with either a second Nanodispenser Chip Centrifuge Spinner and chip or with a Nanodispenser Chip Centrifuge Balance (Figure 44).

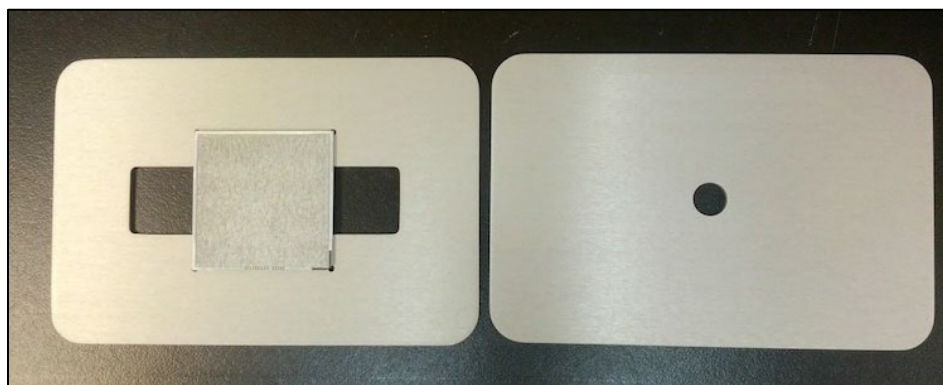


Figure 44. Nanodispenser Chip Centrifuge Spinner and chip (left) and Nanodispenser Chip Centrifuge Balance (right).

- Determine the next step of the protocol by following the definitions in Table 9.

Table 9. Determining the next step of the protocol after dispense.

Chip type	Dispense type	Next step
SmartChip Panel	Sample dispense	Run your reactions on the SmartChip ND cycler*
MyDesign chip	Sample dispense	Repeat Sections VIII.A–D for assay dispense†
	Assay dispense	Run your reactions on the SmartChip ND cycler*

*Refer to the [SmartChip ND Real-Time PCR Cycler and Software User Manual](#)

†Dispensed MyDesign chips can optionally be stored for up to 4 hr at 4°C. After storage and immediately prior to use, allow sealed chips to warm to room temperature and then spin for 5 min at 3,220g.

F. Run PCRs on the SmartChip ND Real-Time PCR Cycler

See the [SmartChip ND Real-Time PCR Cycler and Software User Manual](#) for complete instructions.

G. Discard the 384-Well Source Plate

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

X. Dispenser 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. Cleaning the SmartChip ND Dispensing Platform

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

- If there is a 384-Well Source Plate in the plate nest, remove and properly dispose of it.
- Visually inspect the dispensing platform for any debris. Moisten a cloth with 70% isopropyl alcohol then wipe down the dispensing platform (chip nest, source plate nest, trough, and the areas on the base platform immediately surrounding them). Refer to Figure 45.



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 Field Support at field_support@takarabio.com.



WARNING: Ensure that no liquid drips below the platform. Moistening the cleaning cloth before wiping the dispensing platform is recommended; do not spray the dispensing platform directly.

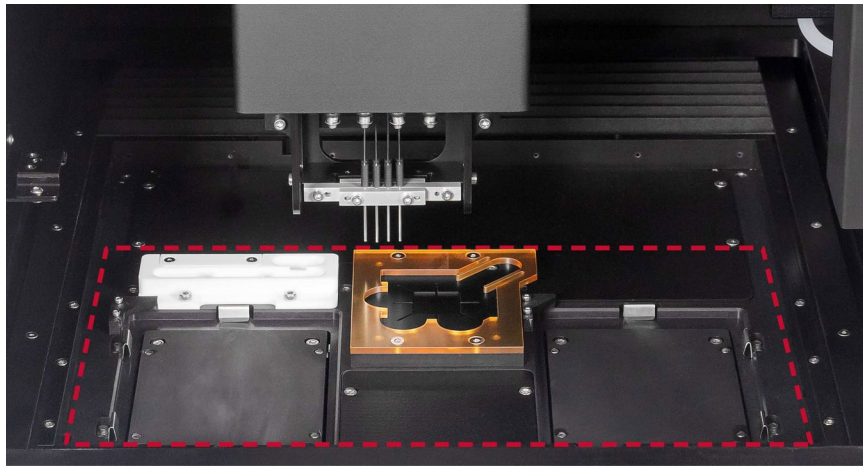


Figure 45. Area of the SmartChip ND dispenser to clean, indicated by the red, dashed rectangle.

B. Daily Maintenance

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

1. Disconnecting and Reconnecting the Tubing Lines from the Caps

The procedure below describes the process of disconnecting the tubing fitting from the bottle caps for the water source bottle, humidifier source bottle, and waste container, as demonstrated in Figure 46.

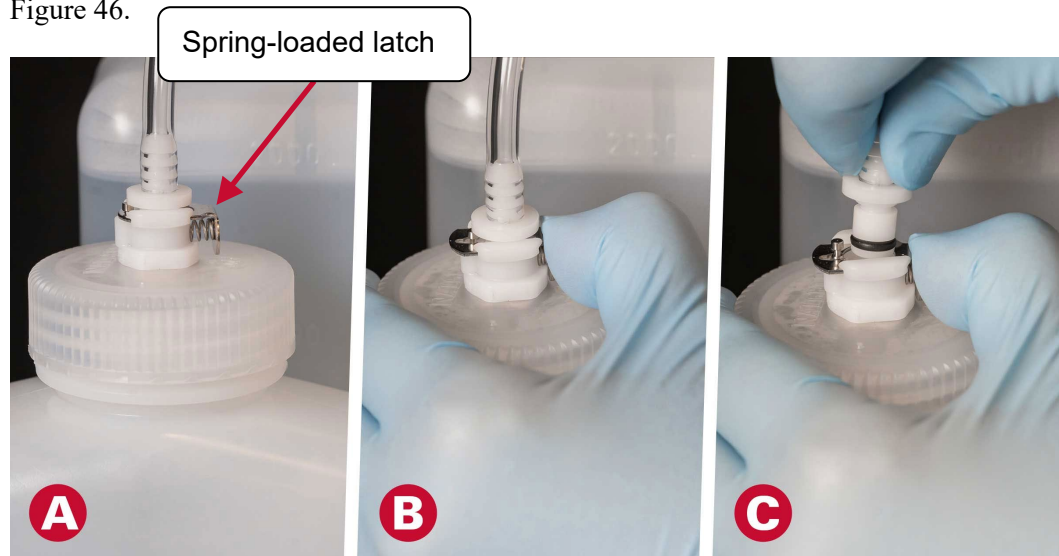


Figure 46. Visual aid for how to remove the tubing connector from the water source bottle, humidifier source bottle, and the waste container.

To disconnect the tubing from the bottle cap:

1. Using your thumb, push in the spring-loaded latch at the side of the connector (Figure 46, Panel A). Figure 46, Panel B shows how the latch will look when it's properly pressed.
2. While holding in the latch, grasp the tubing at its connector and pull up to remove the connector from the bottle cap (Figure 46, Panel C).
3. Release the pressure on the latch.
4. Remove the bottle from the instrument. The tubing will remain in place attached to the instrument.

To reconnect the tubing to the bottle:

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

2. Water Source Bottle Refill Procedure

It is recommended to refill the bottle with deionized, filtered water if the water level is <800 ml.

1. Fill a clean secondary container with deionized, filtered water.
2. Disconnect the humidifier bottle via the fitting at the top of the bottle. To make room to remove the water source bottle, place the humidifier bottle next to the machine on the benchtop.
3. Disconnect the water source bottle and remove the bottle from the instrument through the gap made from removing the humidifier bottle. Place the bottle on the benchtop next to the machine.



Figure 47. Removing the water bottle from the SmartChip ND dispenser.

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

Optional: If there is concern about bubbles in the water source tubing, go to **User Utilities** and click [Water prime] to prime tubing with water and flush out bubbles.

3. Humidifier Source Bottle Refill Procedure

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

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



Figure 48. Visualization of the recommended fill volume of the humidifier source bottle. The water level should be below where the bottle begins to curve to the top.

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

4. Bleach Source Bottle Refill Procedure

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

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

Follow these steps to replace the solution:

1. Prepare a secondary bottle with 500 ml of fresh 0.3% reagent-grade sodium hypochlorite.
2. Disconnect the bleach bottle via the fitting at the top of the bottle (Figure 49) by twisting the fitting to unlock it.
3. Pull the fitting out and place the bottle next to the machine on the benchtop.

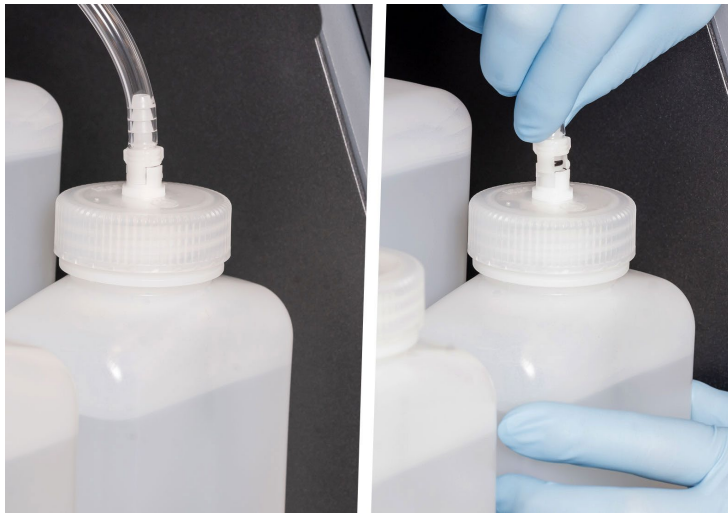


Figure 49. Disconnecting the bleach bottle via the line fitting.

4. Remove the cap from the bleach bottle and properly dispose of any residual sodium hypochlorite solution.
5. Place the bottle back onto the bench, leaving the cap ajar. Fresh solution can be poured through this gap.
6. Refill the bleach bottle with the fresh sodium hypochlorite solution prepared in Step 1.
7. Screw the cap of the bleach bottle back on.
8. Place the bleach source bottle back in its proper position on the bleach scale and connect the tubing labeled "Bleach" onto the bleach bottle cap fitting.
9. Go to **User Utilities** and click [Bleach prime] to prime tubing with fresh 0.3% sodium hypochlorite solution. See [Appendix A, Section D](#) for more details.

5. Emptying the Waste Container

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

1. Locate the cap on the waste container and press the quick-release buttons to release each tube one-by-one from the cap (Figure 50).



Figure 50. Disconnecting the tubing from the waste container cap. The red arrow is pointing in the image to the tubing connectors which attach to the cap.

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

C. Shutdown Procedure (<1 month)

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

1. Check that all nanowell chips and source plates have been removed from the stage module.
2. Follow the SmartChip ND dispenser cleaning procedure described above in [Section X.A.](#)
3. Disconnect the water, humidifier, and bleach bottles following the instructions above in [Section X.B.](#)
4. In **User Utilities**, run the [Bleach prime] ([Appendix A, Section D](#)) followed by the [Water prime] ([Appendix A, Section D](#)).
5. Dispose the contents of the water, humidifier, and bleach bottles.
6. Reconnect the water, humidifier, and bleach bottles.
7. Safely dispose of the contents of the waste bottle using the procedure from [Section B.5.](#)

D. System Shutdown Procedure (>1 month)

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

8. Exit the qPCRStudio software (see [Section V.E.](#)).
9. Turn off the SmartChip ND by toggling the power switch on back of the system.

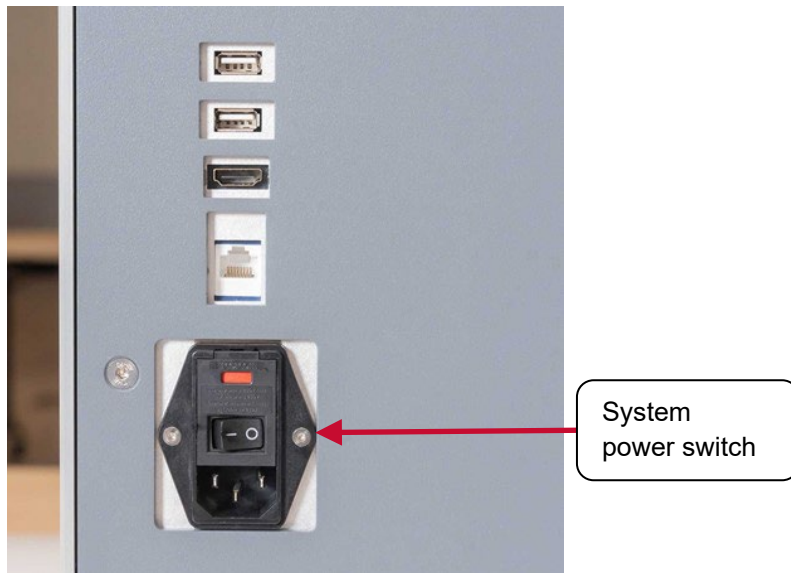


Figure 51. Location of the system power switch.

E. Restarting the SmartChip ND Dispenser from a Complete Shutdown


1. Turn on the SmartChip ND by toggling the power switch in the back of the system.
2. Refill humidifier, water, and bleach source bottles per [Section X.B](#) (1–3), "Daily Maintenance".
3. Start up the qPCRStudio software by touching the touch power button for >1 sec.
4. Run the Daily Warmup per directions in the qPCRStudio Software.

F. Annual Preventative Maintenance

The SmartChip ND should be examined and calibrated every six months by a Takara Bio service engineer.

G. Troubleshooting

Table 10. Troubleshooting guide.

Problem	Possible Explanation	Solution
<p>Water is seen under the front right corner of the instrument.</p> 	<p>In the rare occasion that there is a leak in the humidifier module, the system has been designed to route the fluid to a safe location at the front right side of the instrument. This may create a small puddle of water in the location of the circle on the image to the left.</p>	<p>Stop using the instrument and contact your authorized Takara Bio service technician or field_support@takarabio.com.</p>

Appendix A. Additional qPCRStudio Software Functions

A. Resuming Previous Jobs

The bottom section of the home screen presents the user with options to resume previous jobs. This section will become populated with previously started jobs as users utilize the software. Selecting a previous job to resume will take the user to the last step reached in the guided dispense workflow for that chosen job, allowing them to pick up where they previously left off.

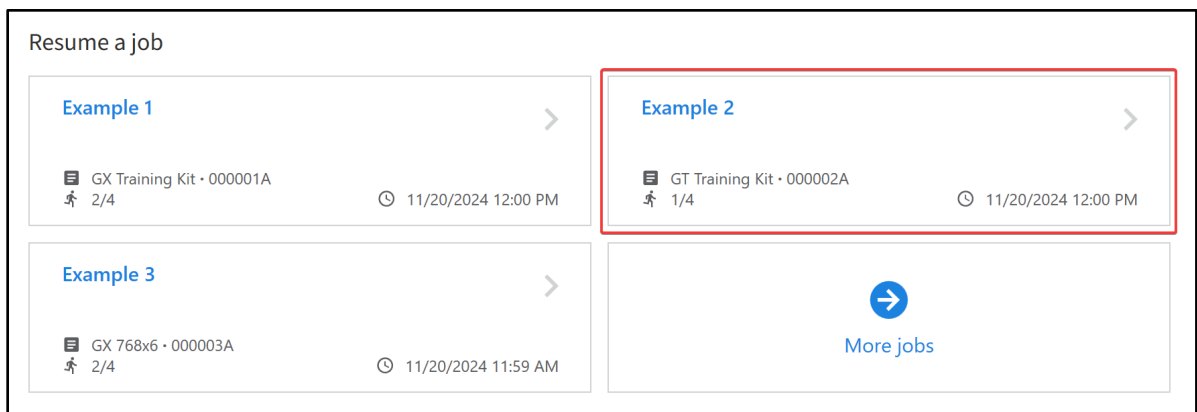


Figure 52. The "Resume a job" section on the qPCRStudio home screen.

B. Creating Custom Applications

Custom applications with user-selectable settings can be created by navigating to the top-right menu button and clicking [App Manager] to bring up the *App Manager* window. Within the *App Manager*

window, choose the options available to create a custom application. Under the options for “SmartChip,” the mode can be changed between gene expression and genotyping, the chip format can be changed between MyDesign and predispensed chips, and dispense volume can also be adjusted based on the available options for the chosen dispense layout. After saving the custom application, the application can be selected within the list of applications on the home screen under the "Start a job" heading.

C. Creating Custom Source Plate Layouts

Custom assay or sample plate layouts can be created by navigating to the top-right menu button and clicking [Source Layout Builder] to bring up the *Source Plate Layout Builder* window. After the sample or assay information is entered in the *Source Plate Layout Builder*, the plate template can be exported to Excel (.XLSX) format for further editing, or the layout can be saved as a .txt file for use in the *App Manager* to create a custom application.

.XLSX format plate template files can also be imported to the *Source Plate Layout Builder*, and groups of Excel cell values can be copied and pasted directly to or from the *Source Plate Layout Builder* by clicking and dragging to select multiple source plate wells and right-clicking to bring up cut, copy, and paste options. For faster operation, the keyboard shortcuts for copy (Ctrl + C) and paste (Ctrl + V) can also be used.

D. User Utilities

Device-related user utilities can be accessed by navigating to the top-right menu button and clicking the **User Utilities** (Figure 53).

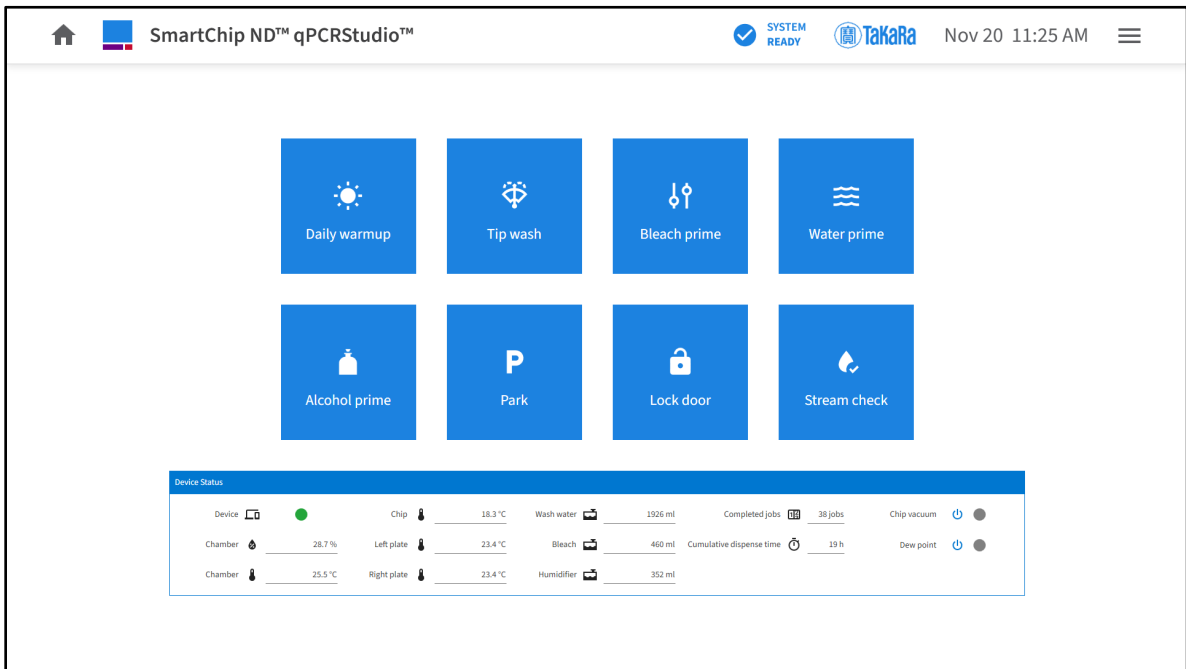


Figure 53. The *User Utilities* window in the qPCRStudio software.

1. Daily Warmup

The Daily warmup:

- Initializes all hardware,
- Primes the water tubing
- Primes the bleach tubing,
- Performs an alcohol tip prime
- Performs a tip wash procedure

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

The reminder to run the daily warmup will be displayed on the Action Center once per day if the daily warmup has not been run for the day but can also be re-run from the User Utilities using the [Daily warmup] button.

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

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

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

2. Tip Wash

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

3. Bleach Prime

When the button is pressed, bleach flows from the bleach bottle through the fluidic line to the tip wash trough for a preset amount of time.

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

4. Water Prime

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

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

5. Alcohol Prime

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

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

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

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

6. Park

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

7. Lock/Unlock Door

This toggle button manually locks or unlocks the door.

8. Stream Check

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

Actions to be taken prior to running stream check:

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



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

3. Observe the streams coming out of the tips; they should be relatively straight in relation to the tips and not changing directions. If the streams do not look straight, perform an alcohol prime to flush the tips out.
4. After the alcohol prime, re-run the stream check to determine if the issue was addressed. If the problem persists, please contact your authorized Takara Bio service technician or Field Support at field_support@takarabio.com.

Appendix B. qPCRStudio Software Alerts

If the SmartChip ND dispenser or qPCRStudio Software does not respond as desired or a warning is displayed, please attempt to rectify the problem using the instructions below, or following the instructions on the screen. If you cannot solve the problem, please contact Field Support at field_support@takarabio.com.

A. Home Bar Alert



Figure 54. Orange "SYSTEM NOT READY" indicator on the home bar.

The orange "SYSTEM NOT READY" indicator on the home bar signifies that alert(s) relevant to system readiness have been raised and have yet to be resolved. Clicking on this indicator will return the user to the *Action Center* screen where they can review and resolve outstanding alert(s).

B. Action Center Alerts

System not ready alert

The "System not ready" alert within the Action Center alerts the user to tasks that need to be completed before the SmartChip ND instrument should be used.



Figure 55. "System not ready" alert in the *Action Center* of the qPCRStudio software.

Water wash low alert

The software is detecting low levels of wash water in the wash water bottle. Check and refill the wash water bottle with water.

Bleach low alert

The software is detecting low levels of bleach in the bleach bottle. Check and refill the bleach bottle with bleach.

Humidifier water low alert

The software is detecting low levels of humidifier water in the humidifier water bottle. Check and refill the humidifier water bottle with water.

Bleach change suggested alert

qPCRStudio will remind users every seven days to empty and refill the SmartChip ND bleach bottle with fresh bleach to maintain optimal wash performance.

Daily warmup needed alert

qPCRStudio will prompt users at the beginning of each day to perform the daily warmup procedure before the SmartChip ND is used for a job. To begin the daily warmup, click the [Begin daily warmup] button and follow the prompts on the screen.

Warranty will expire soon alert

The warranty on the SmartChip ND system lasts for the first 12 months after purchase. As the warranty expiration date approaches, qPCRStudio will recommend the user contact Field Support at field_support@takarabio.com to purchase a service contract for continued support.

Preventative maintenance alert

Nine months after installation or the last preventative maintenance visit, qPCRStudio will recommend that routine maintenance be performed by a Takara Bio Field Support Application Scientist. Please schedule routine maintenance by contacting Field Support at field_support@takarabio.com.

Firmware mismatch alert

Please contact Field Support at field_support@takarabio.com to resolve this alert.

C. Possible Error Messages

1. Workflow Errors

Chip Vacuum Error—can occur if the chip is not sitting flush inside the chip nest or if there is debris under the chip. Please check that the chip is in good contact with the chip nest base and that the chip nest surface is free of debris. If this error persists, please contact your authorized Takara Bio service technician or field_support@takarabio.com as there could be a leak in the vacuum hardware. Generally, it is safe to proceed if the chip has been verified to be sitting fully flat inside the chip nest.

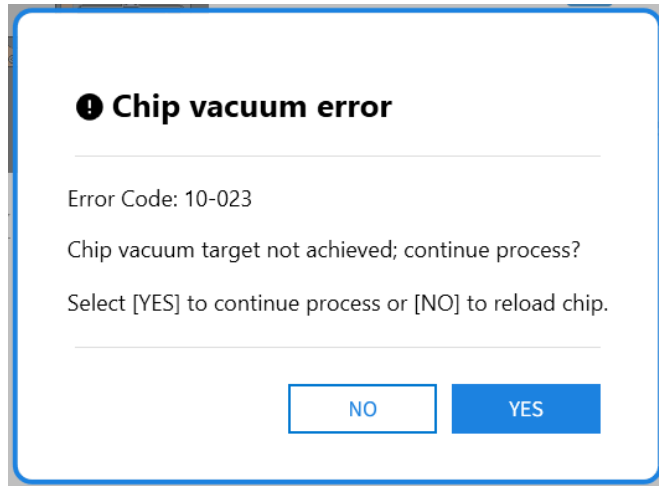


Figure 56. Chip vacuum alert message.

Door Is Open Error — the door open error will can occur if the door is open or if it is not completely closed when starting a dispense. If this error occurs, please open the door all the way and close it again, then retry the dispense.

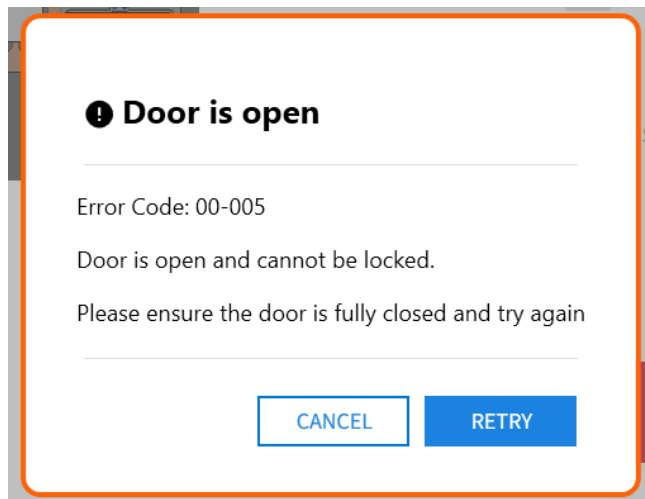


Figure 57. Door open message when starting a dispense.

System Abort During Dispense— the system will reset itself and wash the tips under the assumption they were used. This prevents any contamination for the next dispense.

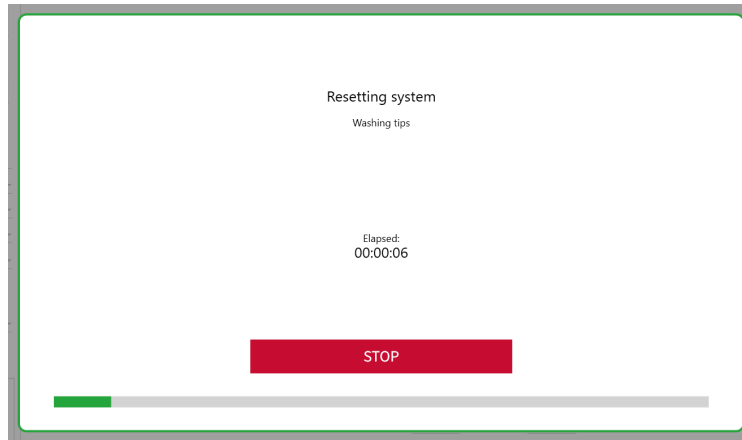


Figure 58. System abort during dispense message.

2. **Additional Potential Errors**

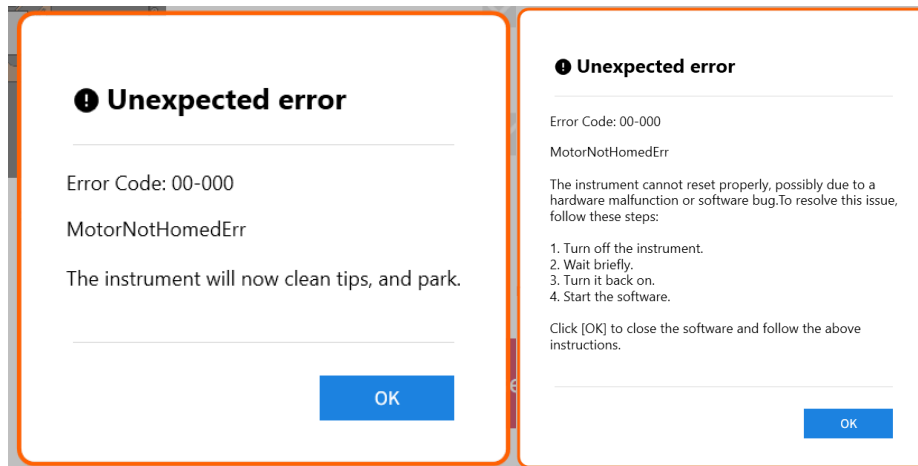


Figure 59. 'MotorNotHomed' error message.

Motor Not Homed Error will only occur if the system did not home properly upon booting. Click [OK] and follow the steps on the screen as listed in Figure 59.

Dewpoint Error—the dewpoint error can occur if the chip nest fails to reach the dewpoint temperature. If this error occurs repeatedly, please contact [Field Support](#), as there could be an issue with the temperature control hardware.

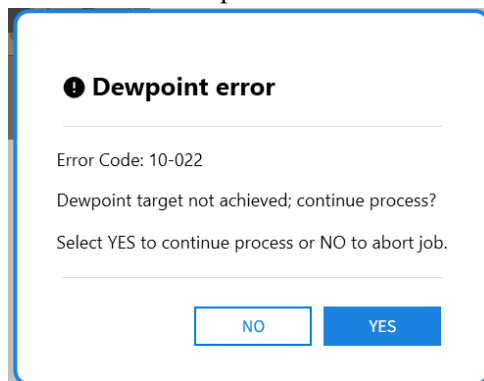


Figure 60. Dewpoint error. The dewpoint error can occur if the chip nest is unable to reach the target temperature prior to starting a dispense.

Appendix C. Preparing Source Plate Files in a Text Editor

Source plate files describe the contents of the 384-well source plates that the SmartChip ND draws from to fill SmartChip Panels or MyDesign chips. These files describe sample or PCR assay attributes and their locations in the plate. This appendix describes how to create these files in a text editor.

To create your own source plate files

Source plate files must be text (*.txt) files. The first part of the file contains header information that should not be modified; the rest of the file is composed of tab-delimited rows of text describing the well contents, with one row for each well. We recommend that you copy the format from the appropriate source plate template file that is installed with qPCRStudio, and then modify the copy (see Step 1, below)

Keep the following in mind when creating a source plate file:

- Valid sample and assay names can contain alphanumeric characters (a-z, 0-9,) hyphens (the - symbol), and underscores (the _ symbol). Do not use forward slashes (/).
- Spaces ([Space bar]) should not be used within the data fields, as it may cause issues that invalidate the file information.
 - Always use [Tab] as a column separator, i.e., a tab-delimited file.
 - For the 6 assays x 768 samples dispense pattern, only one file is used. All 768 samples should be included in that file.

NOTE: We strongly recommend that you do not use Notepad to create or edit source plate files. Tab-delimited formatting is difficult to visualize in Notepad, making it easy to introduce formatting errors while editing. Microsoft Excel or text editors such as Notepad++ display tab-delimited text better.

1. In qPCRStudio, navigate to the top-right menu button and click [Source Layout Builder] to bring up the *Source Plate Layout Builder* window. Select your desired SmartChip format, mode, and layout settings, as well as your desired source plate. Click [Save Layout] to export the corresponding .txt source plate template file to any directory of your choosing.
2. In MS-Excel or a text editor of your choice (see **NOTE** above), open the source plate template file exported in Step 1.
3. Add your source plate information below the text "Begin sample (assay) information" and after the header (column labels).

The source plate description information at the top of the file, the header (column label) text, and the numbers in the "Source" column are required by the software. Type your information below the header using tabs to separate columns of information (see "Attributes for Sample Source Plate Files" and "Attributes for Assay Source Plate Files", Table 11–12, below)

NOTES:

- **Do not edit the "Header" text in the template file.** The software will not find your information if you change the headings (column labels) in your source plate files.
- **Do not change the information in the "Source" column.** This column gives the locations of your sample mixtures or assays in the 384-well source plate and cannot be changed. The SmartChip ND requires that samples and assays be in certain wells of the source plate in order to properly fill the different layout options for SmartChip Panels or MyDesign chips.

- Save your source plate file. When creating an application in the *App Manager* (see [Appendix A, Section B](#)), this file can be selected as your assay/sample layout file to provide custom source plate information in your applications. Once the application is created and saved, it is safe to delete this source plate file.

The sample and assay attributes that can be entered for the two types of analysis (gene expression and SNP genotyping) are shown below.

Gene expression analysis source plates

Table 11. Attributes for sample source plate files for gene expression analysis.

SampleName	Concentration	Source
(Required)	(Optional)	(Do not change!)

The list below explains the purpose of each column/header:

- SampleName: (Required)** The name of the sample.
- Concentration: (Optional)** This is the concentration information of the sample
- Source:** This is a numerical code assigned to each well of the 384-well source plate. These must be in order, so should NOT be modified.

Example:

SampleName	Concentration	Source
Sample_A1	500 pg	1
Sample_A2		2
Sample_B1	1 ng	3

Table 12. Attributes for assay source plate files for gene expression analysis.

AssayName	Source	AssayID	Tm	IsHousekeeping
(Required)	(Do not change!)	(Required)	(Optional)	(Optional)

The list below explains the purpose of each column/header:

- AssayName: (Required)** The name of the assay.
- Source:** This is a numerical code assigned within the software to each well of the 384-well source plate. These should NOT be modified.
- AssayID: (Optional)** This is the ID value assigned to the assay.
- Tm: (Optional)** the expected amplicon melting temperature for the sample, which can be used at the end of the assay to measure specificity
- IsHousekeeping: (Optional)** This field indicates whether or not the assay is a reference gene product assay. Accepted values are either 'yes' or 'no' (without quotes).

Example:

AssayName	Source	AssayID	Tm	IsHousekeeping
GAPDH	1	24	72	yes
Assay_Y2	2	27	68	no
Assay_Z3	3	42		no

SNP genotyping source plates

Table 13. Attributes for sample source plate files for SNP genotyping.

SampleName	Concentration	Source	Gender	Population	Custom 1	Custom 2
(Required)	(Optional)	(Do not change!)	(Optional)	(Optional)	(Optional)	(Optional)

The list below explains the purpose of each column/header:

- **SampleName: (Required)** The name of the sample.
- **Concentration:** (Optional) This is the sample DNA concentration information.
- **Source:** This is a numerical code assigned within the software to each well of the 384-well source plate. These should NOT be modified.
- **Gender:** (Optional) This field can be used to associate the gender information of the sample DNA with the sample in the results files.
- **Population:** (Optional) This field can be used to associate population information of the sample DNA with the sample in the results files.
- **Custom1, Custom2:** (Optional) These fields can be used to record additional information you want to associate with the samples through to the results files.

Table 14. Attributes for assay source plate files for SNP genotyping.

ID	Source	Name	Species	Gene Symbol	Category ID
(Required)	(Do not change!)	(Required)	(Optional)	(Optional)	(Optional)

The list below explains the purpose of each column/header:

- **ID: (Required)** The ID value assigned to the assay.
- **Source:** This is a numerical code assigned within the software to each well of the 384-well source plate. These should NOT be modified.
- **Name: (Required)** The name of the assay.
- **Species:** (Optional) The species the sample/gene is taken from.
- **Gene symbol:** (Optional) The symbol assigned to the target gene.
- **Category ID:** (Optional)

Appendix D. Workflow Guidelines for 768-Sample Dispense

A 768-sample dispense can be performed either by loading one 384-well sample source plate at a time and replacing it after a dispense or by loading two 384-well sample source plates into the two SmartChip ND dispenser source plate nests at the same time.

A. One 384-well Sample Source Plate At A Time

1. Start a new job using a 768 sample dispense application. On the *Job Setup* step, ensure ‘Source Format’ is set to ‘Single Plate’.
2. On the *Source Plate Layout* step of the job, enter source plate well labels for assay, sample 1, and sample 2 source plates by either clicking on the wells and typing in label names or pasting from

- Excel. Pre-existing, reusable source plate TXT files can also be used by first creating a new 768 sample dispense application.
3. Proceed with assay dispense ([Section IX.C](#)).
 4. Label the 384-well plates to identify "Sample plate #1" and "Sample plate #2" (as designated in the *Source Plate Layout* job step).
 5. Load the 384-well plates with samples by referring to the sample source plate map (Step 1).
 6. After each 384-well plate is filled, seal with adhesive film from the Nanodispenser 384-Well Source Plate and Seal and centrifuge at 3,220g for 30 sec at room temperature.
 7. After loading the chip, remove the seal from Sample Plate #1 and load the plate into the SmartChip ND plate nest designated by "Sample #1" on the 'Sample Dispense Run' step.
 8. Confirm that consumables are loaded and click [Start dispense] to dispense Sample Plate #1.
 9. Immediately after this dispense completes, remove Sample Plate #1 from the plate nest and discard appropriately. Remove the seal from Sample Plate #2 and load it into the plate nest designated by "Sample #2" on the *Sample Dispense Run* step.
 10. Confirm that consumables are loaded and click [Start dispense] to dispense Sample Plate #2.
 11. After the Sample Plate #2 dispense completes, select the large blue arrow in the bottom navigation bar to proceed to the *Dispense Run Summary* window and continue with the protocol in [Section IX.D](#).

B. Two 384-well Sample Source Plates at The Same Time

1. Start a new job using a 768 sample dispense application. On the *Job Setup* step, ensure "Source Format" is set to 'Double plates'.
2. On the *Source Plate Layout* step of the job, enter source plate well labels for assay, sample 1, and sample 2 source plates by either clicking on the wells and typing in label names or by pasting from Excel. Pre-existing, reusable source plate TXT files can also be used by first creating a new 768 sample dispense application.
3. Proceed with assay dispense ([Section IX.C](#)).
4. Label the 384-well plates to identify "Sample plate #1" and "Sample plate #2" (as designated in the *Source Plate Layout* job step).
5. Load the 384-well plates with samples by referring to the sample source plate map (Step 1).
6. After each 384-well plate is filled, seal with adhesive film from Nanodispenser 384-Well Source Plate and Seals and centrifuge at 3,220g for 30 sec at room temperature.
7. After loading the chip, remove the seal from Sample plate #1 and load the plate into the SmartChip ND dispenser plate nest designated by "Sample #1" on the 'Sample Dispense Run' step.
8. Remove the seal from Sample plate #2 and load the plate into the plate nest designated by "Sample #2" on the 'Sample Dispense Run' step.
9. Confirm that consumables are loaded and click [Start Dispense] to dispense both plates.
10. After the sample dispense completes, select the blue right arrow icon in the bottom navigation bar to proceed to the *Dispense Run Summary* window and continue with the protocol in [Section IX.D](#).

Appendix E. Guidelines for Partially Filled 384-Well Source Plates or Plates Filled by Automation

A. Partially Filled 384-Well Source Plates

The SmartChip ND dispenser collects source material for dispense in a 2 x 4 pattern across the 384-well plate—two columns and four rows—and it is recommended that it not be programmed to aspirate from empty source wells to protect the functionality of the instrument.

If the number of assays and/or samples dictates a 384-well plate source map with empty source wells, we strongly suggest adding filtered water into all wells the SmartChip ND dispense tips might interact with. These cases might include:

- When dispensing samples or assays from a 96-well plate into the 384-well plate using an automated pipette.
- If you have a number of samples that does not exactly match any of the preset dispense patterns. For example, if you have 100 samples and are using a MyDesign chip, you should choose the 108 samples option and fill the final 8 "sample" wells with filtered water.

B. Plates Filled by Automation

Figures 61–64 will help determine which wells should be filled when using automation. This is also covered in the onsite training by the Field Application Scientist upon receipt of the SmartChip ND system.

NOTE: If you have any questions about customizing the number of samples or assays or how they're distributed in the source plate beyond what is covered in this section, please contact [field support](#).

Example source well plate map for automated pipette use

In Figure 61, there are 67 samples (indicated by the purple boxes) planned to be dispensed by an automated pipette into the 384-well source plate in alternating columns and rows, out to Column 17, Rows A, C, and E.

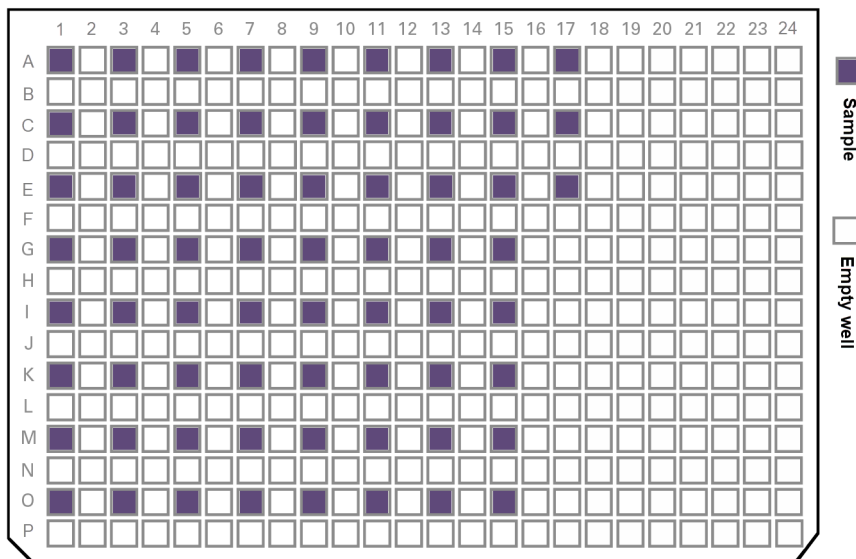


Figure 61. A 384-well plate containing 67 samples as will be dispensed using automation.

Filtered water should be added to empty wells that the SmartChip ND will aspirate to in a 2 x 4 grid; that grid is overlaid on the plate in Figure 62.

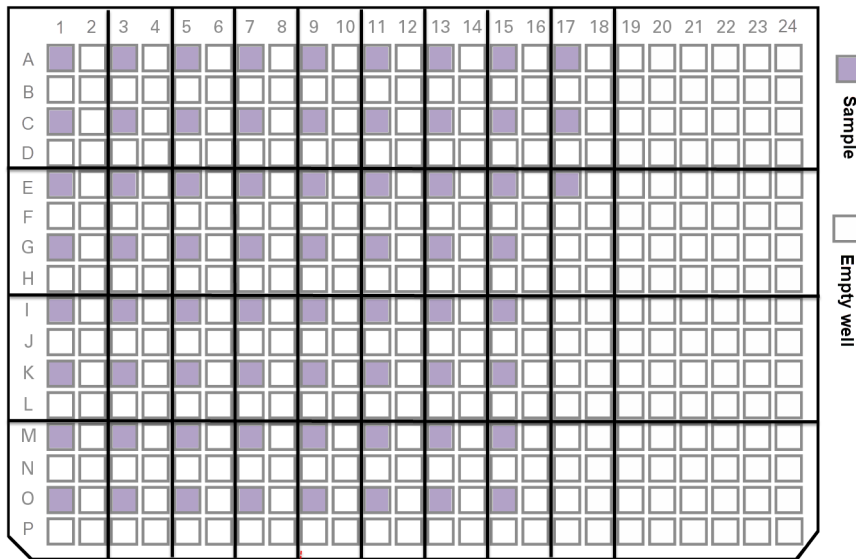


Figure 62. A 384-well plate containing 67 samples with the SmartChip ND aspiration grid overlaid.

Each grid area consists of eight wells. In the example in Figure 62, there are 34 grid areas that contain samples, which translates to 272 wells (34 x 8) in those grid areas.

Since 272 wells doesn't match one of the preset combinations for sample dispense, the dispense coverage would need to be rounded up to the max 384 wells (the '384 samples x 12 assay' dispense preset would therefore need to be selected when preparing to dispense).

The final plate map would therefore resemble Figure 63.

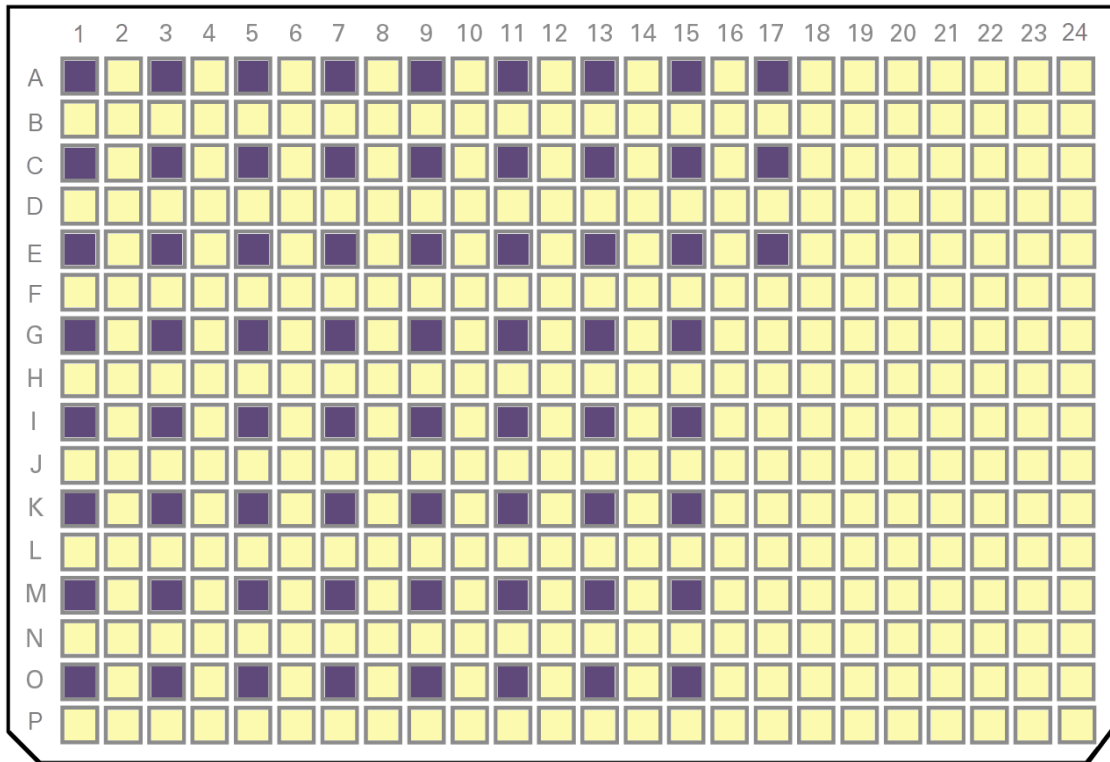


Figure 63. The 384-well source plate fill map for 67 samples (purple wells) dispensed by an automated pipette + filtered water (yellow wells) to accommodate the SmartChip ND best practice.

Appendix F. Instrument Certification and Standards Information

The SmartChip ND system fulfills the following requirements:

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

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

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

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

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