Takara Bio USA, Inc.

SmartChip™ MultiSample NanoDispenser and SmartChip Dispenser Software User Manual

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I. Introduction

A. Thank You for Your Order!

Congratulations on the purchase of your SmartChip MultiSample NanoDispenser (MSND). The SmartChip MSND is designed to load samples into SmartChip Panels, and to load both samples and real-time PCR assays or PCR primers into SmartChip MyDesign Chips.

B. About this Manual

This manual provides instructions for the safe operation and maintenance of the SmartChip MSND. This manual also includes instructions for using the SmartChip Dispenser Software.

Symbols and conventions

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

Table I. User manual symbols and conventions.

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

C. Technical Support

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

D. SmartChip MultiSample NanoDispenser Safety Information

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

Operating conditions:

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

Operate the SmartChip MSND only inside an appropriate building. Do not operate the SmartChip MSND outside or in wet environments.

Instrument use:

WARNING: Use of the SmartChip MSND may cause exposure to toxic or biohazardous chemicals, thereby presenting a hazard. Wear appropriate personal protective equipment (PPE), which should, at minimum, include gloves, eye protection, and lab coat at all times in the laboratory.

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.

Certification and standards information:

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

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

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

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

Moving and lifting the system:

WARNING: If you need to move the system after it has been installed, use proper lifting techniques and appropriate moving equipment. More than one person may be required, particularly when moving the Stage Module.

Warning labels on the instrument:

Please note the warning label on the instrument.

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

II. System Description: Component Overview

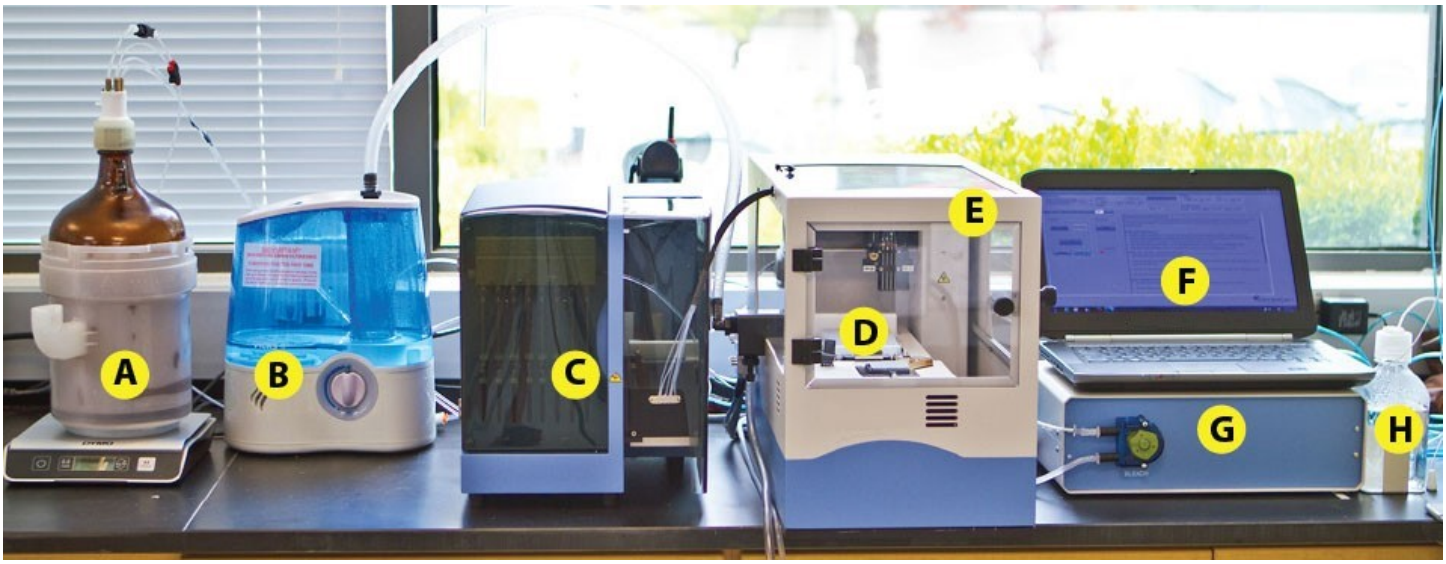


Figure 1. The SmartChip MSND.

The SmartChip MSND includes the following components:

A. Pressure Reservoir and Electronic Scale
B. Humidifier
C. Fluidic Module
D. SmartChip Stage Module
E. Environmental Chamber
F. Laptop Computer
G. Peristaltic Pump Control Box
H. Wash Bottle

Other items not shown include the Fluidic Harness and Power Cord, Waste Container, SmartChip Dispenser Software, User Manual (this document), Digital Pressure Regulator (DPR), Tool Set, Blotter, Chip Spinner and Balance Plate, and SmartChip Source Plate Layout Guides.

Pressure Reservoir and Electronic Scale

The Pressure Reservoir contains helium-pressurized, deionized, and degassed Milli-Q water (or equivalent) which occupies all fluid paths in the Fluidic Module. The liquid is used to draw and push air gaps and reagents through the harness and tip. The Pressure Reservoir sits on an Electronic Scale (Figure 2) which monitors water level so that users can make sure there is enough water prior to starting a chip-dispense operation

Figure 2. Pressure Reservoir seated on the Electronic Scale.

Humidifier

The Humidifier maintains the relative humidity between 30–70% in the Stage Module to minimize reagent evaporation during the dispensing process.

Fluidic Module

The Fluidic Module is a hydro-pneumatic system that controls the aspiration and dispensing of samples and reagents in the Stage Module. A tubular harness connects the two modules (Figure 3). Helium pressure and solenoid valves are used to closely control liquid dispensing. The Fluidic Module also regulates helium to the Pressure Reservoir through a Digital Pressure Regulator.

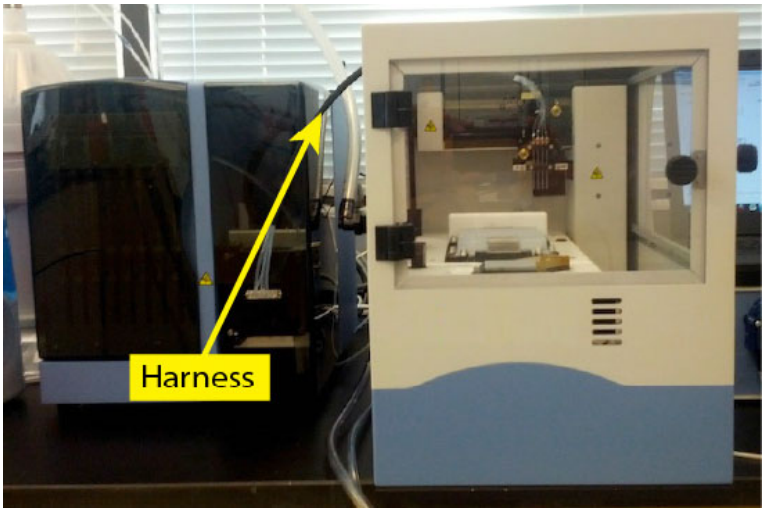


Figure 3. Harness connecting the Fluidic and Stage Modules.

SmartChip Stage Module

The Stage Module houses the head, tips, SmartChip Dispensing Platform, Multi-well Plate Nest, Wash Station, and Tip Mount used for aspirating reagents and dispensing them into a MyDesign chip (Figure 4). A single interface cable facilitates the mechanical control between the two modules. An Environmental Chamber surrounds the Stage Module to maintain optimal humidity levels during reagent dispensing.

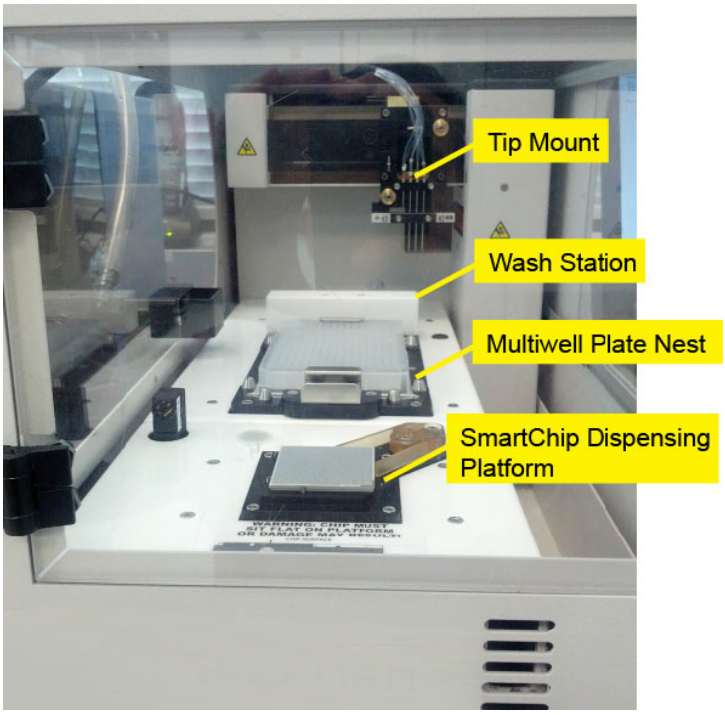


Figure 4. Stage Module in the Environmental Chamber.

Peristaltic Pump Control Box and Environmental Controller

The Peristaltic Pump Control Box includes one peristaltic pump, which pumps and drains Wash Solution and waste into and out of the SmartChip MSND during the tip washing cycles. The Peristaltic Pump Control Box is connected to the Fluidic Module through two of the module's I/O channels.

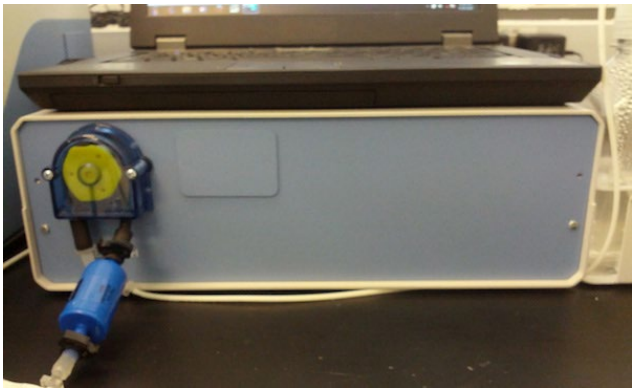


Figure 5. Peristaltic Pump Control Box.

There are two PI controllers inside the Pump Control Box that monitor the enclosure temperature, relative humidity (RH), and chip temperature. They adjust the RH and chip temperature to minimize evaporation during sample dispensing.

The rear of the Pump Control Box has the connections to other components of the system, as shown below (Figure 6).

Figure 6. Connections on the rear of the Peristaltic Pump Control Box.

The connections, starting from the top left, are:

- **I/O:** connection to the Peristaltic Pump
- **TEC:** connection to the Stage Module
- **VACUUM:** connection to the helium supply
- **SENSORS:** connection to the temperature and humidity sensors in the Stage Module
- **MSND 1:** connection to the Fluidic Module
- **MSND 2:** connection to the Fluidic Module
- **SCALE:** connection to the Electronic Scale
- **COMPUTER:** USB connection to computer
- **HUMIDIFIER:** power connection to the humidifier

Wash Bottle

The Wash Bottle contains 0.2% hypochlorite solution which is used during the tip cleaning steps of the dispensing protocol to prevent cross-contamination. The hypochlorite solution is pumped from the reservoir to the wash stage through a mini-peristaltic pump. The pump and reservoir are connected with Flexelene tubing.

A. MultiSample NanoDispenser Specifications and Lab Requirements

Table II. MSND specifications and lab requirements.

Category	Specification
Dispense volume	50 nl or 100 nl per nanowell
Software	SmartChip Dispenser Software
Laptop computer	Windows 7, 2 GB memory, 120 GB storage, 1 GB network adapter, USB ports for memory sticks, CD/DVD burner
Power requirements (for different power supply types)	**120 VAC/60 Hz mains:** one 120-V, 15- or 20-A circuit. Three NEMA 5–15 receptacles are required for the Fluidic Module, Pump Box, and Laptop Computer. (The Humidifier plugs into the Pump Box, and thus a separate receptacle is not required.) **220–240 VAC/50 Hz mains:** one 10-A circuit to power a 230:115 step-down transformer and NEMA 5–15 power strip (transformer and power strip are supplied with the system). Transformer adapters will be supplied for Continental Europe (Schuko type), UK, and China installations. **100 VAC/50–60 Hz mains:** one 15-A circuit to power a 100:120 step-up transformer and NEMA 5–15 power strip (transformer and power strip are supplied with the system). Transformer suitable for Japanese power receptacles.
Fuses	Dispenser; 5 x 20 mm, T5H 1.6 watts/6.3 A max 250 V
Environmental conditions	Ambient temperature: 15–30°C Relative humidity, non-condensing: 30–70% Altitude: <2,000 m from sea level Pollution degree: 2 or less
Dimensions	Laptop Computer: 13” W x 2” H x 10” D (35 cm x 5 cm x 25 cm) Fluidic Module: 11” W x 13” H x 18” D (28 cm x 33 cm x 45 cm) Stage Module: 11” W x 16” H x 24” D (27 cm x 40 cm x 60 cm) Peristaltic Pump Control Box: 10” W x 15” H x 21” D (26 cm x 38 cm x 51 cm)
Bench space	Bench space required for Dispenser, Pump Box, CPU, Pressure Reservoir, transformer (if required): 70” W x 30” D x 24” H (180 cm x 75 cm x 60 cm) *Note: Bench space must be capable of supporting 110 pounds (50kg)*
Floor space	Humidifier: 16” W x 32” H x 26” D (41 cm x 81 cm x 66 cm) Helium Source: 10” diameter cylinder (or equivalent) x ~60” H (25 cm x 125 cm) Waste Container: 8 3/4” W x 14 1/8” H x 6” D (22 cm x 36 cm x 15 cm)
Weight	143 pounds (65 kg)
Performance	Takara Bio Standard Positive Control DNA Test: C_t SD <0.25
Run time	48 samples <12 minutes; 384 samples <60 minutes

B. Setup and Installation

Your Takara Bio Service Engineer will unpack and install your SmartChip MSND and explain the basic operation of the system. They will use material from the SmartChip MSND Starter Kit to qualify the instrument after installation and will leave reusable and/or remaining materials at your site. Table III below lists the SmartChip MSND Starter Kit components and Cat. Nos.

Table III. SmartChip MSND Starter Kit components.

Component	Takara Bio Cat. No.
SmartChip Intermediate Film (pack of 10)	640031
SmartChip Cycling Film (pack of 10)	640033
Nanodispenser Alignment Chip (1)	640041
Nanodispenser Alignment Chip Film (pack of 10)	640030
Blotting Paper (pack of 10)	640021
SmartChip MSND Tube Protection Bags (pack of 10)	640034
Imitation Master Mix with UV Dye & ROX (45 ml)	640026
MSND 384-Well Source Plate and Seals (20 Pack)	640018
MSND 384-Well Source Plate and Seals (120 Pack)	640037

The computer that runs the SmartChip MSND is equipped for Wi-Fi access, but it is disabled. If you choose to activate Wi-Fi, we recommend that you seek support from your institution's IT personnel to avoid interfering with instrument operation.

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

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

Helium

- Purity: 99.9% or greater.
- Capacity: approximately 223 standard cubic feet (reported at 15.6°C and 1 atmosphere [1.01325 Bar]). This capacity is sufficient for six months or more of typical usage.
- Pressure: 2,264 psi (150 Bar), with regulation from 0 to 200 psig (0–15 Bar). Typical usage pressure is 30 psig (2 Bar). Use a regulator (e.g., Concoa regulator, Cat. No. 3124311-01-580).
- Fittings: must accommodate the 3.2 mm outer diameter of the flexible urethane tube fittings (push-to-connect fittings). Acceptable thread forms are 1/8" NPT (female) or M5 straight thread (female).

Wash Bottle

- 1-L container for 0.2% hypochlorite solution

Reagents for SmartChip MSND Reservoirs

- **Pressure Reservoir:** deionized filtered water (Milli-Q or Elga system or equivalent; 0.2-µm filtration)
- **Wash Bottle:** 0.2% hypochlorite (made from reagent-grade sodium hypochlorite in deionized, filtered water)

Other reagents and materials

- Prepared Sample/PCR Reagent Mixtures (for SmartChip MyDesign Chips you will also need PCR Assays). Instructions for preparing samples and reagents for dispensing with the SmartChip MSND are provided with Seq-Ready™ TE Panels, SmartChip Panels, and MyDesign Chips
- MSND 384-Well Source Plate and Seals (Takara Bio, Cat. No. 640018 or 640037)
- (For RNA analysis) RNAse Blaster Solution (Takara Bio, Cat. No. 636839)
- DNA decontamination solution such as DNAZap (Thermo Fisher Scientific, Cat. No. AM9890)
- 70% isopropanol

Equipment

- Ice bucket and/or cold rack
- Calibrated pipette and nuclease-free, aerosol-resistant tips (8-channel and repeating pipettes are very useful in this procedure)
- Vortex
- Centrifuge with rotor capable of spinning microwell plates at 3,220*g*

D. Required Materials from Takara Bio

To order, contact your Takara Bio representative or visit our website at takarabio.com.

For all applications

Table IV. Materials from Takara Bio required for all applications.

Cat. No.	Product name	Description
640018 640037	MSND 384-Well Source Plate and Seals (20 Pack) MSND 384-Well Source Plate and Seals (120 Pack)	These specific 384-well plates are the required container for solutions that will be dispensed using the SmartChip MultiSample NanoDispenser
640021	Blotting Paper (pack of 10)	Small round pieces of blotting paper ideally suited to blotting filled chips

For expression and genotyping analysis using SmartChip MyDesign Chips

Table V. Materials from Takara Bio required for expression and genotyping analysis using SmartChip MyDesign Chips.

Cat. No.	Product name	Description
640032, 640036	SmartChip MyDesign Kit	Empty SmartChip nanowell chip. You add both nucleic acid samples and PCR assays using the SmartChip MSND.Single chip or 20-pack available.
640031	SmartChip Intermediate Film (pack of 10)	SmartChip sealing film used to temporarily seal chips between SmartChip MSND dispensing steps
640033	SmartChip Cycling Film (pack of 10)	Optical chip sealing film for real-time PCR cycling in the Smartchip cycler

For expression and genotyping analysis using predispensed SmartChip Panels

Table VI. Materials from Takara Bio required for expression and genotyping analysis using predispensed SmartChip Panels

Cat. No.	Product name	Description
Various	SmartChip Panel	SmartChip Panels containing PCR assays. You add experimental samples and PCR reagents using the SmartChip MSND. Custom and fixed-content SmartChip Panels are available.
640033	SmartChip Cycling Film (pack of 10)	Optical chip sealing film for real-time PCR cycling in the SmartChip cycler

III. System Description: SmartChip Technology and PCR Workflow

SmartChip technology distinguishes Takara Bio's PCR platform from other systems. Each SmartChip has a 72 x 72 array of nanowells and can accommodate up to 5,184 100-nl real-time PCRs in a single run. There are two types of chips:

- **SmartChip Panels:** contain PCR assays dispensed into the chips at Takara Bio. You add your experimental samples and PCR reagents to these chips using the SmartChip MSND.
- **SmartChip MyDesign Chips:** provided empty. You add experimental samples, PCR reagents, and PCR assays to these chips using the SmartChip MSND.

After the chip is loaded with samples and PCR primers or assays, they are run on the SmartChip Real-Time PCR Cycler. After thermal cycling, the real-time PCR data are analyzed using the SmartChip qPCR software.

A. Expression Analysis

For mRNA expression analysis, the SmartChip Real-Time PCR System has been tested with cDNA synthesized from total RNA using the PrimeScript™ 1st strand cDNA Synthesis Kit (Cat. No 6110A or 6110B) and SmartChip TB Green® Gene Expression Master Mix (Takara Bio, Cat. No. 640210) or SmartChip™ Probe qPCR Master Mix (Cat. No. 640209). The SmartChip system can be used with other fluorescent dyes; contact Takara Bio technical support for current information.

The SmartChip system also supports green intercalating dye-based real-time PCR for the analysis of microRNA and long noncoding RNA and probe-based detection using SmartChip Probe qPCR Master Mix.

- **SmartChip MyDesign Chips** are provided empty. Use the SmartChip MSND to add both PCR assay(s) and experimental cDNA 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.

B. SNP Genotyping

The SmartChip system can be used for SNP genotyping using TaqMan SNP Genotyping Assays (Thermo Fisher Scientific), BHQ*plus* assays (LGC Biosearch), rhAmp assays (IDT), and, with minor modifications, KASP (LCG Genomics).

- **SmartChip MyDesign Chips** are provided empty. Use the SmartChip MSND 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 MSND to add your experimental DNA samples to the SmartChip Panel.

C. Software Files for Real-Time PCR Using the SmartChip System

The SmartChip Real-Time PCR System needs information about your experimental samples, your PCR assays, and how to run the PCRs. This section describes the information and files needed by the SmartChip MSND and the files created by the SmartChip Dispenser Software for use by the SmartChip cycler.

1. Sample Information

The SmartChip MSND aspirates samples from a 384-well plate and dispenses them into the nanowells of the SmartChip Panel or MyDesign Chip. The SmartChip MSND requires that samples be located in specific wells, depending on the quantity of samples and the number of PCR assays. We refer to this plate as a "Source Plate".

You will need to enter sample information and locations into the SmartChip Dispenser Software; this information is stored in Sample Source Plate files.

2. PCR Assay Information

The required PCR assay information varies depending on the type of chip you are using.

SmartChip MyDesign Chips

SmartChip MyDesign Chips are supplied empty. You add PCR Assays and Sample/PCR Reagent mixtures to the chips.

You will need to enter your PCR assay information and locations in the 384-well assay source plate into the SmartChip Dispenser Software; this information is stored in an Assay Source Plate file.

- You can create Assay Source Plate files by entering your PCR assay information and locations into the *Assay Source Plate* tab of the SmartChip Dispenser Software. If you are filling many SmartChip MyDesign Chips with the same set of PCR assays, you can open a saved Assay Source Plate file that contains your assay set.
- If you are using an automated system to fill your Assay Source Plates, you can prepare Assay Source Plate files in a text editor. To simplify the process of entering the data, use the Assay Source Plate template file corresponding to the SmartChip configuration you are using. (See Appendix A for instructions.)

Assay Source Plate files can include the following information:

- For gene or microRNA expression: assay names and IDs, amplicon melting temperature (T_m), and designation as a housekeeping assay
- For genotyping: assay names and IDs, gene, gene symbol, category ID, or species

Predispensed SmartChip Custom Panels (for genotyping or mRNA/microRNA expression)

Predispensed SmartChip Custom Panels contain the PCR assays that you've selected; the assay information is in protocol files on the CD that shipped with your SmartChip Panels. The protocol files contain the following PCR assay information for use by the qPCR software:

- **Assay Map file:** PCR assay locations on the SmartChip Custom Panel
- **Assay Attributes file:**
  - For SmartChip Panels for mRNA or microRNA expression: assay names and IDs, amplicon melting temperature (T_m), and designation of reference assays
  - For SmartChip Genotyping Panels: information from the Assay Information File(s) (AIF) provided with the TaqMan SNP genotyping assay(s)

You do not need the protocol files to dispense samples into your chip, but you will need it to run your reactions on the SmartChip cycler.

Predispensed SmartChip Panels (for mRNA, microRNA, or long noncoding RNA expression)

Predispensed SmartChip Panels contain a fixed set of PCR assays for gene expression or microRNA or long noncoding RNA analysis. The PCR assay information is in protocol files that are installed with the software. The protocol files contain the following PCR assay information for use by the qPCR software:

- **Assay Map file:** PCR assay locations on the SmartChip Panel
- **Assay Attributes file:** assay names and IDs, amplicon melting temperature (T_m), and designation of reference assays

You do not need PCR assay information to dispense your sample into the chip, but you will need it to run your reactions on the SmartChip cycler.

3. Files Generated by the SmartChip Dispenser Software

The SmartChip Dispenser Software creates an XML SmartChip Layout file (`*.xml`) from sample and PCR assay information you have entered. The file contains information about your samples and, if you are using a SmartChip MyDesign Chip, about the PCR assays in your chip. The SmartChip Layout file is required by the SmartChip cycler to run and analyze your experiment. (See "Generating and Saving Your SmartChip Layout File" in Section VII.D for more information.)

D. SmartChip Real-Time PCR Workflow Overview

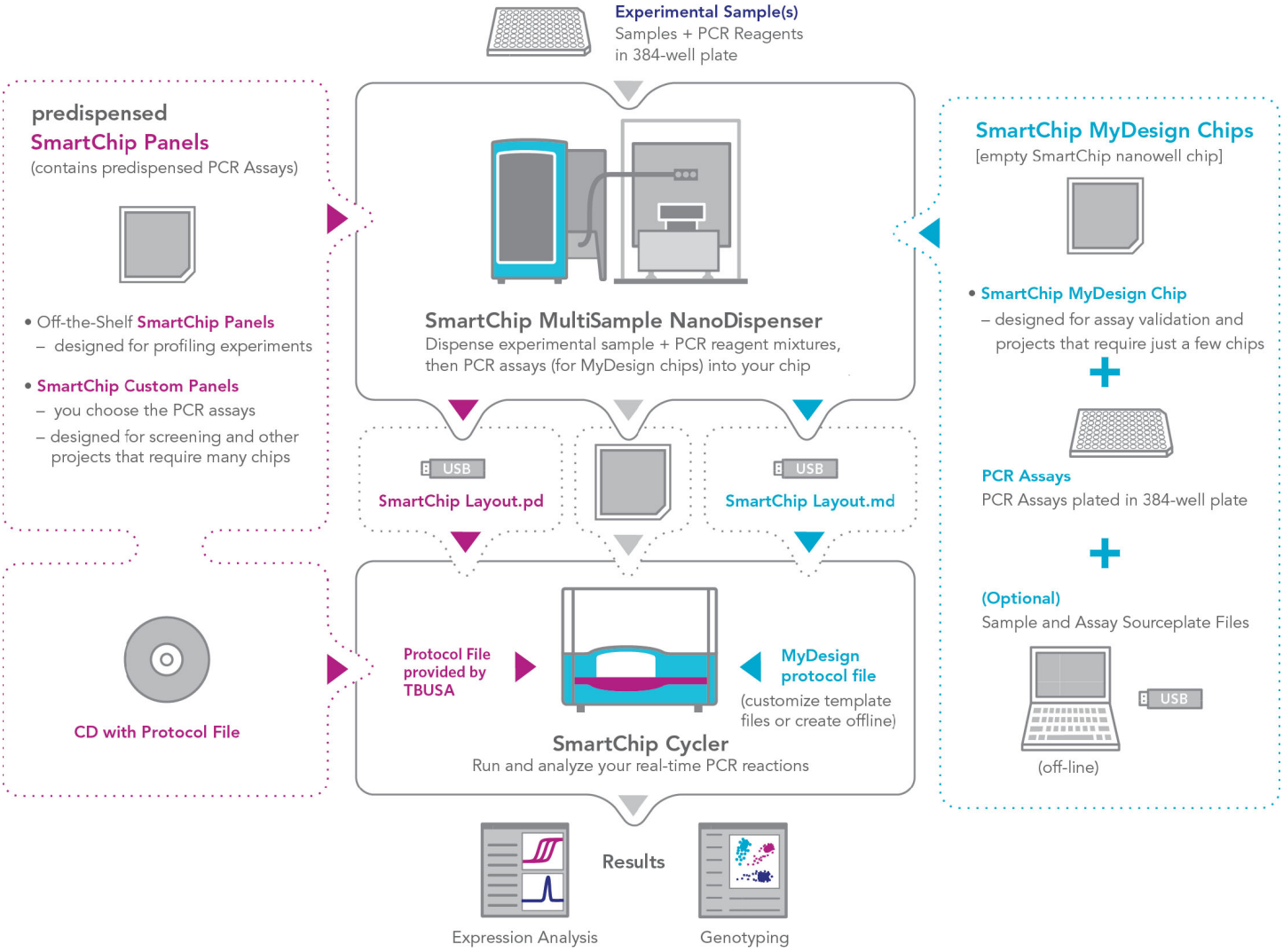


Figure 7. SmartChip Real-Time PCR workflow overview.

IV. Protocol: Quick Guide

Print the Quick Guide below (Table VII) for easy reference in the laboratory.

Table VII. Quick Guide.

For empty SmartChip MyDesign Chips	For predispensed SmartChip Panels
Prepare the SmartChip MultiSample NanoDispenser (MSND).	
Prepare the Sample (and Assay) Source Plate(s).	
For **SmartChip MyDesign Chips:** 1. Prep sample mixtures and plate in a 384-well plate 2. Dilute the PCR assays and plate in a 384-well plate	For predispensed **SmartChip Panels**: 1. Prep sample mixtures and plate in a 384-well plate
Enter information about your experiment in the SmartChip Dispenser Software. 1. On the *Setup* tab, enter information about your experiment. 2. On the *Sample Source Plate* tab, enter your sample information. Open a Sample Source Plate file from an earlier experiment, enter sample information by typing or copying from Excel, or import a file you created from a sample layout template. 3. On the *Assay Source Plate* tab, enter sample information as necessary	
For **SmartChip MyDesign Chips:** 1. Reuse an Assay Source Plate file, enter assay information by typing or copying from Excel, or import an assay layout file	For predispensed **SmartChip Panels:** 1. No action is required (**SmartChip Panels** contain predispensed PCR assays)
4. Click the [Generate SmartChip Layout File] button to create the layout file for the SmartChip cycler. Save it to a USB memory stick or network drive so that you can access it from the thermal cycler.	
For **SmartChip MyDesign Chips:** 1. A `SmartChip Layout.md` file is created	For predispensed **SmartChip Panels:** 1. A `SmartChip Layout.pd` file is created
Dispense into your SmartChip Panel or MyDesign Chip. 1. Place the chip and Sample Source Plate into the instrument and dispense samples and PCR reagents.	
For **SmartChip MyDesign Chips:** 1. Seal the chip with SmartChip Intermediate Film and centrifuge briefly 2. Place your Assay Source Plate into the instrument and dispense PCR assays into the chip 3. Seal with SmartChip Cycling Film and centrifuge	For predispensed **SmartChip Panels:** 1. Seal the chip with SmartChip Cycling Film and centrifuge
Run your PCRs on the SmartChip cycler. (See the SmartChip Real-Time PCR System / SmartChip qPCR Software User Manual for instructions.)	

V. Protocol: Preparing the SmartChip MultiSample NanoDispenser (MSND)

A. Power on the System

> **IMPORTANT:** Make sure that the Pump Box is connected to the proper USB port on the computer with a USB cable.

1. Power on the Fluidic Module and the Pump Box using the switches on the back of the components.
2. Power on the computer and start the SmartChip Dispenser Software. It may take ~5 min for the SmartChip MSND dew point sensors to stabilize and the system to become available.

B. Check System Containers

1. Check the helium tank pressure. The regulator should have a supply input (on the side closer to the helium tank) of >500 psi (3.5 MPa) and an output (on the side closer to the SmartChip MSND) of ~30–40 psi (0.24 MPa). If the helium tank pressure drops below 500 psi, replace the tank.
2. Check the amount of water in the Pressure Reservoir as described below.
    a. Open the top of the protective cover on the Pressure Reservoir (Figure 8). Be careful not to misplace the O-ring or damage the tubes coming from the lid.

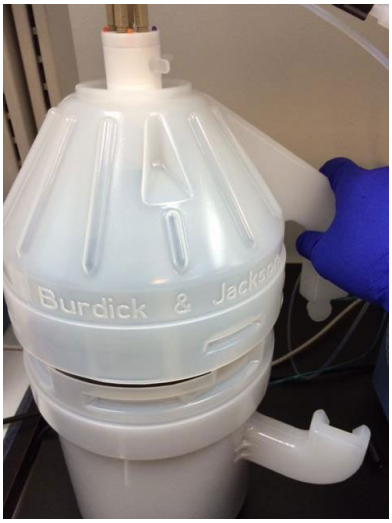


Figure 8. Pressure Reservoir.

    b. Tilt the bottle sideways to check the amount of water in the Reservoir. It should be at least half full of deionized filtered water at the beginning of a run.
    c. If needed, add water to the Reservoir (see “Refilling the Pressure Reservoir” in Section C below).
3. Check the Waste Container. If full, dispose of waste appropriately and replace the Waste Container with an empty one.

4. Check the Wash Bottle. If there is less than ~1 inch (2.5 cm) of liquid in the Wash Bottle, add 0.2% reagent-grade sodium hypochlorite solution to the 500 ml mark (Figure 9). Replace the 0.2% sodium hypochlorite solution when it is more than a week old.

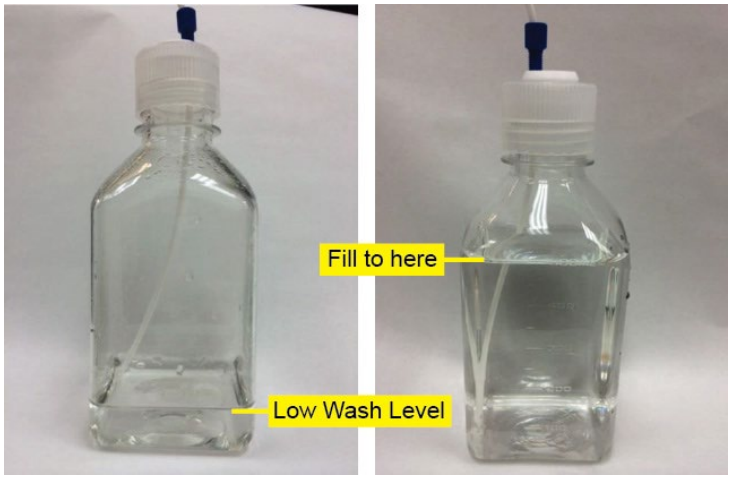


Figure 9. Wash Bottle.

5. Check the Humidifier Reservoir. If the level of water in the Humidifier Reservoir is less than 2 inches (5 cm) from the bottom of the Reservoir (Figure 10), add water (See "Adding Water to the Humidifier Reservoir" in Section V.D below).

Figure 10. Humidifier Reservoir.

6. Check the system humidity. Close all of the doors to the Environmental Chamber. Rotate the Humidifier control switch all the way to the right (clockwise), to the maximum setting. For optimal performance, the humidity needs to be between 30–70%.
7. Perform the Daily Warmup. (See "Running the Daily Warmup" in Section V.E below.)
8. Perform the Tip Clean procedure. (See "Running the Tip Clean Procedure" in Section V.F below.)

C. Refilling the Pressure Reservoir

1. Put on clean gloves.
2. Vent the helium by closing the stopcock on the helium input line and opening the vent stopcock Figure 11).

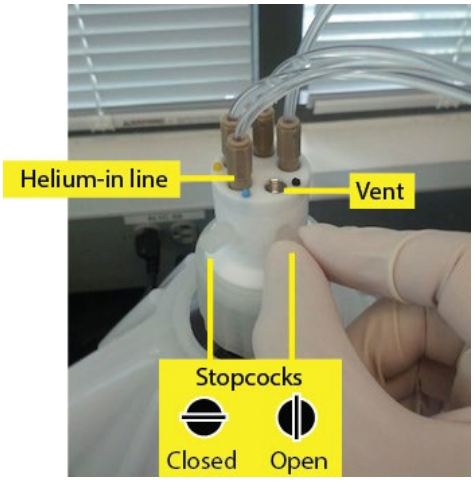


Figure 11. Venting the helium.

3. Open the top of the protective cover (Figure 12). There is no need to remove the entire tubing harness from the Reservoir.

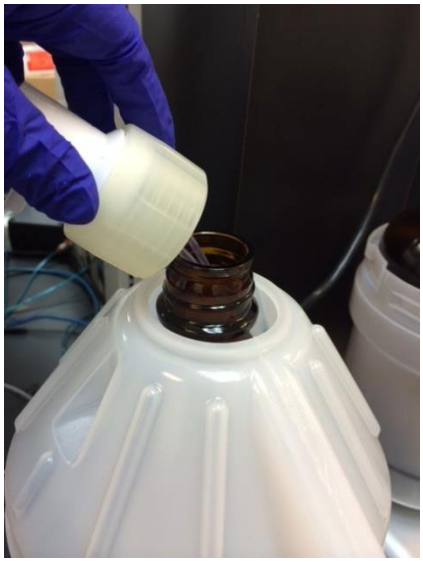


Figure 12. Opening the top of the protective cover.

4. Using a graduated cylinder, fill the bottle with deionized filtered water to the top of the lower part of the protective cover (Figure 13).

Figure 13. Fill the Reservoir with water.

5. Reattach the cap, replacing the tubes inside the Reservoir.
6. Reattach the top of the protective cover.
7. Let the Reservoir liquid degas for 30 min. You should see helium bubbling through the water during this period.
8. Close the system by opening the stopcock on the helium input line and closing the vent stopcock.

D. Adding Water to the Humidifier Reservoir

1. Unplug the hose adapter from the top of the Humidifier (Figure 14).

Figure 14. Unplugging the hose adapter from the Humidifier.

2. Fill the Reservoir with deionized water (Figure 15). The photo on the right shows the filled Reservoir.

Figure 15. Filling the Reservoir with deionized water.

3. Close the cap securely and place the Reservoir back onto the Humidifier base unit (Figure 16).

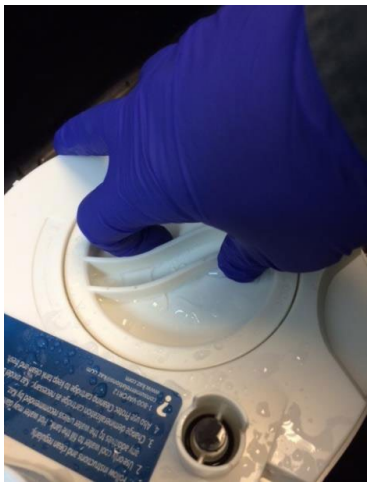


Figure 16. Closing the Reservoir.

4. Replace the hose adapter.

E. Running the Daily Warmup

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

This procedure takes approximately 8 minutes.

1. Click the *Run* tab in the SmartChip Dispenser Software.

2. Click the [Daily Warmup] button in the **Instrument preparation** section of the *Startup* tab (Figure 17). The SmartChip MSND will do the following:
    a. Display a dialog in the software indicating that the system is being brought up to pressure.
    b. Send the head to the Purge position on the SmartChip MSND platform.
    c. Prime the syringe path once.
    d. Purge the syringe valves to remove any air that may be trapped in the syringe valves.

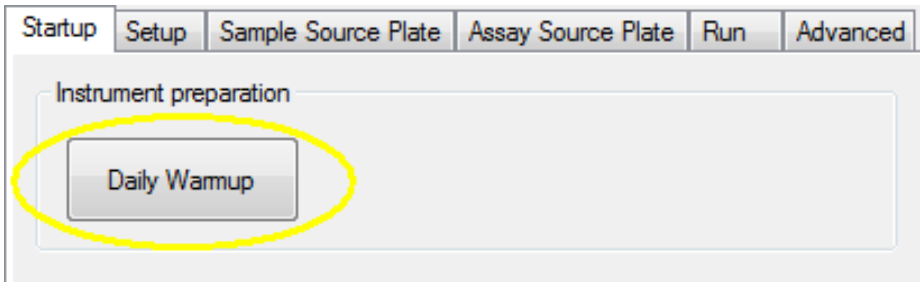


Figure 17. [Daily Warmup] button in the Instrument preparation section.

3. During the Daily Warmup, monitor the syringes in the Fluidic Module for trapped bubbles (Figure 18).

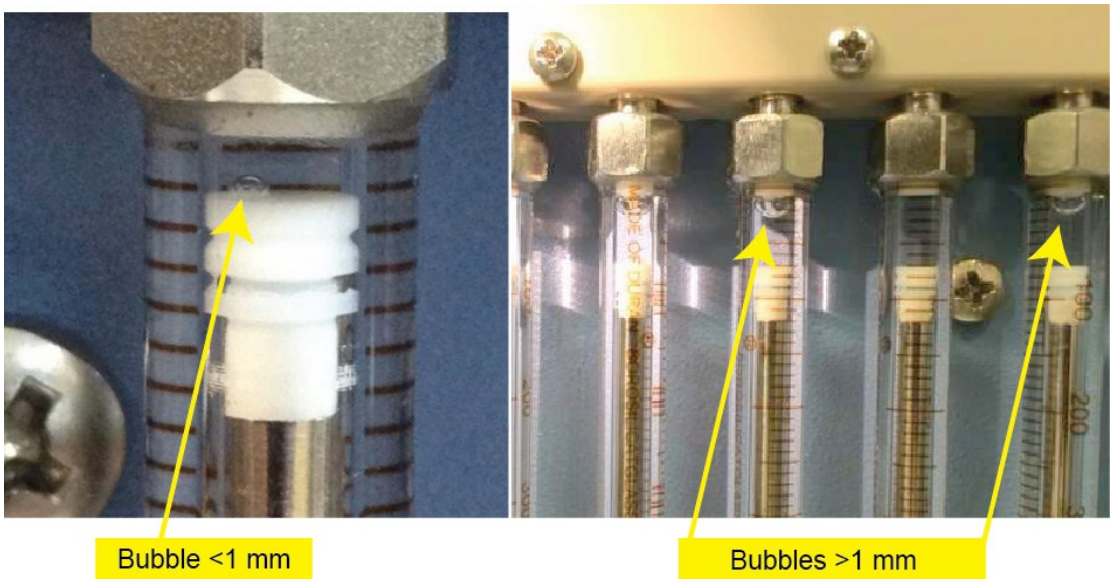


Figure 18. Monitor the Fluidic Module for trapped bubbles. The bubble on the left is an acceptable size, while the bubbles on the right are too large.

Small bubbles are acceptable, but larger bubbles are not. If bubbles larger than 1 mm are observed:

    a. Allow the Daily Warmup procedure to finish.
    b. Repeat the first portion of the Daily Warmup procedure.
        i. Click the [Daily Warmup] button.

ii. When the **Elapsed time** is 55 sec, click the [STOP] button (Figure 19).

Figure 19. *Daily Warmup* runtime dialog box.

iii. If bubbles persist, repeat Step b above until all bubbles larger than 1 mm are purged from the syringe bank.

NOTE: If large bubbles are still present after three Daily Warmup cycles, we recommend an isopropyl alcohol wash (see Appendix C).

F. Running the Tip Clean Procedure

The cleaning process takes about two minutes.

1. Click the *Advanced* tab in the SmartChip Dispenser Software.
2. Click the [Tip Clean] button in the **Manual Control** section (Figure 20).

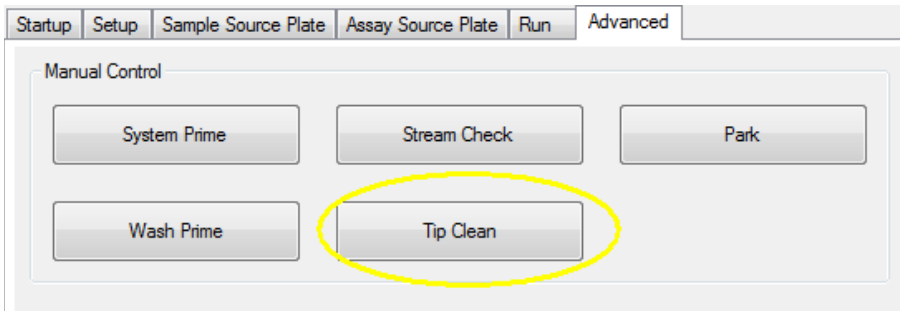


Figure 20. The [Tip Clean] button in the Manual Control section.

VI. Protocol: Preparing 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 transferred using the SmartChip MSND.

IMPORTANT: Only nontreated NUNC Polypropylene 384-Well Plates (Takara Bio Cat. No. 430-000044-3) are validated for use on the SmartChip MSND.

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 Panels

IMPORTANT: Avoid introducing dust and debris to solutions that will be dispensed with the SmartChip MSND. 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. Preparing Source Plates for SmartChip MyDesign Chips

1. Pipette the Sample/PCR Reagent Mixtures containing cDNA (for expression analysis) or DNA sample (for genotyping) and real-time PCR master mix into a 384-well Sample Source Plate.

   Prepare your Samples and Assays using the SmartChip MyDesign Kit User Manual provided with your SmartChip MyDesign Chips.

   To make it easier to manually pipette into the 384-well Source Plates:

   - Use the provided SmartChip Plate Layout Guides. Place the guide in the plate lid, under your Source Plate.
   - See the *Sample Source Plate* and *Assay Source Plate* tabs in the SmartChip Dispenser Software.

   Table VIII below shows recommended volumes per well and the number of wells in your Sample Source Plate.

Table VIII. Recommended volumes per well and number of wells in Sample Source Plate.

SmartChip layout		Recommended volume and # of wells in Source Plate	
# of assays	# of samples	Sample Source Plate	Assay Source Plate
12	384	11.7 µl/well	17.9 µl in each of 4 wells
24	216	12.4 µl/well	17.9 µl in each of 2 wells
36	144	13.2 µl/well	20.3 µl/well
48	108	14.0 µl/well	17.9 µl/well
54	96	14.4 µl/well	17.1 µl/well
72	72	15.6 µl/well	15.6 µl/well
80	64	16.1 µl/well	15.1 µl/well
96	54	17.1 µl/well	14.4 µl/well
120	42	18.9 µl/well	13.7 µl/well
144	36	20.3 µl/well	13.2 µl/well
216	24	17.9 µl in each of 2 wells	12.4 µl/well
248	20	19.4 µl in each of 2 wells	12.2 µl/well
296	16	16.1 µl in each of 4 wells	12.0 µl/well
384	12	17.9 µl in each of 4 wells	11.7 µl/well

2. Pipette your Assay/PCR Reagent Mixtures into a 384-well Assay 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.

3. After filling, seal the plate(s) with adhesive film and centrifuge at 3,220*g* for 5 min at room temperature.

B. Preparing Source Plates for Predispensed SmartChip Panels

1. Pipette Sample/PCR Reagent Mixtures containing cDNA (for expression analysis) or DNA sample (for genotyping) and real-time PCR master mix into a 384-well Sample Source Plate.

   Prepare your samples and assays using the protocol provided with your SmartChip Custom Panels.

   To make it easier to manually pipette into the 384-well Source Plates:

   - Use the provided SmartChip Plate Layout Guides. Place the guide in the plate lid, under your Source Plate.
   - Use the *Sample Source Plate* and *Assay Source Plate* tabs in the SmartChip Dispenser Software.
   - See Appendix B: Source Plate Layouts.

   Tables IX and X below show recommended volumes per well and number of wells for the Sample Source Plates.

Table IX. Sample Source Plates for expression analysis: predispensed SmartChip Panels.

SmartChip layout		Minimum–recommended volume/well, # of wells	Total Sample Volume	
# of assays	# of samples		Minimum (µl)	Recommended (µl)
12	96	15–18 µl per well, 1 well	15	18
24	48	21–25 µl per well, 1 well	21	25
48	24	21–25 µl per well, 2 wells	42	50
96	12	21–25 µl per well, 4 wells	84	100
192	6	23–27 µl per well, 7 wells	158	190
384	3	23–27 µl per well, 14 wells	316	379

Table X. Sample Source Plates for SNP genotyping: predispensed SmartChip Panels.

SmartChip layout		Minimum–recommended volume/well, # of wells	Total Sample Volume	
# of assays	# of samples		Minimum (µl)	Recommended (µl)
12	384	10.5–12.6 µl per well, 1 well	10.5	12.6
24	216	11.6–14.0 µl per well, 1 well	11.6	14.0
48	108	14.3–17.1 µl per well, 1 well	14.3	17.1
72	72	16.9–20.3 µl per well, 1 well	16.9	20.3
96	54	19.6–23.5 µl per well, 1 well	19.6	23.5
120	42	22.6–27.1 µl per well, 1 well	22.6	27.1
192	24	20.9–25.1 µl per well, 2 wells	41.8	50.1
384	12	20.9–25.1 µl per well, 4 wells	83.5	100.2

NOTE: Use Source Plates immediately or store on ice and centrifuge just before use.

2. After filling, seal plate(s) with adhesive film and centrifuge at 3,220*g* for 5 min at room temperature.

VII. Protocol: Programming the SmartChip MultiSample NanoDispenser (MSND)

The SmartChip Dispenser Software controls all SmartChip MSND operations: dispensing your samples/assays into the nanowells of SmartChip MyDesign Chips or Panels and warming up and cleaning the instrument.

For real-time PCR applications, such as expression and genotyping analysis, SmartChip Dispenser Software uses information you provide about the chip contents and their locations on the chip to create the SmartChip Layout file that is needed to run the real-time PCRs on the SmartChip cycler. You can input detailed information such as your SmartChip type and number, the number of experimental samples—plus names and concentrations, and the PCR assays (if you are using a SmartChip MyDesign Chip).

A. *Setup* Tab: Entering Information About Your Experiment

1. Click the *Setup* tab (Figure 21).

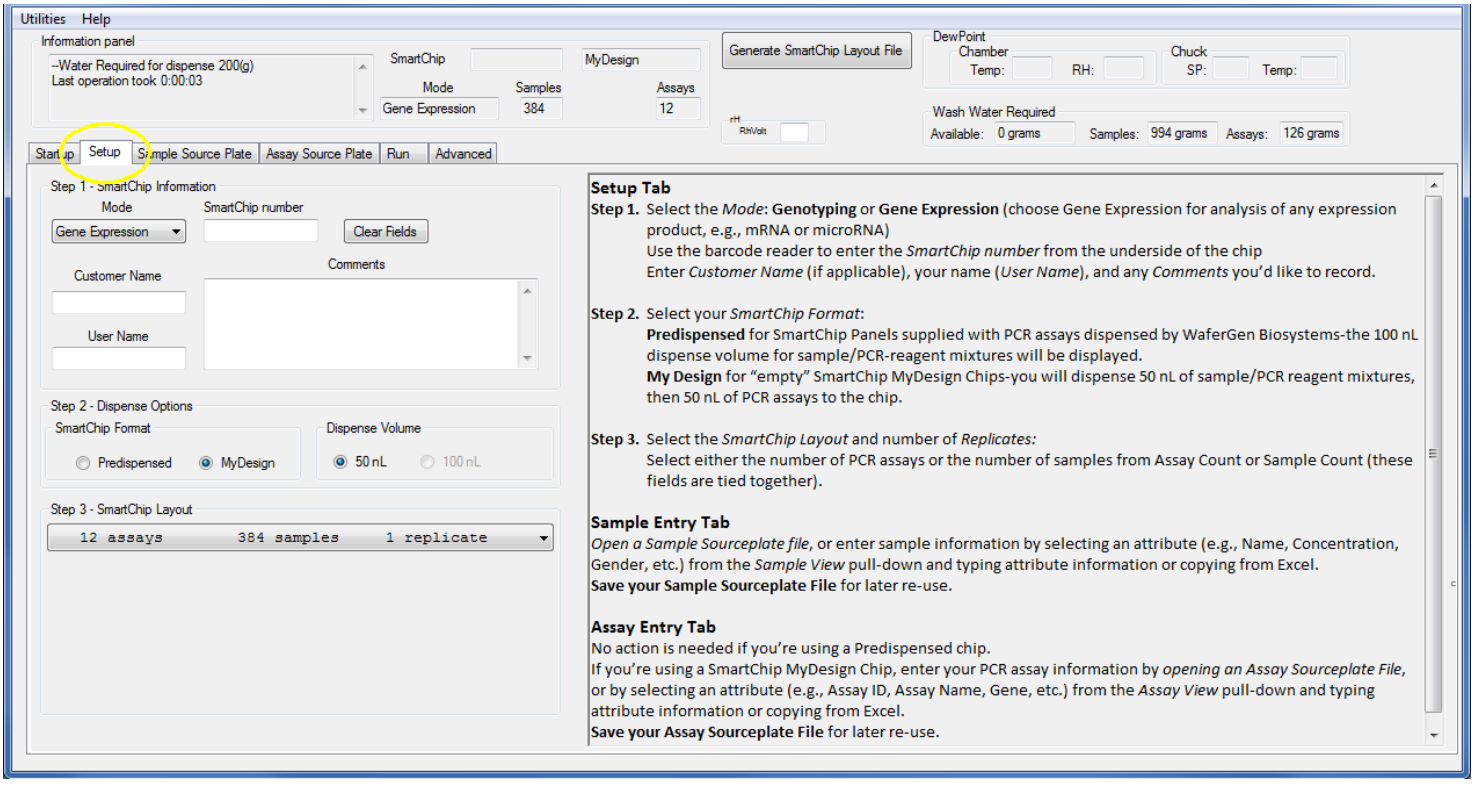


Figure 21. *Setup* tab in the SmartChip Dispenser Software.

2. Enter information about the chip as described below.
   a. Select the type of experiment from the **Mode** list (Figure 22). For microRNA analysis, select **Gene Expression**.

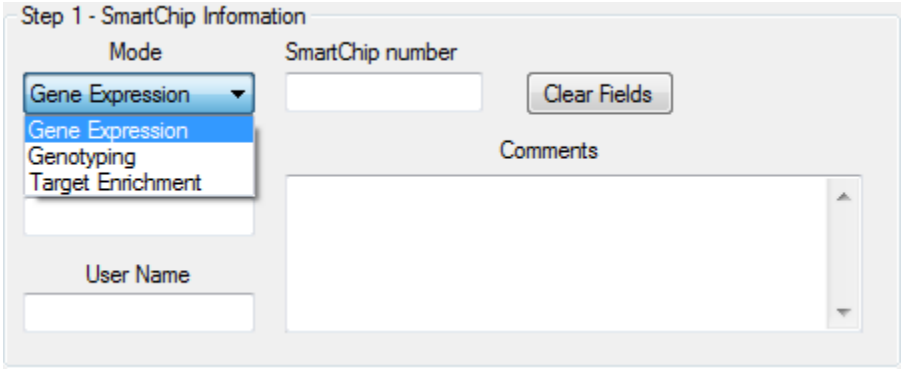


Figure 22. Mode list.

   b. (Optional, but highly recommended for real-time PCR applications): Enter the 'SmartChip number'. Type the number in or place your cursor in the "SmartChip number" field and use the bar code reader to scan the 2-D barcode on the back of the chip. The SmartChip number can be used later to identify the SmartChip Layout file for this chip.

3. (Optional) Enter relevant information in the "Customer Name", "User Name", and "Comments" fields.
4. Select the **SmartChip Format** in the **Step 2—Dispense Options** section (Figure 23).
   - Select **Predispensed** if you are using SmartChip Panels. The "Dispense Volume" field displays "100 nL" for this selection.
   - Select **MyDesign** if you are using MyDesign Chips. The "Dispense Volume" field displays '50 nL' corresponding to 50 nl of Sample/PCR Reagent Mixture, then 50 nl of Assay/PCR Reagent Mixtures.

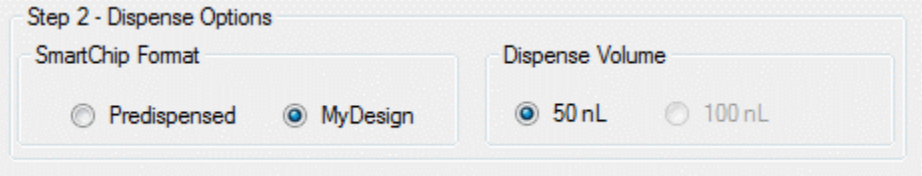


Figure 23. SmartChip Format options.

5. Select the **SmartChip Layout** (Figure 24).

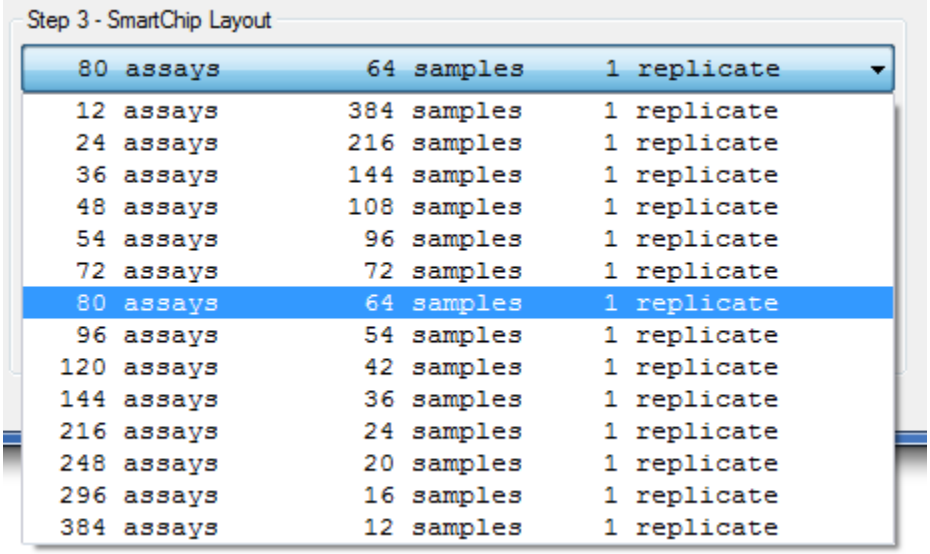


Figure 24. SmartChip Layout options.

B. *Sample Source Plate* Tab: Entering Your Sample Information

1. Click the *Sample Source Plate* tab (Figure 25). A Sample Source Plate map that corresponds to the SmartChip Layout (selected on the *Setup* tab) is shown. You can use this map as a guide for placing sample mixtures into your Sample Source Plate.

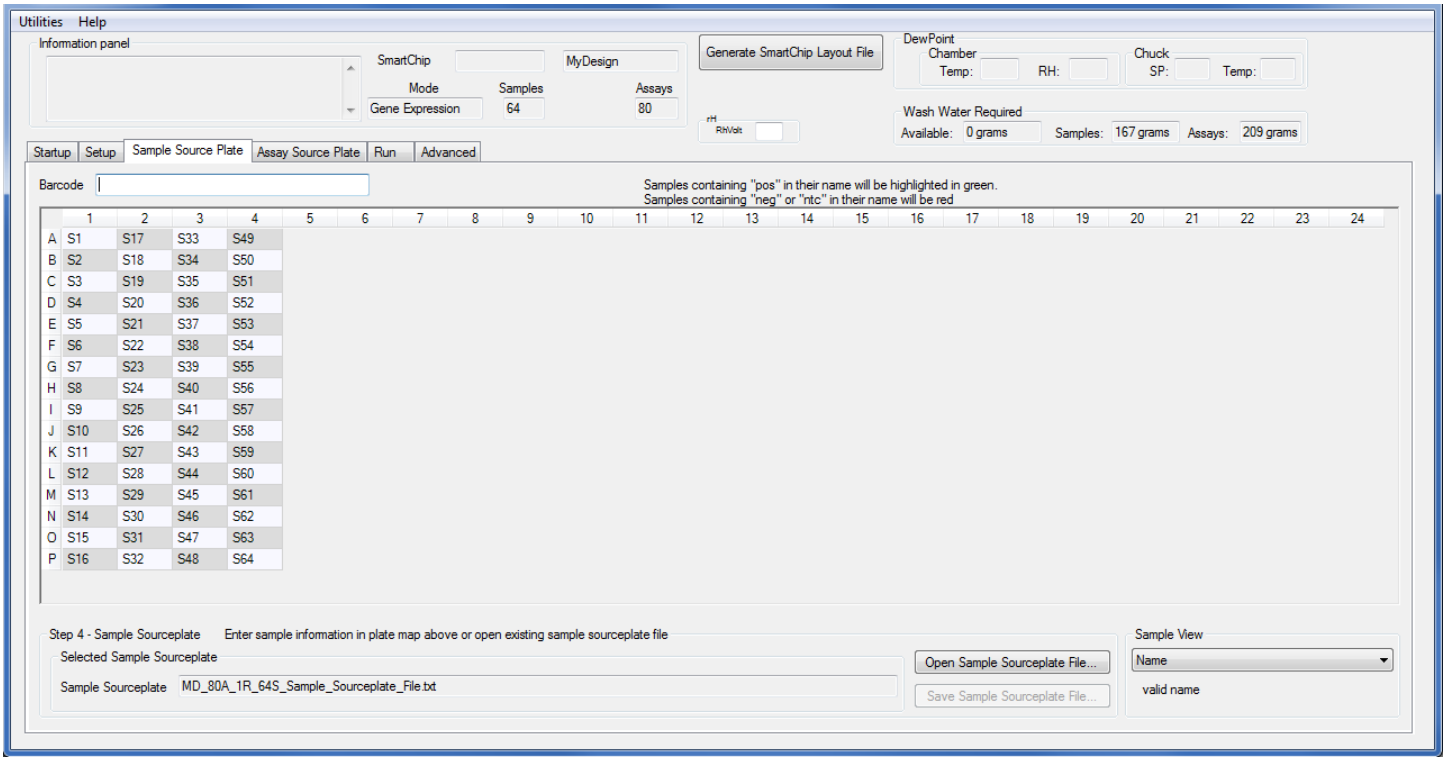


Figure 25. *Sample Source Plate* tab.

2. Add information about the samples in the Sample Source Plate grid, using one of the methods below:
   - Load a Sample Source Plate file (Figure 26). If you have a file describing the location of samples in your Source Plate, open it by selecting [Open Sample Source Plate File…], navigating to the file location, selecting it, and clicking [Open].

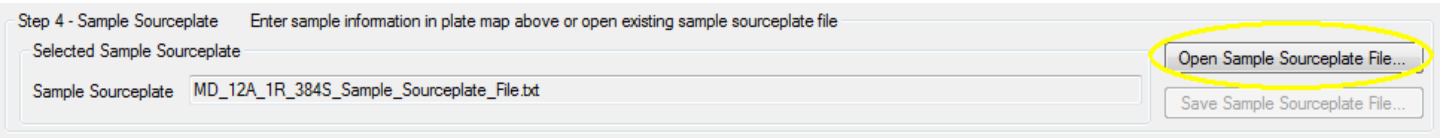


Figure 26. Loading a Sample Source Plate file.

- Manually enter sample information as described below:
  i. Select the sample attribute from the **Sample View** list (Figure 27). The attributes listed depend on the selection in the **Mode** list in the *Setup* tab.

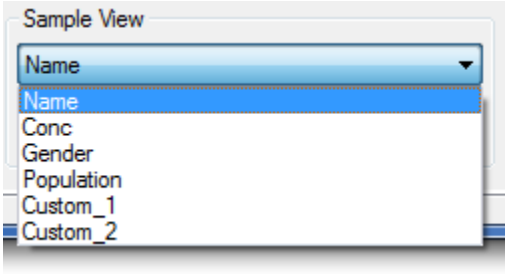


Figure 27. Sample View list.

  ii. Enter information for the selected attribute into the Sample Source Plate grid. Place your cursor in the cell you want to edit and type the values or copy and paste from Excel.

3. Click the "Barcode" field and then scan the barcode on the Sample Source Plate.
4. Click the [Save Source Plate File] button. You can reuse this Sample Source Plate file in subsequent experiments if desired.

Tips for working with the Sample Source Plate grid

NOTE: The functions described below are also available in the Assay Source Plate grid.

- Right-click a cell to copy the contents of the cell.
- After copying, left-click a cell or a range of cells to paste the content.
- Hold the mouse over the cell to see all the information entered about the well (Figure 28).

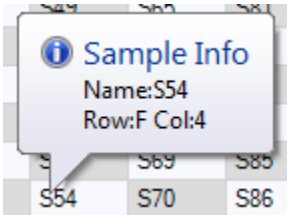


Figure 28. Well information.

- Double-click the border of the column header to expand the column wide enough to view the contents (Figure 29).

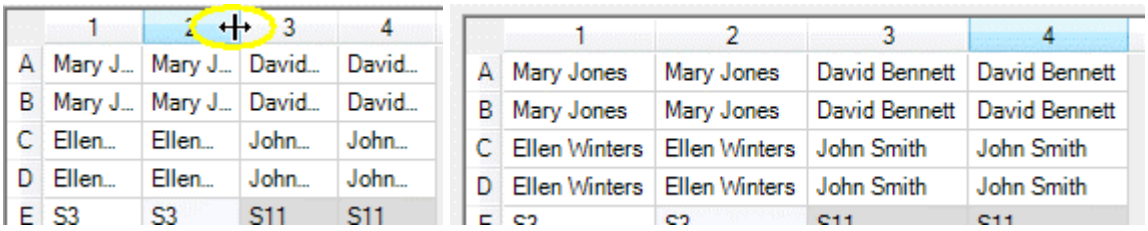


Figure 29. Increasing the width of a column.

C. *Assay Source Plate* Tab: Entering PCR Assay Information (MyDesign Chips Only)

1. Click the *Assay Source Plate* tab (Figure 30). An Assay Source Plate map that corresponds to the SmartChip Layout (selected on the *Setup* tab) is shown. You can use this map as a guide for placing assay mixtures into your Assay Source Plate.

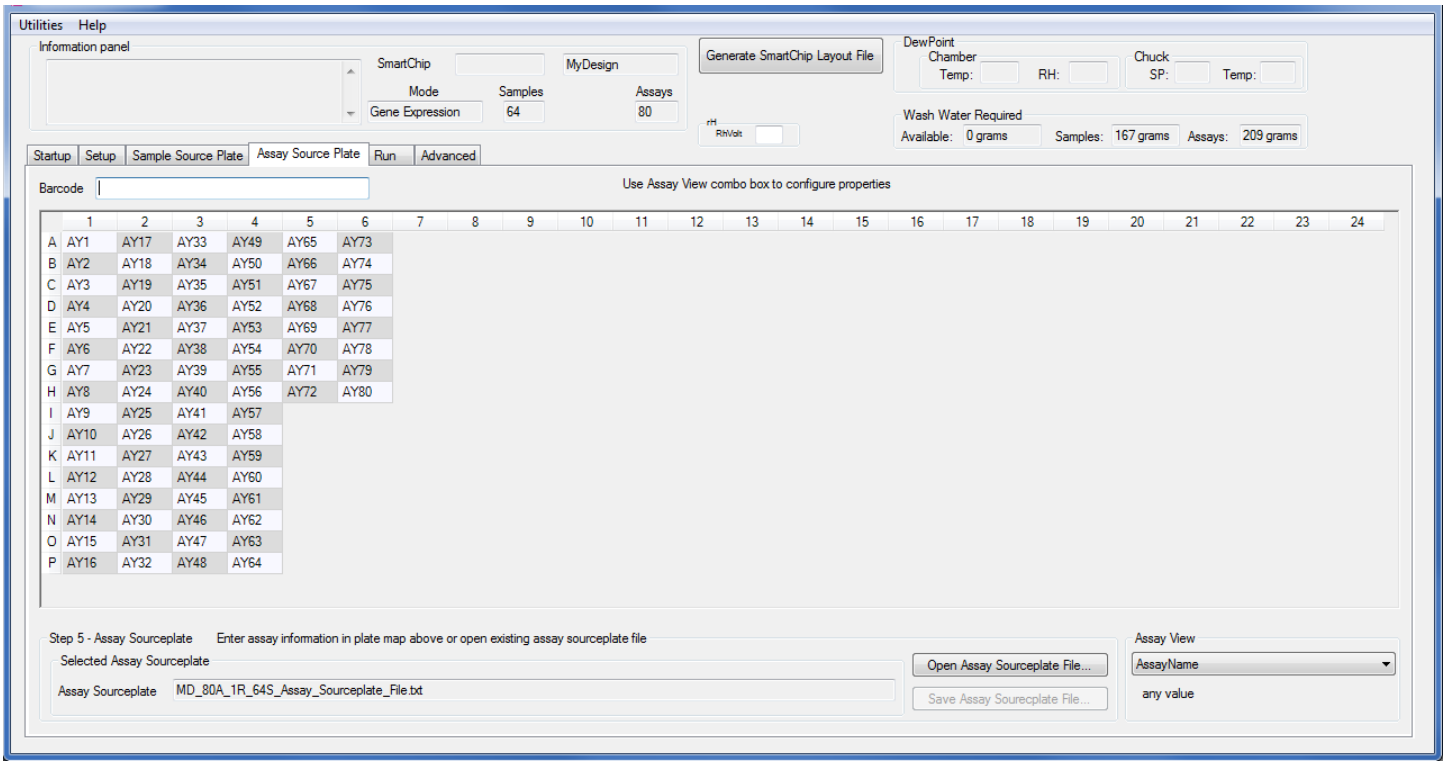


Figure 30. Assay Source Plate tab.

2. Enter information about the PCR assays in the Assay Source Plate grid, using one of the methods below:
   - Load an Assay Source Plate file (Figure 31). If you have a file describing the location of samples in your Source Plate, open it by selecting [Open Assay Source Plate File], navigating to the file location, selecting it, and clicking [Open].

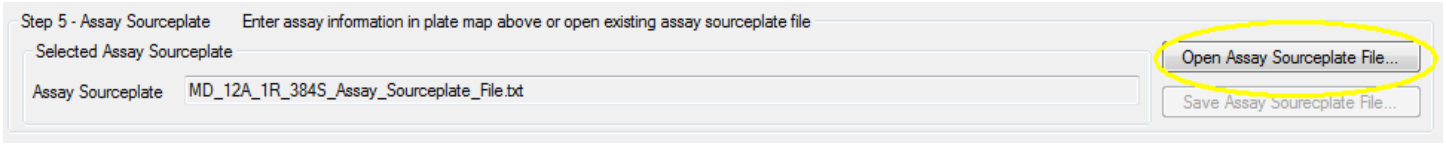


Figure 31. Loading an Assay Source Plate file.

- Manually enter assay information as described below:
  i. Select the assay attribute from the **Assay View** list (figure 32).

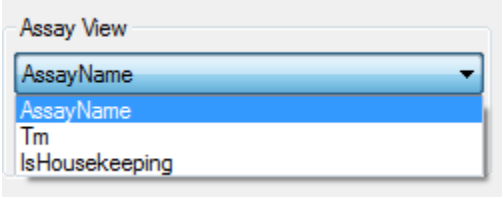


Figure 32. Assay View list.

  ii. Enter the information for the selected attribute into the Assay Source Plate grid. Place your cursor in the cell you want to edit and type the values or copy and paste from Excel.

3. Click the "Barcode" field and then scan the barcode on the Assay Source Plate.
4. Click the [Save Assay Source Plate File] button. You can reuse this file in subsequent experiments if desired.

D. Generating and Saving Your SmartChip Layout File

1. Click the [Generate SmartChip Layout File] button (Figure 33). The SmartChip Layout file is needed by the qPCR software to run your PCRs on the SmartChip cycler. The extension on the SmartChip Layout file indicates whether the file contains only sample layout information or both sample and PCR assay layout information:
   - ***.pd extension:** the file contains only sample information and is for use with a predispensed SmartChip Panel.
   - ***.md extension:** the file contains sample and PCR assay information and is for use with a SmartChip MyDesign Chip.

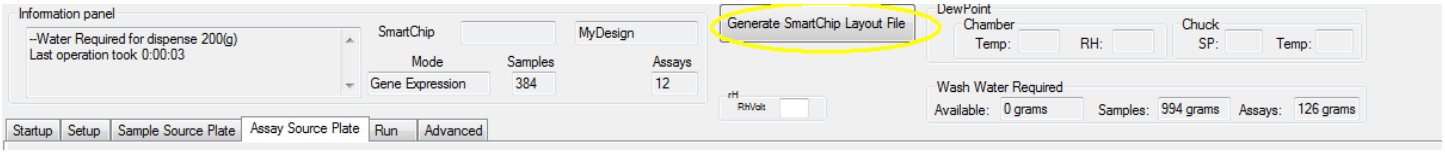


Figure 33. Generating a SmartChip Layout file.

2. Enter information in the *Save SmartChip Layout File* dialog as described below.
   a. Edit the file name, if desired. The default name includes the SmartChip number, the date, and the time.
   b. Choose a location to save the file. This file is needed to run your reactions on the SmartChip cycler, so you may want to save it to a USB memory stick or a network folder. If you do not specify a location for the file, the default location is:
      - **Windows 7:** `C:\ProgramData\WaferGen\SmartChip Dispenser`
      - **Windows XP:** `C:\Documents and Settings\All Users\Application Data\WaferGen\SmartChip Dispenser`

VIII. Protocol: Dispensing the Reagent Mixtures into Your Chip

A. Placing the Chip in the SmartChip MSND

1. Remove the protective film from your SmartChip Panel or MyDesign Chip.
2. Visually inspect the Dispensing Platform and clean it if there is any debris.
3. Place the chip on the platform inside the Stage Module, as described below (Figure 34).
    a. Stretch the arms of the clip apart and angle the chip onto the Dispensing Platform, with the beveled corner on the lower right and the edges of the chip pressed against the three alignment pins.
    b. Carefully release the clip that holds the chip in position.

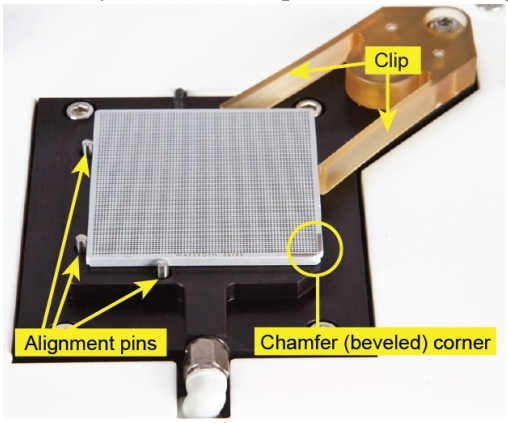


Figure 34. Placing the chip on the Dispensing Platform inside the Stage Module.

B. Placing the Sample Source Plate in the SmartChip MSND

1. If you haven't already done so, centrifuge your Sample Source Plate at 3,220*g* for 5 min at 20–25°C.
2. Remove the adhesive film.
3. Place the Sample Source Plate on the Plate Nest with the A1 position in the top, back right corner (Figure 35).

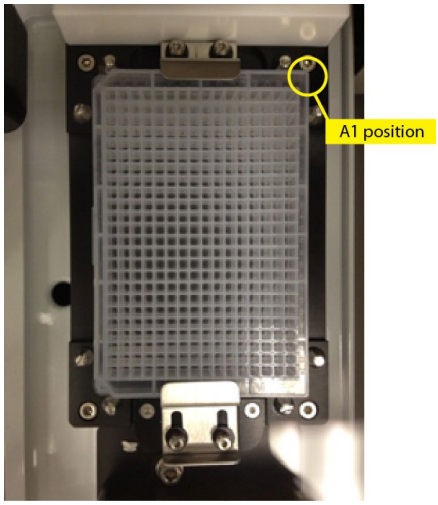


Figure 35. Sample Source Plate on the Plate Nest.

4. Close the Environmental Chamber doors.

C. Dispensing the Sample/PCR Reagent Mixture, Blotting, Sealing, and Spinning the Chip

IMPORTANT: Do not open the door of the Stage Module while the SmartChip MSND is dispensing. If the door is open, the chip can become contaminated. Additionally, when the door is open, evaporation from the nanowells can occur, resulting in changes to concentrations of the reagents in the chip.

IMPORTANT: Do not touch the Barcode Reader while the SmartChip MSND is dispensing. Operating the reader while the system is dispensing could interfere with the instrument run.

1. Click the *Run* tab in the SmartChip Dispenser Software and then click the [Dispense Samples] button (Figure 36). If the humidity is not high enough, the [Dispense Samples] button flashes and a message appears (Figure 37). When the humidity reaches the correct level, the [Start dispense] button turns green (Figure 38). Click the button to begin dispensing.

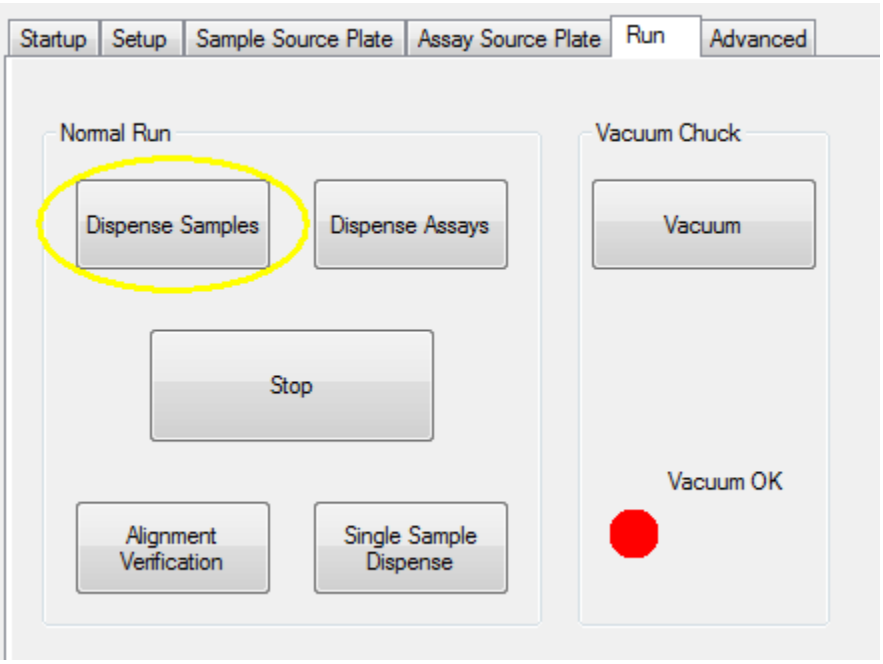


Figure 36. *Run* tab.

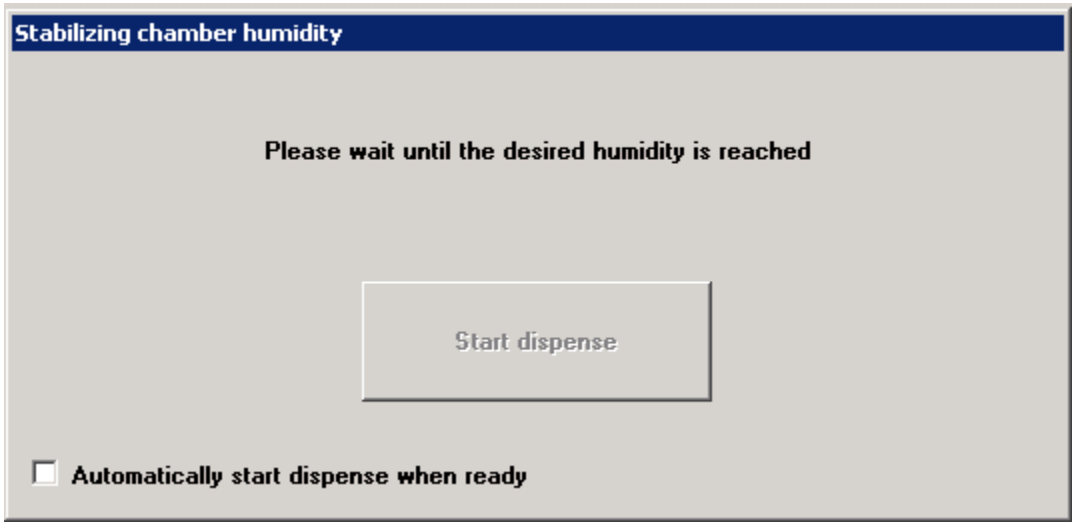


Figure 37. Dialog box while chamber humidity is stabilized.

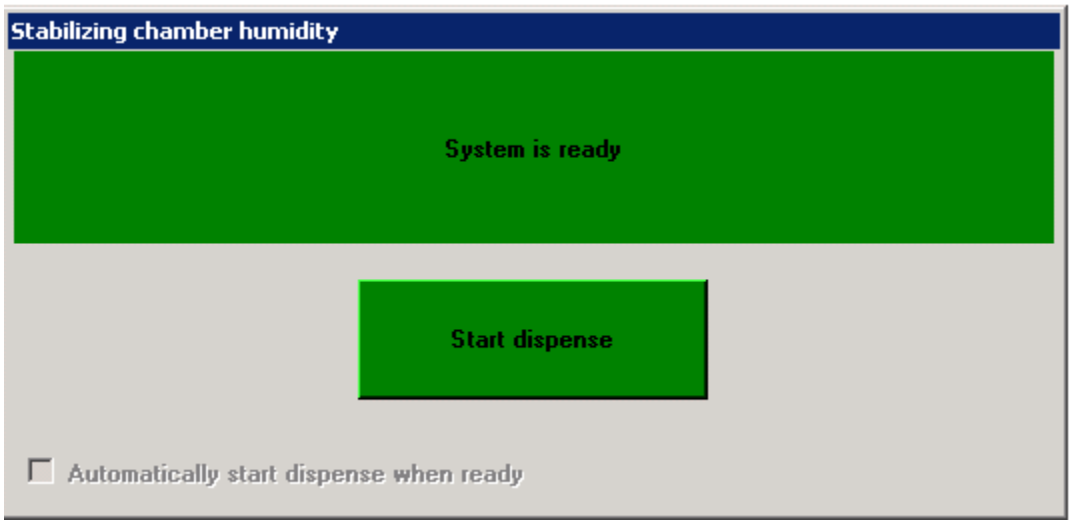


Figure 38. System is ready for dispensing.

2. After dispensing is complete, promptly blot the chip for 2 sec, as described below.
    a. Place the chip, wells facing up, on a clean lab wipe.
    b. Gently place a piece of SmartChip 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 SmartChip 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, then gently remove the Blotting Paper and dispose of it in a biohazard container.

! **IMPORTANT:** When filling SmartChip MyDesign Chips, be sure the seal the chips with SmartChip Intermediate Film after the Sample/PCR Reagent Mix has been dispensed. This film is removable so that you can unseal the chip to add the PCR Assay Mixture.

3. Quickly seal the loaded chip with the appropriate film as described below.

   For predispensed **SmartChip Panels**, use SmartChip cycling Film. The **SmartChip Panel** is completely filled. Go to Step 4.

   For **SmartChip MyDesign Chips**, use **SmartChip Intermediate Film** (not shown) if you will be dispensing into the chip a second time (i.e., you have dispensed samples into the chip and will dispense PCR assays next). Note that you will seal the chip with SmartChip Cycling Film after the PCR assay dispense step.

   i. Remove the backing from the adhesive film. With **SmartChip Cycling Film**, remove the backing only from the center of the film (Figure 39).

Figure 39. Removing the backing from the SmartChip Cycling Film.

   ii. Leave the blue backing around the periphery of the film in place.

   iii. 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. For **SmartChip Cycling Film**, place the film in the orientation shown below (Figure 40).

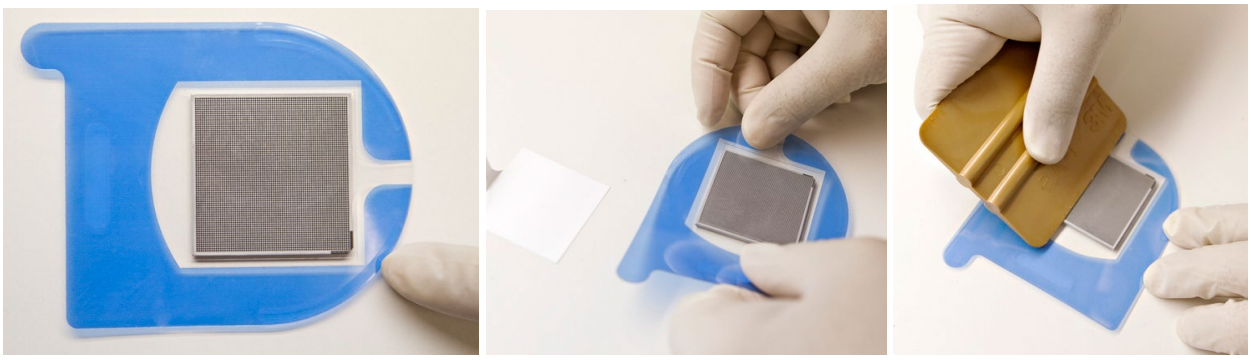


Figure 40. Placing SmartChip Cycling Film on the chip.

IMPORTANT: With SmartChip Cycling Film, be sure to place the film on the chip in the orientation shown in the photos above. The chamfered corner of the chip is at the bottom right in the photo (our scientist is pointing to it in the photo on the right (Figure 40).

iv. Use your fingers to press and smooth the seal, starting at the center of the chip and moving toward the edges.

v. To make sure that a strong seal is achieved, apply pressure with the seal applicator from the center of the chip outward. Repeat 8 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 will.

4. Using the Chip Spinner, centrifuge the chip at 3,220*g* for 15 min at 20–25°C, as described below.
   a. Place your chip(s) in the Chip Spinner(s).
   b. Counterbalance with either the Balance Plate or with a second Chip Spinner and chip (Figure 41).

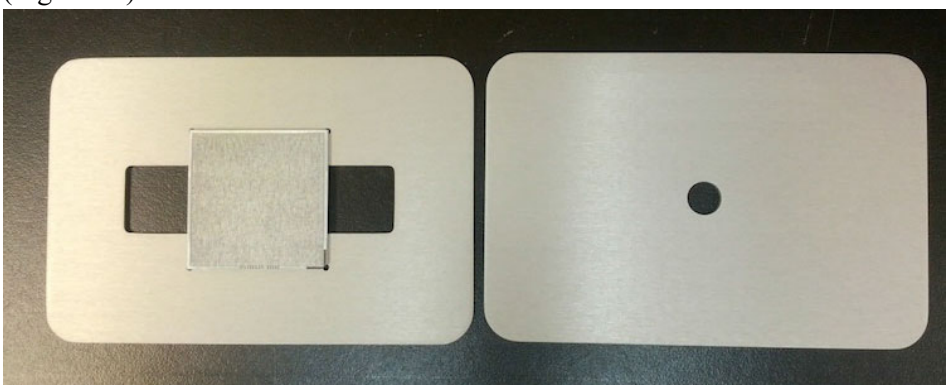


Figure 41. Chip Spinner (left) and Balance Plate (right).

5. Follow the instructions below for the type of chip you are using.
   - For a **SmartChip Panel**, run your PCRs on the SmartChip cycler. See the SmartChip Real-Time PCR System / SmartChip qPCR Software User Manual for complete instructions on thermal cycling the filled SmartChip Panel.
   - For a **SmartChip MyDesign Chip**, dispense your PCR Assays into your chip by following the instructions below.

> **!** **IMPORTANT:** SmartChip Human microRNA Panels should be thermal cycled immediately. SmartChip mRNA or genotyping chips can 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, then spin for 5 min at 3,220*g*.

6. Clean the SmartChip MSND as described below.
   a. After each dispensing, remove the 384-well plate from the plate nest and properly dispose of it.
   b. Inspect the dispensing platform for any debris and wipe down with 70% isopropanol.
   c. After the final dispense of the day, perform the Tip Clean procedure. (See "Running the Tip Clean Procedure" in Section V.F.)

D. Dispensing Assay/PCR Reagent Mixtures into the SmartChip MyDesign Chip

This procedure to dispense Assay/PCR Reagent Mixtures into SmartChip MyDesign Chips is very similar to that used to dispense Sample/Real-Time PCR Mixtures.

1. Carefully remove the SmartChip Intermediate Film from the chip containing your Sample and PCR Reagent Mixtures.
2. Load your chip into the SmartChip MSND. Follow the instructions in "Placing the Chip in the SmartChip MSND" in Section VIII.A.
3. Load your Assay Source Plate into the SmartChip MSND. Follow the instructions in "Placing the Sample Source Plate in the SmartChip MSND" in Section VIII.B.
4. Dispense the Assay/PCR Reagent Mixtures into the chip and prepare the chip for thermal cycling. Follow the instructions in "Dispensing the Sample/PCR Reagent Mixture, Blotting, Sealing, and Spinning the Chip" in Section VIII.C.
5. Clean the SmartChip MSND following the instructions in Section VIII.C, Step 6 (above).
6. Run your PCRs on the SmartChip cycler. See the SmartChip Real-Time PCR System / SmartChip qPCR Software User Manual for complete instructions on thermal cycling the filled SmartChip MyDesign Chip.

IX. Maintenance

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

A. Daily Maintenance

Daily Maintenance procedures help ensure optimal instrument operation and prevent problems. They are described in "Protocol: Preparing the SmartChip MultiSample NanoDispenser (MSND)" (Section V).

B. Complete Shutdown Procedure

Follow these instructions to completely shut down the SmartChip MSND if the instrument will not be used for more than a week.

1. Visually inspect the dispensing platform for any debris and wipe down with 70% isopropanol.
2. Perform the Tip Clean procedure. See "Running the Tip Clean Procedure" in Section V.F.
3. Exit the SmartChip Dispenser Software by clicking the [Close] button () at the top right of the window.
4. Turn off the Peristaltic Pump Control Box using the switch on the back left side of the box.
5. Turn off the Fluidic Module using the power switch on the rear of the box.
6. Power down the computer that controls the SmartChip MSND.
7. Remove all SmartChip nanowell chips and Source Plates from the Stage Module, and clean the dispensing platform of any debris.
8. Empty the waste container, rinse, and allow it to dry.

C. Cleaning the Humidifier

Empty and clean the Humidifier weekly.

1. Rinse the humidifier chamber with a 1:10 dilution of commercial bleach (0.6% sodium hypochlorite).
2. Rinse 3 times with filtered, deionized water.
3. Let the humidifier dry.
4. Refill with deionized water.

D. Verifying the Dispensing Tip Alignment

Verify the alignment of the dispensing tip every ~20 runs of the SmartChip MSND, or approximately every two weeks.

The alignment verification run dispenses fluorescent Imitation Master Mix into the Nanodispenser Alignment Chip (Takara Bio, Cat. No. 640041) provided with the instrument. After dispensing, look at the chip under UV light and magnification to check dispensing consistency.

1. Prepare a Source Plate containing the Imitation Master Mix as described below.
    a. Pipette 15.5 µl of Imitation Master Mix with UV Dye & ROX (Takara Bio, Cat. No. 640026) into all of the wells in Columns 1 and 2 of a 384-well plate.
    b. Put the plate into the plate nest, with the filled wells closer to the wash trough.

2. Seal the bottom of the SmartChip Alignment Chip with Nanodispenser Alignment Chip Film, as described below.
    a. Hold the Alignment Chip Film by the tab and pull it apart. Carefully peel off the backing completely.
    b. With the bottom of the Alignment Chip facing up and the beveled corner in the lower left as you face the chip, apply the film to the back of the chip so that the tab is on the right side (Figure 42).

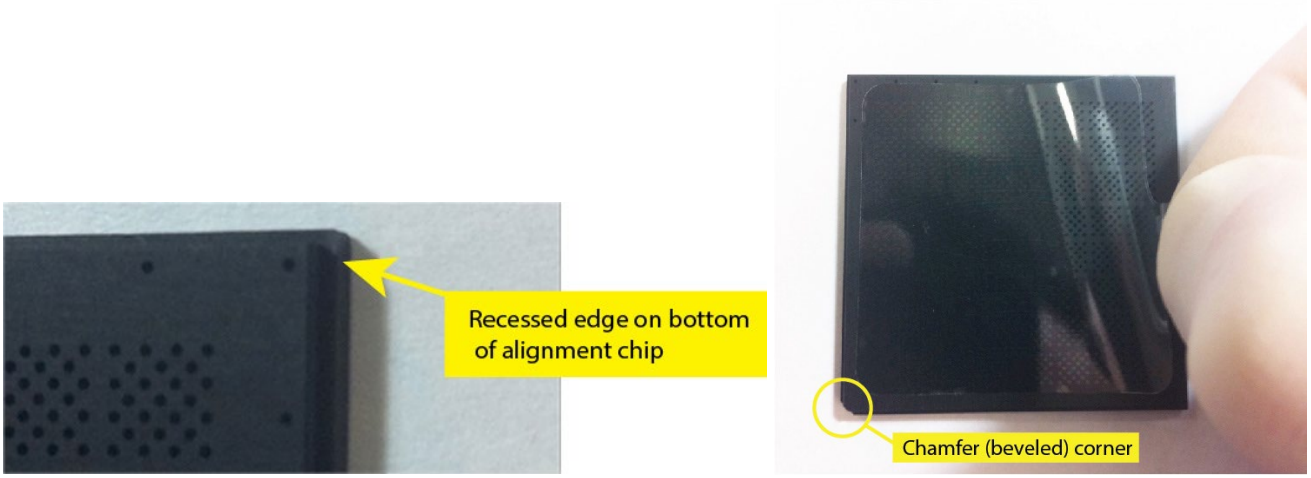


Figure 42. Sealing the Alignment Chip with Nanodispenser Alignment Chip Film.

    c. Remove any air trapped between the film and the Alignment Chip by pushing the bubbles toward the outer edges of the film.
3. Position the chip on the SmartChip MSND as described below.
    a. Turn the chip over so that the film is on the bottom and place the chip on the dispensing platform of the SmartChip MSND, with the chamfer (beveled corner) on the lower right. Be sure that the chip is flat, the chamfer is in the lower right corner, and the chip sits snugly against the three alignment posts (Figure 43).

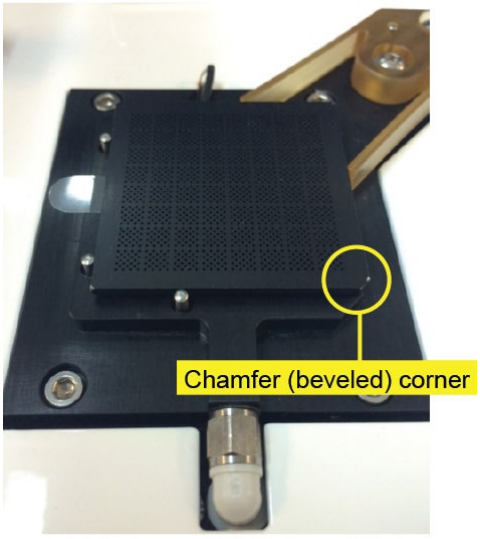


Figure 43. Position the sealed Alignment Chip on the SmartChip MSND.

    b. Close the front and side doors.

4. Perform the alignment verification using the SmartChip Dispenser Software. Click the *Run* tab and then click the [Alignment Verification] button (Figure 44) to start dispensing.

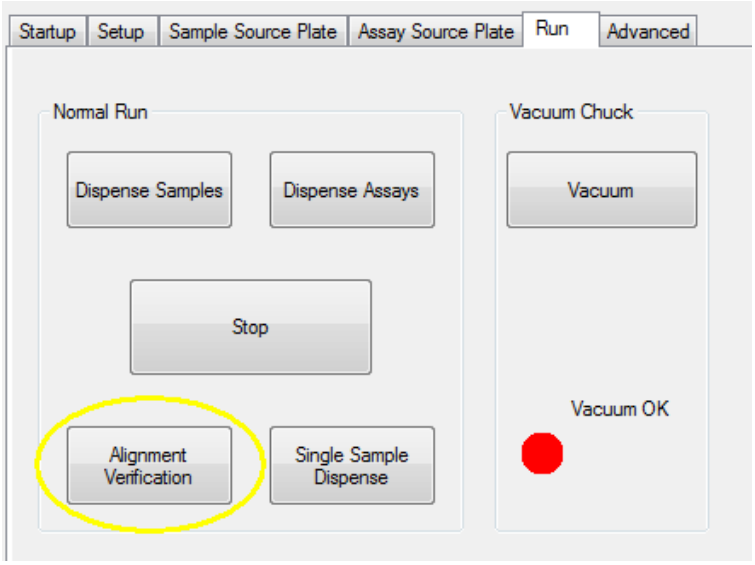


Figure 44. *Run* tab and [Alignment Verification] button.

5. When dispensing is complete, evaluate the results. Inspect the Nanodispenser Alignment Chip under UV light and magnification and note any fluorescent artifacts on the surface of the chip.

NOTE: Droplets on the upper edge of nanowell walls will sometimes appear to be outside of the nanowell. By changing the angle of illumination with the UV light, you should be able to better discriminate whether droplets are inside or outside of the nanowells.

- A few small artifacts on the surface of the chip are normal. Because the wells in the Nanodispenser Alignment Chip are smaller than the wells on a standard SmartChip nanowell chip, the presence of a few small drops on the chip surface does not indicate that the system is out of alignment.
- If the number of fluorescent droplets outside the nanowells exceeds 16 (>1%), it may indicate that the system is out of alignment. Repeat the Daily Warmup procedure and Alignment Verification. If the problem persists, contact Takara Bio technical support.
- Droplets may be present on the chip surface in a consistent direction (e.g., always on the right side of the wells), which may indicate misalignment. See Figure 45 (below) for an acceptable alignment verification run and one that warrants a call to Takara Bio technical support.

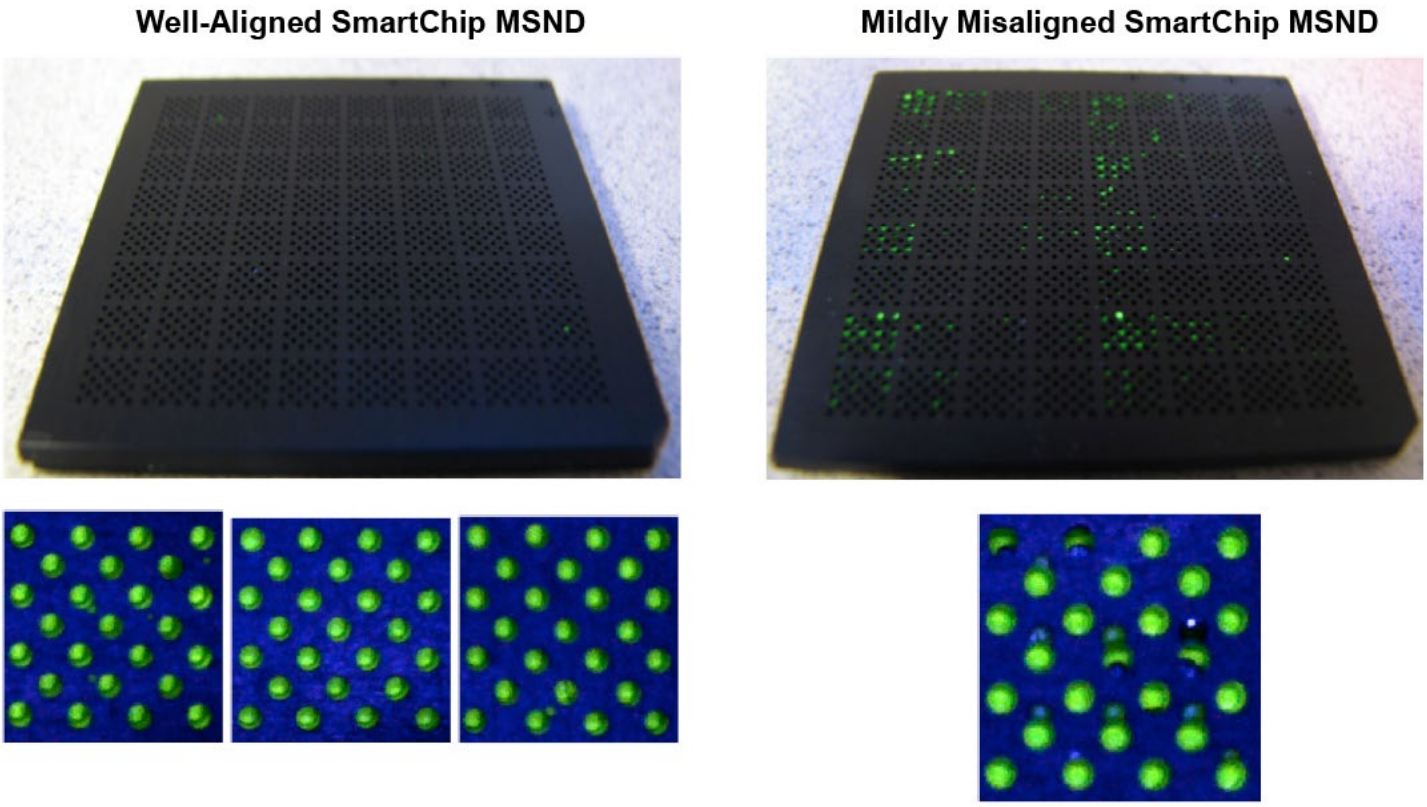


Figure 45. Alignment Verification run results. In the photos on the left, some random droplets are visible. This is expected. In the photos on the right, drops are consistently on the top side of the nanowells. This indicates misalignment.

6. Clean the Nanodispenser Alignment Chip as follows:
    a. Use the tab to carefully peel the film from the chip.
    b. Thoroughly rinse the chip, first with deionized water, then with methyl, ethyl, or isopropyl alcohol.
    c. Dry the chip with compressed air.

E. Common Replacement Parts

- External fuse (installed in the Power Entry Module): 5 x 20 mm 5A Time Lag (Slo-Blo)
- Replace Wash Tubing in the Peristaltic Pump Box with Flexelene tubing (Eldon James Company, Cat. No. FX1-2W)

F. Bi-Annual Preventative Maintenance

Have the SmartChip MSND examined and calibrated every six months by a Takara Bio service engineer.

X. Troubleshooting

If the SmartChip MSND or SmartChip Dispenser Software does not respond as desired or a warning is displayed, please attempt to rectify the problem using the tables below. If you cannot solve the problem, contact Takara Bio technical support.

Table XI. Problem: Hanging drop, drop dispersion, or improper dispensing.

Possible cause	Solution
Trapped air in the system fluidics	A large volume of air in the tube will act like a spring. When the microsolenoid valve is actuated, the air will absorb the pulse, leaving a drop hanging on the end of the tip. Inspect the tube between the tip and the 2 x 6 manifold (where not covered). Look for air or bubbles within the tube. If air is found within the tube, move the tips to the wash position and prime the system by clicking the [Daily Warmup] button.
Faulty tip connection	Inspect the slip fits on the tips and on the 2 x 6 manifold. The tubing must fit snugly to the stainless steel tube. If the tube can be removed easily, cut off a small section of the tube and reseat the tube.
Crimped fluidic tubing	Inspect the tubing for crimps or bends. Remove crimped/bent sections if feasible or replace the harness.
Plugged tip	Back-flush the tips by clicking the [Tip Clean] button in the SmartChip Dispenser Software. If the tip is plugged with a soluble material, aspirating and dispensing reagent capable of dissolving the material may clear the blockage. If these measures fail, it may be necessary to replace the tip, or the blockage could be upstream of the tip. Contact Takara Bio technical support.
Leaking inlet valve	Let the system sit idle for 5 min and inspect the dispensing tips. If there are water bubbles at any of the tips, the corresponding solenoid is leaking. Contact Takara Bio technical support.
Incompatible buffers and samples	Extremes of fluidic properties, such as viscosity, may result in poor performance. Contact Takara Bio technical support.
Tubing in the Pump Box is cracked	Replace existing Wash tubing with Flexelene tubing (Eldon James Company, Cat. No. FX1-2W).

Table XII. Problem: Low Pressure error displayed.

Possible cause	Solution
Pressure is too low	Check that the helium supply regulator is set to 30 psig (2 bar) maximum for a standard system. Contact Takara Bio technical support.

Table XIII. Problem: Dispensing head does not home.

Possible cause	Solution
No communication with the instrument	Cycle the power on the Fluidic Module and restart the computer.

Table XIV. Problem: Low or partial dispenses.

Possible cause	Solution
Microsolenoid valve failure	Contact Takara Bio technical support.
Relative height of system components	The relative height of the Pressure Reservoir, Stage Module, and Fluidic Module must be the same as when the system was installed. If you change the height of any component and are seeing low or partial dispenses, contact Takara Bio technical support.
Crimped tubing	See “Hanging drop” (Table XI) above.
Pressure too low	Check that the helium supply regulator is set to 30 psig (2 bar) maximum for a standard system. Contact Takara Bio technical support.

Table XV. Problem: Apparent low sample concentration.

Possible cause	Solution
Syringe thumbscrew is loose	Tighten the syringe thumbscrew until finger tight. The thumbscrew is located at the bottom of the syringe in the Fluidic Module.
Tip is plugged	See “Hanging drop” (Table XI) above.
Air bubble in the syringe path	Prime the syringe path by performing the Daily Warmup.
Microsolenoid valve is leaky	See “Hanging Drop > Leaking inlet valve” (Table XI) above.
Syringe valve is blocked or leaky	See “Hanging Drop > Leaking inlet valve” (Table XI) above.
Low liquid level in the Pressure Reservoir	Check the level of the system liquid in the Pressure Reservoir. Make sure that the end of the tubing is submerged in the water. Add deionized, degassed water as needed (see “Power on the System”, Section V.A).

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

Possible cause	Solution
No power	Inspect the power cables and connections.
Syringe is not initialized	Cycle the power on the Fluidic Module and restart the computer.
Obstruction	Verify that the syringes are not obstructed.

Table XVII. Problem: Persistent soft clicking. The digital pressure regulator in the Fluidic Module has a soft click during normal operation to maintain pressure.

Possible cause	Solution
Helium leak in the system	Place soapy water around the following gas connections in the system: • 5-port cap connection to the Pressure Reservoir • All 5-port cap ports • Inlet and outlet of pressure relief valve • Gas outlet connection at the back of the Fluidic Module • Gas inlet connection at the back of the Fluidic Module • Helium tank connections Bubbles are an indication of a leak at that connection. Tighten the fitting.
	Check for holes in the tubing from the gas outlet of the Fluidic Module into the helium input ports (normally the green and blue ports of the Pressure Reservoir).
	Check that the O-ring underneath the 5-port cap is seated correctly.
	Ensure that the stopcock for the vent port on the Pressure Reservoir is in the closed position (i.e., the back port is closed on a standard pressure bottle).
	Ensure that the ferrule orientations for the helium input ports on the Pressure Reservoir are correct.

Table XVIII. Problem: Loud digital pressure regulator chattering.

Possible cause	Solution
Gas path blocked at the Pressure Reservoir inlet	Ensure that the stopcock for the standard helium input port on the Pressure Reservoir is in the open position (this is the blue port on a standard reservoir bottle and should be in the vertical position). Verify that the input pressure from the helium tank regulator is in the appropriate range and not too high.

Table XIX. Problem: Soft digital pressure regulator clicking and fluid leak observed.

Possible cause	Solution
Fluid leak in the system downstream of the helium input	Locate the source of the leak. Tighten the fittings and tubing at the source of the leak. Possible sources include the following: • Liquid output ports on the Pressure Reservoir (red and yellow ports on a standard bottle). Check that the fittings are tight and that the ferrule orientations are correct. • 8-port manifold • Hole in tubing • Connections at the microsolenoid valve • Anywhere in the syringe or pressure paths

A. Technical Support

If you require technical support, please contact your authorized Takara Bio service technician, or contact us directly at: techUS@takarabio.com.

Appendix A: Preparing Source Plate Files in a Text Editor

Source Plate files describe the contents of the 384-well Source Plates that the SmartChip MSND 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.

Source Plate files must be text (*.txt) files, with descriptive header information followed by a single line of tab-delimited text describing the contents of each well.

NOTE: Keep the following in mind when creating a Source Plate file:

- Spaces will create invalid information.
- Always use <tab> as a column separator in Source Plate files.
- Valid Sample and Assay names can contain a–z, 0–9, and _. Do not use the / symbol.

To create your own Source Plate files, we recommend that you copy the format from the appropriate Source Plate template file that is installed with the SmartChip Dispenser Software.

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

1. In Excel, open the Source Plate template file corresponding to the type of Source Plate you want to document.

   The template files are found in the following directories:

   - **Windows 7:** `C:\ProgramData\WaferGen\SmartChip Dispenser`
   - **Windows XP:** `C:\Documents and Settings\All Users\Application Data\WaferGen\SmartChip Dispenser`

   There are folders for Assay and Sample Source Plate files, and inside each of those are folders named `Templates`. The filename conventions for the Source Plate template files are:

   - MD or PD for MyDesign or predesigned, respectively
   - Number of PCR Assays in the SmartChip layout (e.g., 12A, 48A, or 72A)
   - Number of replicate PCR Assays (e.g., 1 for genotyping, 4 for expression analysis)
   - Number of Samples (e.g., 3S or 12S)
   - Source Plate type (e.g., Sourceplate_File or Assay_Sourceplate_File)
   - File extension (*.txt)

2. 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” or “Well” 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” below.

- **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. In addition, this text is case-sensitive.
- **Do not change the information in the "Source" or "Well" column.** This column gives the locations of your Sample mixtures or Assays in the 384-well Source Plate and cannot be changed. The SmartChip MSND 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.

3. Save your Source Plate file in the `Assay Source Plate files` or `Sample Source Plate files` folder. Do not save the file in the `Templates` folder. Give the file a descriptive name. The Sample and Assay attributes that can be entered for different types of chips are shown in the tables below.

Table XX. Attributes for Sample Source Plate files for expression analysis.

Expression analysis			
Header (description)	SampleName (sample name)	Concentration (sample concentration)	Source (location in the plate)
Data	Fill in (required)	Fill in (optional)	(Do not change!)

Table XXI. Attributes for Sample Source Plate files for SNP genotyping.

SNP genotyping							
Header (description)	SampleName (sample name)	Concentration (sample DNA concentration)	Source (location in the plate)	Gender (sample gender)	Population (sample population)	Custom 1	Custom 2
Data	Fill in (required)	Fill in (optional)	(Do not change!)	Fill in (optional)	Fill in (optional)	Fill in (optional)	Fill in (optional)

Table XXII. Attributes for Assay Source Plate files for expression analysis.

SNP genotyping					
Header (description)	AssayName (assay name)	Source (location in the source plate)	AssayID (assay ID)	Tm (amplicon T_m)	IsHousekeeping (reference gene product assay)
Data	Fill in (required)	(Do not change!)	Fill in (required)	Fill in (optional)	Fill in yes or no (optional)

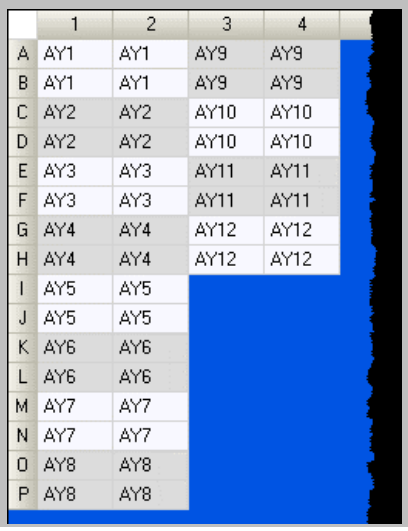
Table XXIII. Attributes for Assay Source Plate files for SNP genotyping.

SNP genotyping					
Header (description)	ID (assay ID)	Source (location in the source plate)	Name (assay name)	Gene Symbol (target gene)	Category ID
Data	Fill in (required)	(Do not change!)	Fill in (required)	Fill in (optional)	Fill in (optional)

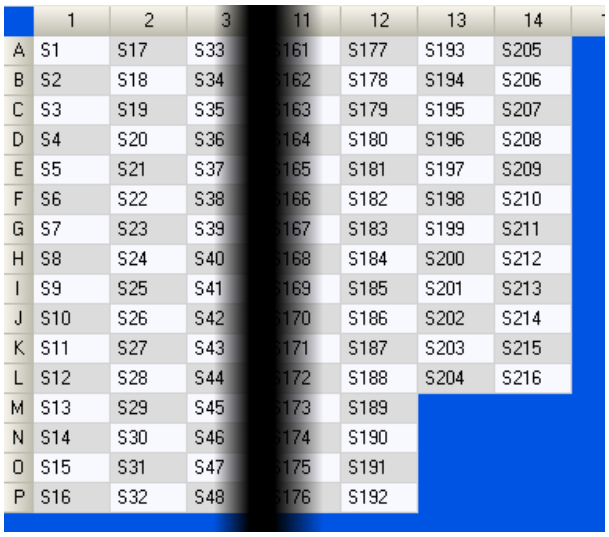
Sample and Assay Source Plates for SmartChip MyDesign Chips

Table XXIV. Sample and Assay Source Plates for SmartChip MyDesign Chips for the following assay x sample formats: 12 x 384 and 24 x 216.

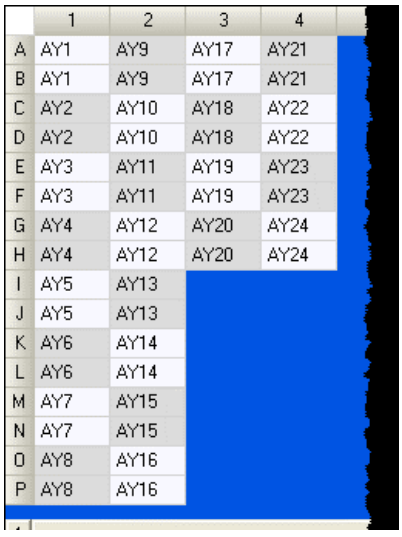
SmartChip Layout		Volume and # of wells in Source Plate	
Assays	**Samples**	**Sample Source Plate**	**Assay Source Plate**
12	384	11.7 µl/ well Load samples starting at the top left of the plate (well A1), first running down the columns, and filling wells in each column from left to right. Fill all of the wells of the 384-well plate.	17.9 µl in each of 4 wells
24	216	12.4 µl/well	17.9 µl in each of 2 wells



	1	2	3	4
A	AY1	AY1	AY9	AY9
B	AY1	AY1	AY9	AY9
C	AY2	AY2	AY10	AY10
D	AY2	AY2	AY10	AY10
E	AY3	AY3	AY11	AY11
F	AY3	AY3	AY11	AY11
G	AY4	AY4	AY12	AY12
H	AY4	AY4	AY12	AY12
I	AY5	AY5		
J	AY5	AY5		
K	AY6	AY6		
L	AY6	AY6		
M	AY7	AY7		
N	AY7	AY7		
O	AY8	AY8		
P	AY8	AY8		



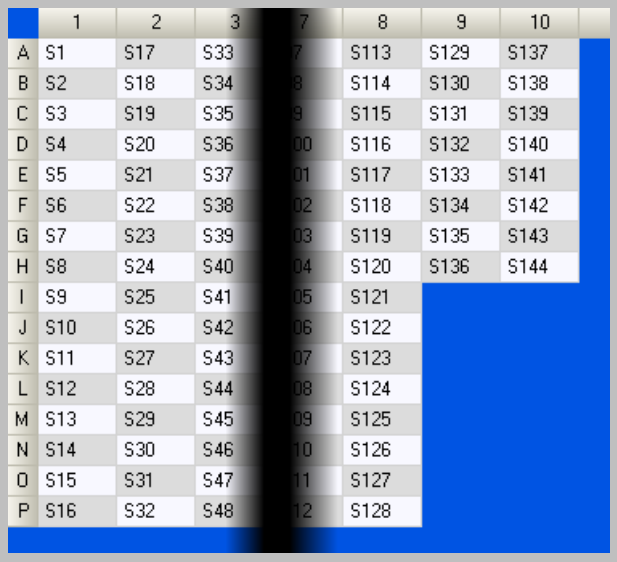
	1	2	3	11	12	13	14
A	S1	S17	S33	S161	S177	S193	S205
B	S2	S18	S34	S162	S178	S194	S206
C	S3	S19	S35	S163	S179	S195	S207
D	S4	S20	S36	S164	S180	S196	S208
E	S5	S21	S37	S165	S181	S197	S209
F	S6	S22	S38	S166	S182	S198	S210
G	S7	S23	S39	S167	S183	S199	S211
H	S8	S24	S40	S168	S184	S200	S212
I	S9	S25	S41	S169	S185	S201	S213
J	S10	S26	S42	S170	S186	S202	S214
K	S11	S27	S43	S171	S187	S203	S215
L	S12	S28	S44	S172	S188	S204	S216
M	S13	S29	S45	S173	S189		
N	S14	S30	S46	S174	S190		
O	S15	S31	S47	S175	S191		
P	S16	S32	S48	S176	S192		



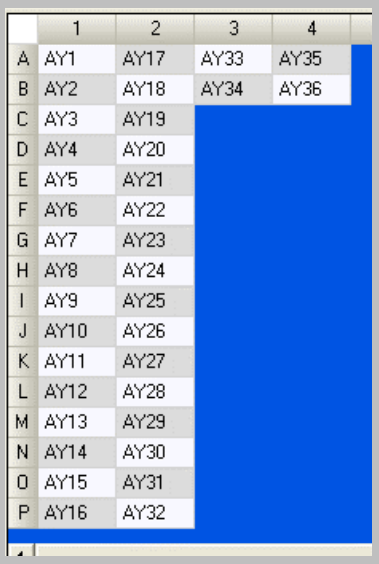
	1	2	3	4
A	AY1	AY9	AY17	AY21
B	AY1	AY9	AY17	AY21
C	AY2	AY10	AY18	AY22
D	AY2	AY10	AY18	AY22
E	AY3	AY11	AY19	AY23
F	AY3	AY11	AY19	AY23
G	AY4	AY12	AY20	AY24
H	AY4	AY12	AY20	AY24
I	AY5	AY13		
J	AY5	AY13		
K	AY6	AY14		
L	AY6	AY14		
M	AY7	AY15		
N	AY7	AY15		
O	AY8	AY16		
P	AY8	AY16		

Table XXV. Sample and Assay Source Plates for SmartChip MyDesign Chips for the following assay x sample formats: 36 x 144 and 48 x 108.

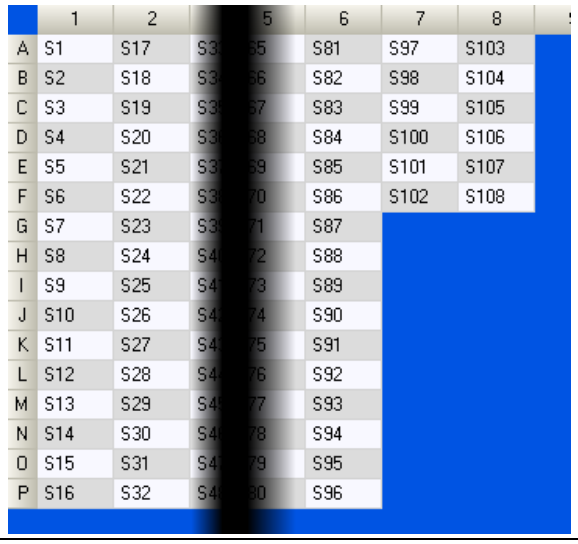
SmartChip Layout		Volume and # of wells in Source Plate	
Assays	Samples	Sample Source Plate	Assay Source Plate
36	144	13.2 µl/ well	20.3 µl/well
48	108	14.0 µl/well	17.9 µl/well



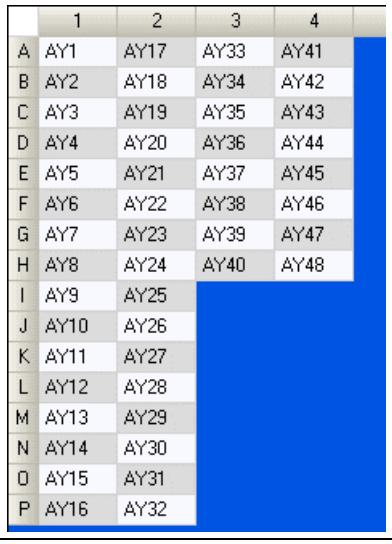
	1	2	3	7	8	9	10
A	S1	S17	S33	7	S113	S129	S137
B	S2	S18	S34	8	S114	S130	S138
C	S3	S19	S35	9	S115	S131	S139
D	S4	S20	S36	00	S116	S132	S140
E	S5	S21	S37	01	S117	S133	S141
F	S6	S22	S38	02	S118	S134	S142
G	S7	S23	S39	03	S119	S135	S143
H	S8	S24	S40	04	S120	S136	S144
I	S9	S25	S41	05	S121		
J	S10	S26	S42	06	S122		
K	S11	S27	S43	07	S123		
L	S12	S28	S44	08	S124		
M	S13	S29	S45	09	S125		
N	S14	S30	S46	10	S126		
O	S15	S31	S47	11	S127		
P	S16	S32	S48	12	S128		



	1	2	3	4
A	AY1	AY17	AY33	AY35
B	AY2	AY18	AY34	AY36
C	AY3	AY19		
D	AY4	AY20		
E	AY5	AY21		
F	AY6	AY22		
G	AY7	AY23		
H	AY8	AY24		
I	AY9	AY25		
J	AY10	AY26		
K	AY11	AY27		
L	AY12	AY28		
M	AY13	AY29		
N	AY14	AY30		
O	AY15	AY31		
P	AY16	AY32		



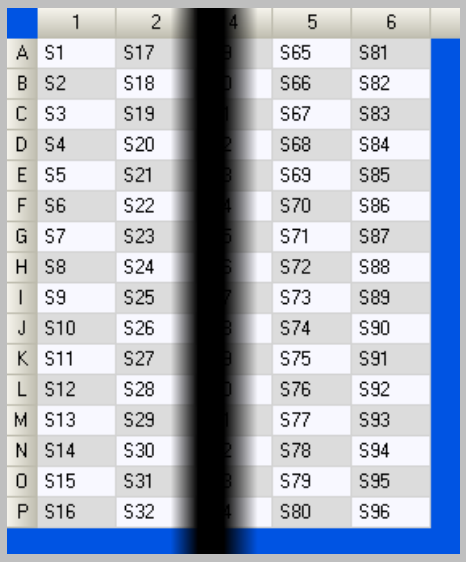
	1	2	5	6	7	8
A	S1	S17	5	S81	S97	S103
B	S2	S18	6	S82	S98	S104
C	S3	S19	7	S83	S99	S105
D	S4	S20	8	S84	S100	S106
E	S5	S21	9	S85	S101	S107
F	S6	S22	70	S86	S102	S108
G	S7	S23	71	S87		
H	S8	S24	72	S88		
I	S9	S25	73	S89		
J	S10	S26	74	S90		
K	S11	S27	75	S91		
L	S12	S28	76	S92		
M	S13	S29	77	S93		
N	S14	S30	78	S94		
O	S15	S31	79	S95		
P	S16	S32	80	S96		



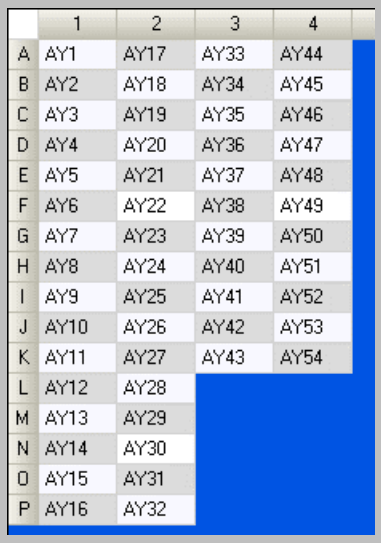
	1	2	3	4
A	AY1	AY17	AY33	AY41
B	AY2	AY18	AY34	AY42
C	AY3	AY19	AY35	AY43
D	AY4	AY20	AY36	AY44
E	AY5	AY21	AY37	AY45
F	AY6	AY22	AY38	AY46
G	AY7	AY23	AY39	AY47
H	AY8	AY24	AY40	AY48
I	AY9	AY25		
J	AY10	AY26		
K	AY11	AY27		
L	AY12	AY28		
M	AY13	AY29		
N	AY14	AY30		
O	AY15	AY31		
P	AY16	AY32		

Table XXVI. Sample and Assay Source Plates for SmartChip MyDesign Chips for the following assay x sample formats: 54 x 96 and 72 x 72.

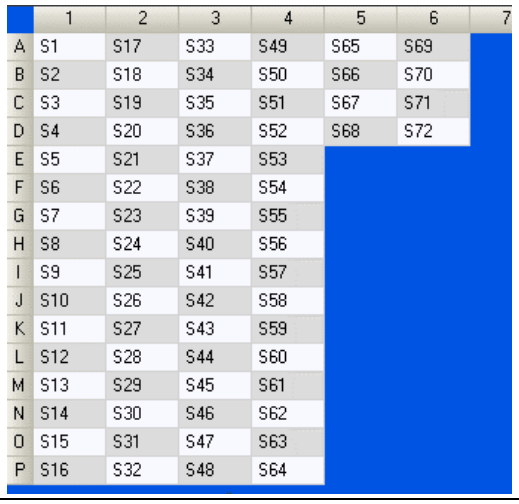
SmartChip Layout		Volume and # of wells in Source Plate	
Assays	Samples	Sample Source Plate	Assay Source Plate
54	96	14.4 µl/ well	17.1 µl/well
72	72	15.6 µl/well	15.6 µl/well



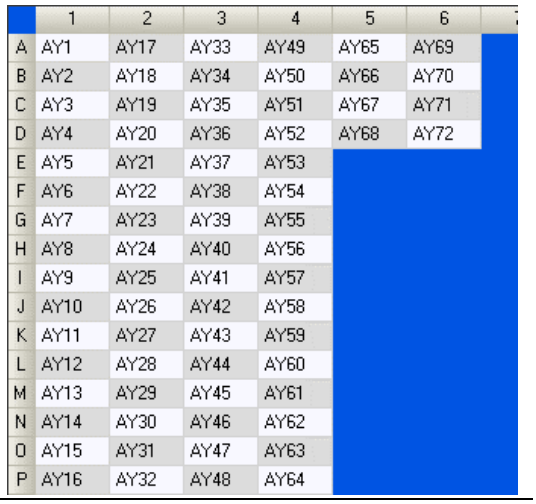
	1	2	4	5	6
A	S1	S17		S65	S81
B	S2	S18		S66	S82
C	S3	S19		S67	S83
D	S4	S20		S68	S84
E	S5	S21		S69	S85
F	S6	S22		S70	S86
G	S7	S23		S71	S87
H	S8	S24		S72	S88
I	S9	S25		S73	S89
J	S10	S26		S74	S90
K	S11	S27		S75	S91
L	S12	S28		S76	S92
M	S13	S29		S77	S93
N	S14	S30		S78	S94
O	S15	S31		S79	S95
P	S16	S32		S80	S96



	1	2	3	4
A	AY1	AY17	AY33	AY44
B	AY2	AY18	AY34	AY45
C	AY3	AY19	AY35	AY46
D	AY4	AY20	AY36	AY47
E	AY5	AY21	AY37	AY48
F	AY6	AY22	AY38	AY49
G	AY7	AY23	AY39	AY50
H	AY8	AY24	AY40	AY51
I	AY9	AY25	AY41	AY52
J	AY10	AY26	AY42	AY53
K	AY11	AY27	AY43	AY54
L	AY12	AY28		
M	AY13	AY29		
N	AY14	AY30		
O	AY15	AY31		
P	AY16	AY32		



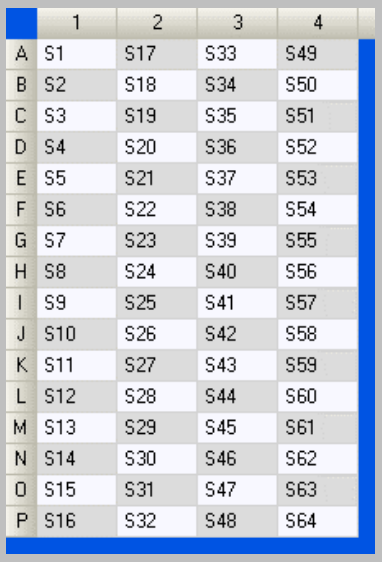
	1	2	3	4	5	6	7
A	S1	S17	S33	S49	S65	S69	
B	S2	S18	S34	S50	S66	S70	
C	S3	S19	S35	S51	S67	S71	
D	S4	S20	S36	S52	S68	S72	
E	S5	S21	S37	S53			
F	S6	S22	S38	S54			
G	S7	S23	S39	S55			
H	S8	S24	S40	S56			
I	S9	S25	S41	S57			
J	S10	S26	S42	S58			
K	S11	S27	S43	S59			
L	S12	S28	S44	S60			
M	S13	S29	S45	S61			
N	S14	S30	S46	S62			
O	S15	S31	S47	S63			
P	S16	S32	S48	S64			



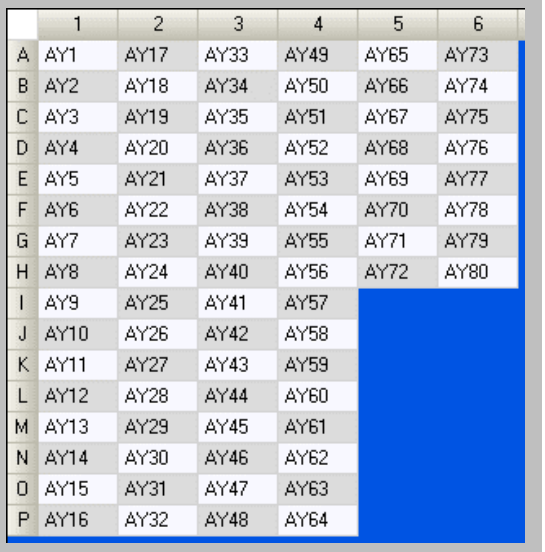
	1	2	3	4	5	6
A	AY1	AY17	AY33	AY49	AY65	AY69
B	AY2	AY18	AY34	AY50	AY66	AY70
C	AY3	AY19	AY35	AY51	AY67	AY71
D	AY4	AY20	AY36	AY52	AY68	AY72
E	AY5	AY21	AY37	AY53		
F	AY6	AY22	AY38	AY54		
G	AY7	AY23	AY39	AY55		
H	AY8	AY24	AY40	AY56		
I	AY9	AY25	AY41	AY57		
J	AY10	AY26	AY42	AY58		
K	AY11	AY27	AY43	AY59		
L	AY12	AY28	AY44	AY60		
M	AY13	AY29	AY45	AY61		
N	AY14	AY30	AY46	AY62		
O	AY15	AY31	AY47	AY63		
P	AY16	AY32	AY48	AY64		

Table XXVII. Sample and Assay Source Plates for SmartChip MyDesign Chips for the following assay x sample formats: 80 x 64 and 96 x 54.

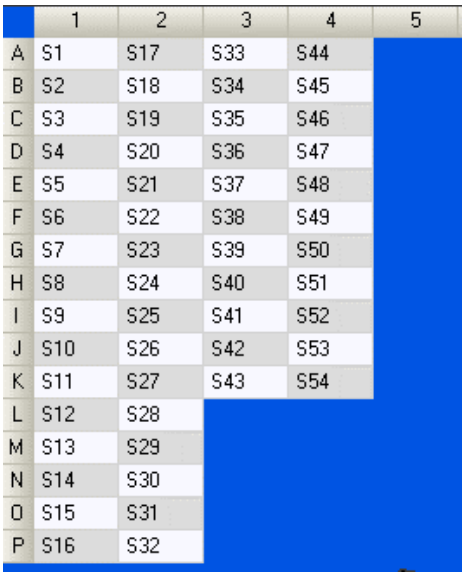
SmartChip Layout		Volume and # of wells in Source Plate	
Assays	Samples	Sample Source Plate	Assay Source Plate
80	64	16.1 µl/ well	15.1 µl/well
96	54	17.1 µl/well	14.4 µl/well



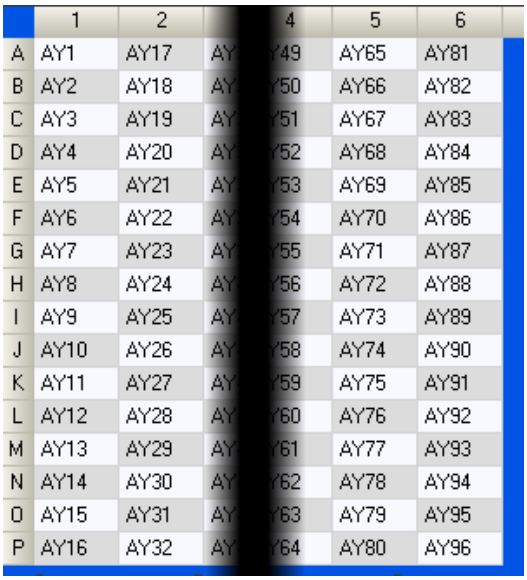
	1	2	3	4
A	S1	S17	S33	S49
B	S2	S18	S34	S50
C	S3	S19	S35	S51
D	S4	S20	S36	S52
E	S5	S21	S37	S53
F	S6	S22	S38	S54
G	S7	S23	S39	S55
H	S8	S24	S40	S56
I	S9	S25	S41	S57
J	S10	S26	S42	S58
K	S11	S27	S43	S59
L	S12	S28	S44	S60
M	S13	S29	S45	S61
N	S14	S30	S46	S62
O	S15	S31	S47	S63
P	S16	S32	S48	S64



	1	2	3	4	5	6
A	AY1	AY17	AY33	AY49	AY65	AY73
B	AY2	AY18	AY34	AY50	AY66	AY74
C	AY3	AY19	AY35	AY51	AY67	AY75
D	AY4	AY20	AY36	AY52	AY68	AY76
E	AY5	AY21	AY37	AY53	AY69	AY77
F	AY6	AY22	AY38	AY54	AY70	AY78
G	AY7	AY23	AY39	AY55	AY71	AY79
H	AY8	AY24	AY40	AY56	AY72	AY80
I	AY9	AY25	AY41	AY57		
J	AY10	AY26	AY42	AY58		
K	AY11	AY27	AY43	AY59		
L	AY12	AY28	AY44	AY60		
M	AY13	AY29	AY45	AY61		
N	AY14	AY30	AY46	AY62		
O	AY15	AY31	AY47	AY63		
P	AY16	AY32	AY48	AY64		



	1	2	3	4	5
A	S1	S17	S33	S44	
B	S2	S18	S34	S45	
C	S3	S19	S35	S46	
D	S4	S20	S36	S47	
E	S5	S21	S37	S48	
F	S6	S22	S38	S49	
G	S7	S23	S39	S50	
H	S8	S24	S40	S51	
I	S9	S25	S41	S52	
J	S10	S26	S42	S53	
K	S11	S27	S43	S54	
L	S12	S28			
M	S13	S29			
N	S14	S30			
O	S15	S31			
P	S16	S32			



	1	2	4	5	6
A	AY1	AY17	AY49	AY65	AY81
B	AY2	AY18	AY50	AY66	AY82
C	AY3	AY19	AY51	AY67	AY83
D	AY4	AY20	AY52	AY68	AY84
E	AY5	AY21	AY53	AY69	AY85
F	AY6	AY22	AY54	AY70	AY86
G	AY7	AY23	AY55	AY71	AY87
H	AY8	AY24	AY56	AY72	AY88
I	AY9	AY25	AY57	AY73	AY89
J	AY10	AY26	AY58	AY74	AY90
K	AY11	AY27	AY59	AY75	AY91
L	AY12	AY28	AY60	AY76	AY92
M	AY13	AY29	AY61	AY77	AY93
N	AY14	AY30	AY62	AY78	AY94
O	AY15	AY31	AY63	AY79	AY95
P	AY16	AY32	AY64	AY80	AY96

Table XXVIII. Sample and Assay Source Plates for SmartChip MyDesign Chips for the following assay x sample formats: 120 x 42 and 144 x 36.

SmartChip Layout		Volume and # of wells in Source Plate	
Assays	Samples	Sample Source Plate	Assay Source Plate
120	42	18.9 µl/ well	13.7 µl/well

	1	2	3	4
A	S1	S17	S33	S38
B	S2	S18	S34	S39
C	S3	S19	S35	S40
D	S4	S20	S36	S41
E	S5	S21	S37	S42
F	S6	S22		
G	S7	S23		
H	S8	S24		
I	S9	S25		
J	S10	S26		
K	S11	S27		
L	S12	S28		
M	S13	S29		
N	S14	S30		
O	S15	S31		
P	S16	S32		

	1	2	3	6	7	8
A	AY1	AY16	AY31	AY76	AY91	AY106
B	AY2	AY17	AY32	AY77	AY92	AY107
C	AY3	AY18	AY33	AY78	AY93	AY108
D	AY4	AY19	AY34	AY79	AY94	AY109
E	AY5	AY20	AY35	AY80	AY95	AY110
F	AY6	AY21	AY36	AY81	AY96	AY111
G	AY7	AY22	AY37	AY82	AY97	AY112
H	AY8	AY23	AY38	AY83	AY98	AY113
I	AY9	AY24	AY39	AY84	AY99	AY114
J	AY10	AY25	AY40	AY85	AY100	AY115
K	AY11	AY26	AY41	AY86	AY101	AY116
L	AY12	AY27	AY42	AY87	AY102	AY117
M	AY13	AY28	AY43	AY88	AY103	AY118
N	AY14	AY29	AY44	AY89	AY104	AY119
O	AY15	AY30	AY45	AY90	AY105	AY120

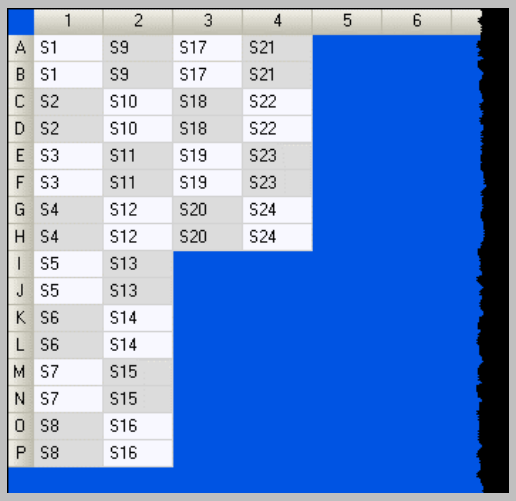
144	36	20.3 µl/well	13.2 µl/well	

	1	2	3	4
A	S1	S17	S33	S35
B	S2	S18	S34	S36
C	S3	S19		
D	S4	S20		
E	S5	S21		
F	S6	S22		
G	S7	S23		
H	S8	S24		
I	S9	S25		
J	S10	S26		
K	S11	S27		
L	S12	S28		
M	S13	S29		
N	S14	S30		
O	S15	S31		
P	S16	S32		

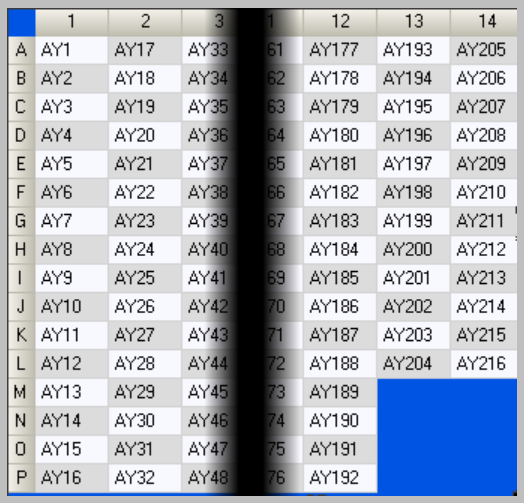
	1	2	7	8	9	10
A	AY1	AY17	A 97	AY113	AY129	AY137
B	AY2	AY18	A 98	AY114	AY130	AY138
C	AY3	AY19	A 99	AY115	AY131	AY139
D	AY4	AY20	A 100	AY116	AY132	AY140
E	AY5	AY21	A 101	AY117	AY133	AY141
F	AY6	AY22	A 102	AY118	AY134	AY142
G	AY7	AY23	A 103	AY119	AY135	AY143
H	AY8	AY24	A 104	AY120	AY136	AY144
I	AY9	AY25	A 105	AY121		
J	AY10	AY26	A 106	AY122		
K	AY11	AY27	A 107	AY123		
L	AY12	AY28	A 108	AY124		
M	AY13	AY29	A 109	AY125		
N	AY14	AY30	A 110	AY126		
O	AY15	AY31	A 111	AY127		
P	AY16	AY32	A 112	AY128		

Table XXIX. Sample and Assay Source Plates for SmartChip MyDesign Chips for the following assay x sample formats: 216 x 24 and 248 x 20.

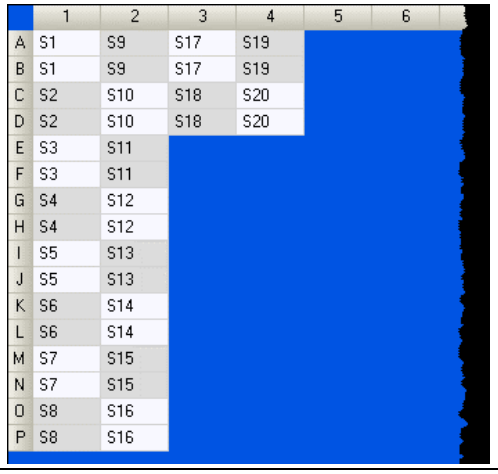
SmartChip Layout		Volume and # of wells in Source Plate	
Assays	Samples	Sample Source Plate	Assay Source Plate
216	24	17.9 µl in each of 2 wells	12.4 µl/well
248	20	19.4 µl in each of 2 wells	12.2 µl/well



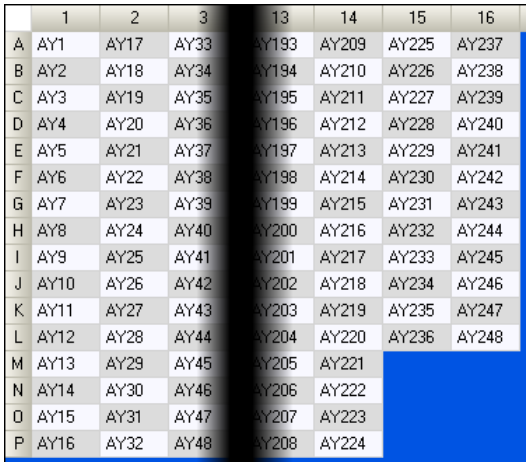
	1	2	3	4	5	6
A	S1	S9	S17	S21		
B	S1	S9	S17	S21		
C	S2	S10	S18	S22		
D	S2	S10	S18	S22		
E	S3	S11	S19	S23		
F	S3	S11	S19	S23		
G	S4	S12	S20	S24		
H	S4	S12	S20	S24		
I	S5	S13				
J	S5	S13				
K	S6	S14				
L	S6	S14				
M	S7	S15				
N	S7	S15				
O	S8	S16				
P	S8	S16				



	1	2	3	12	13	14
A	AY1	AY17	AY33	AY177	AY193	AY205
B	AY2	AY18	AY34	AY178	AY194	AY206
C	AY3	AY19	AY35	AY179	AY195	AY207
D	AY4	AY20	AY36	AY180	AY196	AY208
E	AY5	AY21	AY37	AY181	AY197	AY209
F	AY6	AY22	AY38	AY182	AY198	AY210
G	AY7	AY23	AY39	AY183	AY199	AY211
H	AY8	AY24	AY40	AY184	AY200	AY212
I	AY9	AY25	AY41	AY185	AY201	AY213
J	AY10	AY26	AY42	AY186	AY202	AY214
K	AY11	AY27	AY43	AY187	AY203	AY215
L	AY12	AY28	AY44	AY188	AY204	AY216
M	AY13	AY29	AY45	AY189		
N	AY14	AY30	AY46	AY190		
O	AY15	AY31	AY47	AY191		
P	AY16	AY32	AY48	AY192		



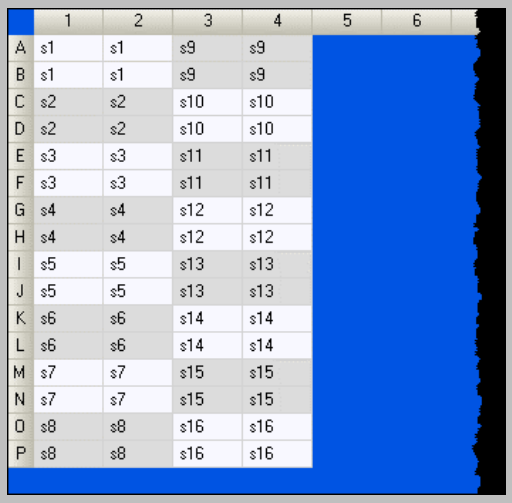
	1	2	3	4	5	6
A	S1	S9	S17	S19		
B	S1	S9	S17	S19		
C	S2	S10	S18	S20		
D	S2	S10	S18	S20		
E	S3	S11				
F	S3	S11				
G	S4	S12				
H	S4	S12				
I	S5	S13				
J	S5	S13				
K	S6	S14				
L	S6	S14				
M	S7	S15				
N	S7	S15				
O	S8	S16				
P	S8	S16				



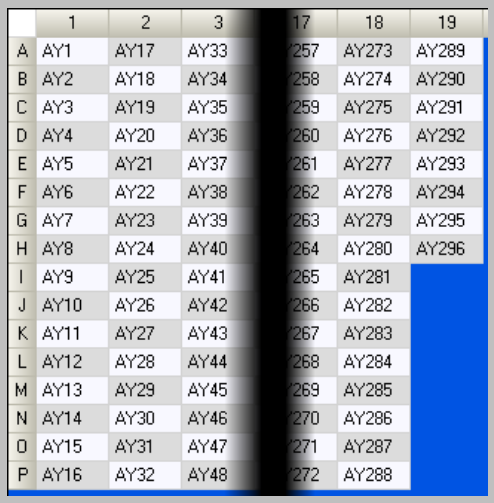
	1	2	3	13	14	15	16
A	AY1	AY17	AY33	AY193	AY209	AY225	AY237
B	AY2	AY18	AY34	AY194	AY210	AY226	AY238
C	AY3	AY19	AY35	AY195	AY211	AY227	AY239
D	AY4	AY20	AY36	AY196	AY212	AY228	AY240
E	AY5	AY21	AY37	AY197	AY213	AY229	AY241
F	AY6	AY22	AY38	AY198	AY214	AY230	AY242
G	AY7	AY23	AY39	AY199	AY215	AY231	AY243
H	AY8	AY24	AY40	AY200	AY216	AY232	AY244
I	AY9	AY25	AY41	AY201	AY217	AY233	AY245
J	AY10	AY26	AY42	AY202	AY218	AY234	AY246
K	AY11	AY27	AY43	AY203	AY219	AY235	AY247
L	AY12	AY28	AY44	AY204	AY220	AY236	AY248
M	AY13	AY29	AY45	AY205	AY221		
N	AY14	AY30	AY46	AY206	AY222		
O	AY15	AY31	AY47	AY207	AY223		
P	AY16	AY32	AY48	AY208	AY224		

Table XXX. Sample and Assay Source Plates for SmartChip MyDesign Chips for the following assay x sample formats: 296 x 16 and 384 x 12.

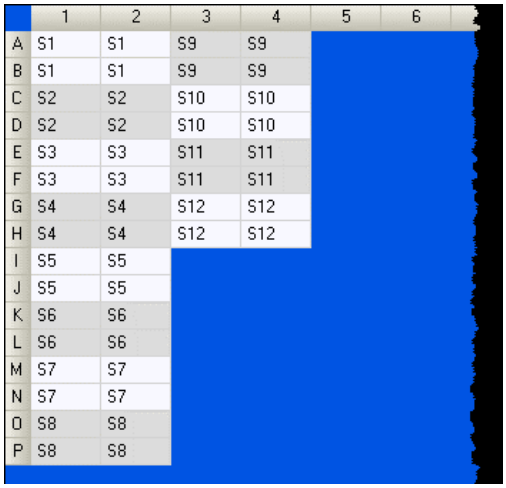
SmartChip Layout		Volume and # of wells in Source Plate	
Assays	Samples	Sample Source Plate	Assay Source Plate
296	16	16.1 µl in each of 4 wells	12.0 µl/well
384	12	17.9 µl in each of 4 wells	11.7 µl/well Load samples starting at the top left of the plate (well A1), running down the columns from left to right. Fill all of the wells of the 384-well plate.



	1	2	3	4	5	6
A	s1	s1	s9	s9		
B	s1	s1	s9	s9		
C	s2	s2	s10	s10		
D	s2	s2	s10	s10		
E	s3	s3	s11	s11		
F	s3	s3	s11	s11		
G	s4	s4	s12	s12		
H	s4	s4	s12	s12		
I	s5	s5	s13	s13		
J	s5	s5	s13	s13		
K	s6	s6	s14	s14		
L	s6	s6	s14	s14		
M	s7	s7	s15	s15		
N	s7	s7	s15	s15		
O	s8	s8	s16	s16		
P	s8	s8	s16	s16		



	1	2	3	17	18	19
A	AY1	AY17	AY33	257	AY273	AY289
B	AY2	AY18	AY34	258	AY274	AY290
C	AY3	AY19	AY35	259	AY275	AY291
D	AY4	AY20	AY36	260	AY276	AY292
E	AY5	AY21	AY37	261	AY277	AY293
F	AY6	AY22	AY38	262	AY278	AY294
G	AY7	AY23	AY39	263	AY279	AY295
H	AY8	AY24	AY40	264	AY280	AY296
I	AY9	AY25	AY41	265	AY281	
J	AY10	AY26	AY42	266	AY282	
K	AY11	AY27	AY43	267	AY283	
L	AY12	AY28	AY44	268	AY284	
M	AY13	AY29	AY45	269	AY285	
N	AY14	AY30	AY46	270	AY286	
O	AY15	AY31	AY47	271	AY287	
P	AY16	AY32	AY48	272	AY288	



	1	2	3	4	5	6
A	S1	S1	S9	S9		
B	S1	S1	S9	S9		
C	S2	S2	S10	S10		
D	S2	S2	S10	S10		
E	S3	S3	S11	S11		
F	S3	S3	S11	S11		
G	S4	S4	S12	S12		
H	S4	S4	S12	S12		
I	S5	S5				
J	S5	S5				
K	S6	S6				
L	S6	S6				
M	S7	S7				
N	S7	S7				
O	S8	S8				
P	S8	S8				

Sample Source Plates for Predispensed SmartChip Expression Panels

Table XXXI. Sample Source Plates for predispensed SmartChip expression panels (mRNA and microRNA) for 3, 6, and 12 samples.

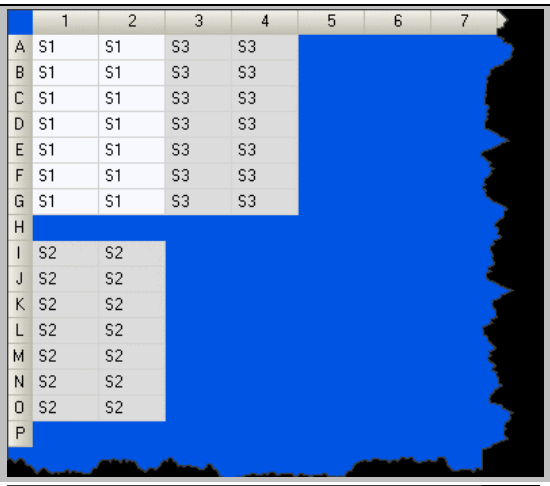
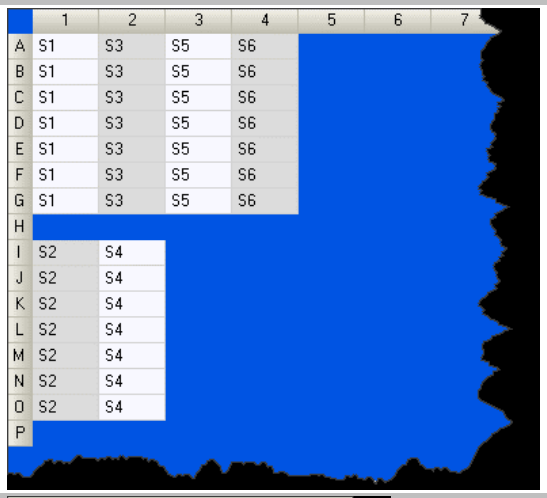
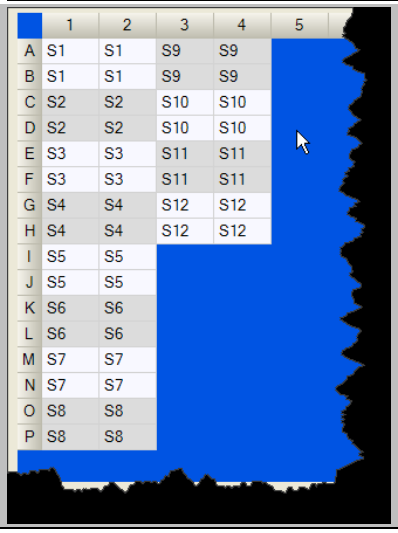
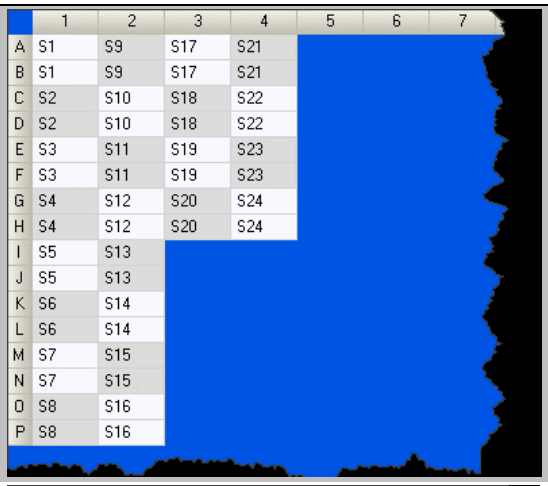
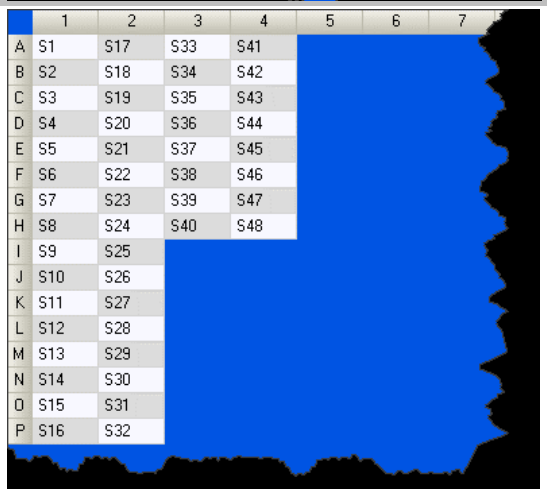
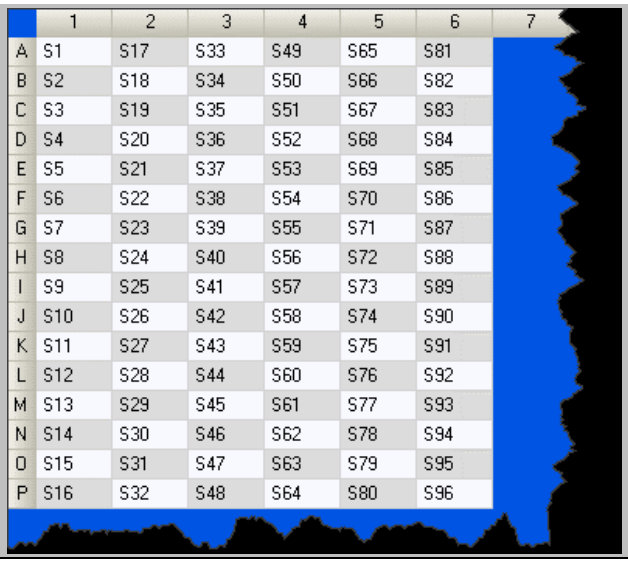
# of samples	Sample/reagent mixture volume and # of wells	Sample/reagent mixture locations in the 384-well plate
3	27 µl in each of 14 wells	
6	27 µl in each of 7 wells	
12	25 µl in each of 4 wells	

Table XXXII. Sample Source Plates for predispensed SmartChip expression panels (mRNA and microRNA) for 24, 48, and 96 samples.

# of samples	Sample/reagent mixture volume and # of wells	Sample/reagent mixture locations in the 384-well plate
24	25 µl in each of 2 wells	
48	25 µl/well	
96	18 µl/well	

Sample Source Plates for predispensed SmartChip SNP Genotyping Panels

Table XXXIII. Sample Source Plates for predispensed SmartChip SNP genotyping panels for 12, 24, and 42 samples.

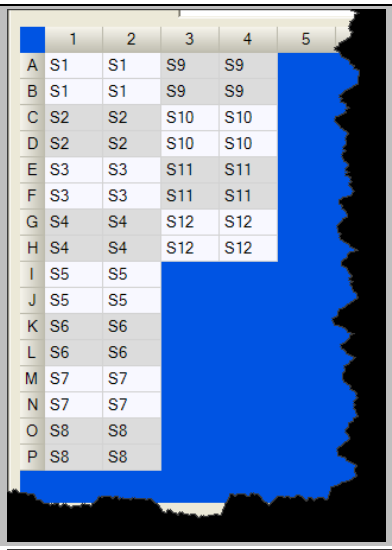
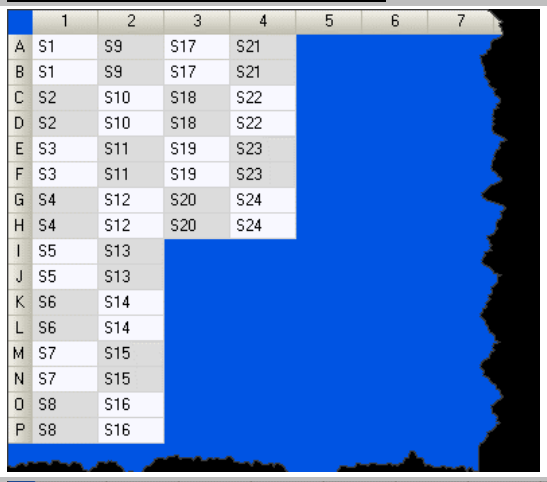
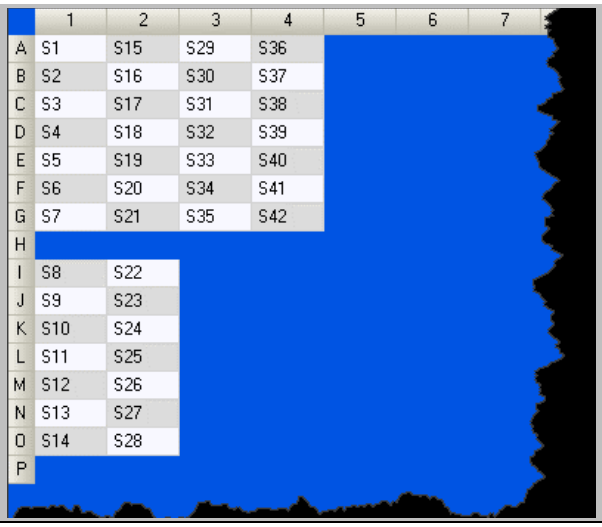
# of samples	Sample/reagent mixture volume and # of wells	Sample/reagent mixture locations in the 384-well plate
12	25 µl in each of 4 wells	
24	25.1 µl in each of 2 wells	
42	27.1 µl/well	

Table XXXIV. Sample Source Plates for predispensed SmartChip SNP genotyping panels for 54, 72, and 108 samples.

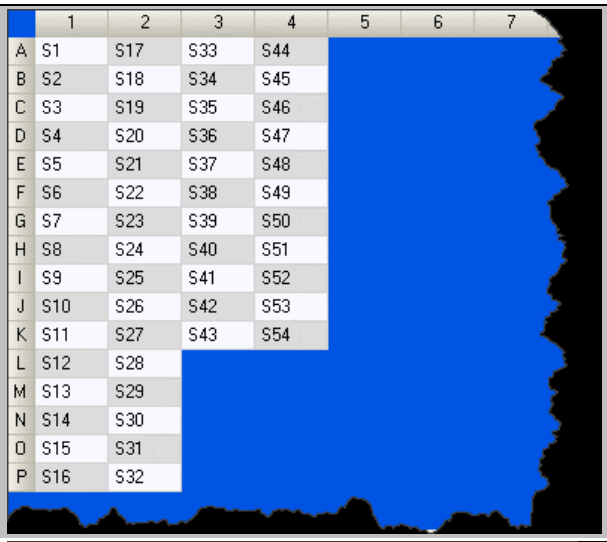
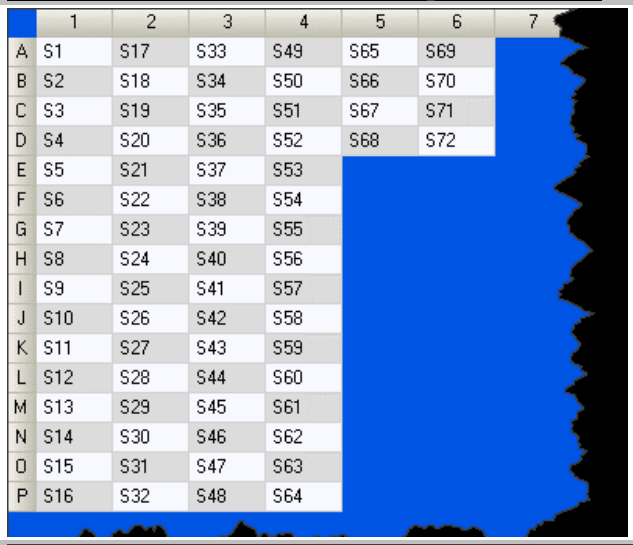
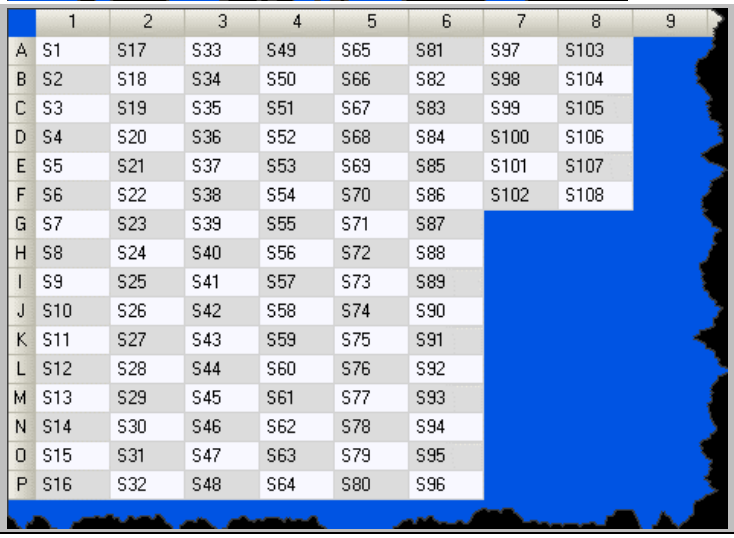
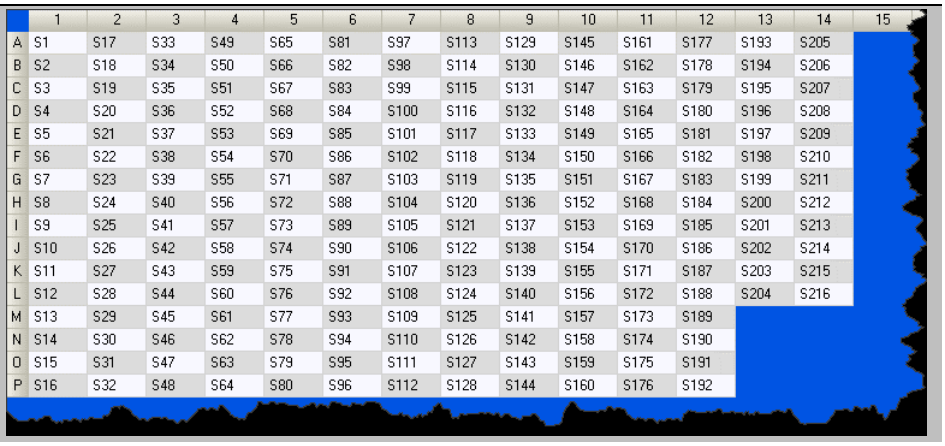
# of samples	Sample/reagent mixture volume and # of wells	Sample/reagent mixture locations in the 384-well plate
54	23.5 µl/well	
72	20.3 µl/well	
108	17.1 µl/well	

Table XXXV. Sample Source Plates for predispensed SmartChip SNP genotyping panels for 216 and 384 samples.

# of samples	Sample/reagent mixture volume and # of wells	Sample/reagent mixture locations in the 384-well plate
216	14.0 µl/well	
384	12.6 µl/well	Load assays starting at the top left of the plate (well A1), running down the columns from left to right. (The loading order follows the pattern shown above.)

Appendix B: Alcohol Wash Procedure

Use this procedure to remove trapped bubbles from the syringes in the fluidic system. You will need ~500 ml of 70% isopropanol and a clean plastic bag to hold the fluidic harness during the procedure.

1. Open the top portion of the protective cover of the Pressure Reservoir, being careful not to damage the tubes coming from the lid.
2. Put on clean gloves.
3. Drain the water from the Pressure Reservoir and replace it with isopropanol, as described below.
   a. Vent the helium by closing the stopcock on the helium input line and opening the vent stopcock (Figure 46).

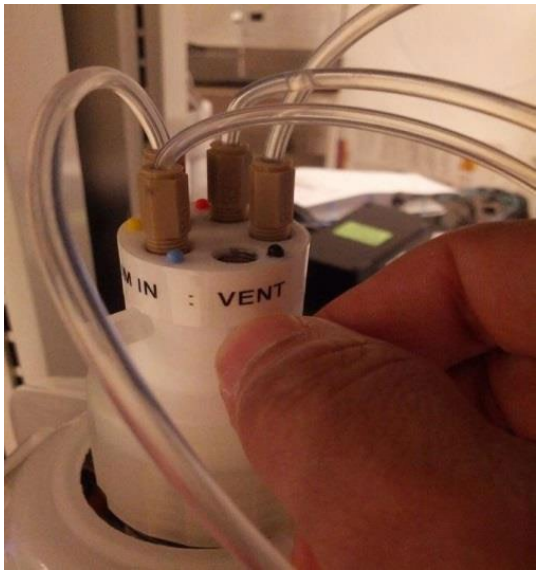


Figure 46. Vent the helium from the Pressure Reservoir.

   b. Carefully remove the cap and tubing from the Pressure Reservoir (Figure 47).

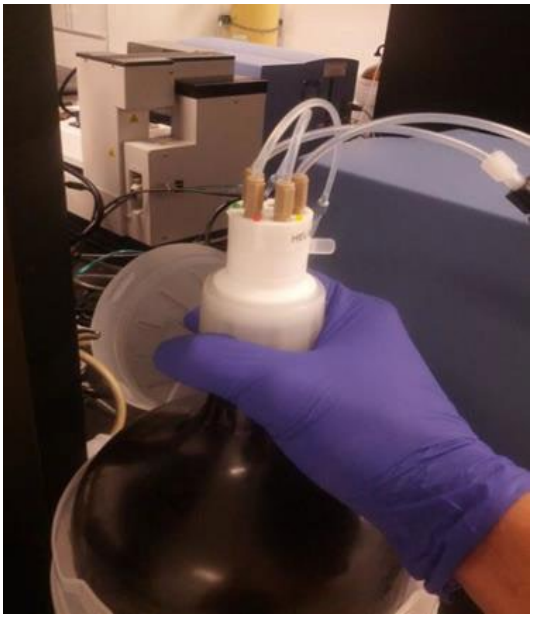


Figure 47. Remove the reservoir cap and tubing.

c. Lift the fluidic harness, open the bag, and place the bag under the harness. Place the fluidic harness inside the bag and set it on the bench (Figure 48).
d. Empty the water from the Pressure Reservoir.

Figure 48. Fluidic harness.

e. Fill the bottle with 500 ml of 70% isopropanol.
f. Reattach the cap and replace the tubes in the reservoir. Avoid touching the tubes when inserting them into the bottom of the reservoir.
g. Reattach the top of the protective cover.
h. Close the system by opening the stopcock on the helium input line and closing the vent stopcock.
i. Reattach the protective cover.

4. Prime the system with 70% isopropanol by running the first portion of the Daily Warmup procedure as described below.
   a. Click the [Daily Warmup] button.
   b. When the **Elapsed time** is 55 sec, click the [Stop] button (Figure 49).

Figure 49. Elapsed time dialog box.

   c. When complete, repeat the prime procedure once more.

5. Run the Daily Warmup procedure.
6. Refill the reservoir with water as described below.
   a. When the Daily Warmup is complete, depressurize the reservoir. Follow the instructions in Step 3 above to remove the fluidic harness. The same plastic bag can be used for this step.
   b. After the fluidic harness has been removed, drain the remaining isopropanol from the reservoir to a waste container.
   c. Rinse the reservoir thoroughly with deionized filtered (0.2 µm) water.
   d. Fill the bottle with deionized filtered water to the top of the bottom section of the protective cover.
   e. Let the reservoir liquid degas for 30 min. You should see helium bubbling through the water during this period.
   f. Prime the system twice, following the instructions in Step 4 above.
7. Repeat the Daily Warmup procedure once more.

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