

nCounter® SPRINT Profiler

User Manual

This manual applies only to the nCounter SPRINT Profiler and not to any other instrument supplied by NanoString Technologies.

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Getting Started

NanoString Technologies designed the nCounter *SPRINT* Profiler instrument for quick installation so that it can be used within 24 hours after delivery. Review this user manual before installing or operating the instrument. Also review any additional documentation provided before performing an nCounter assay, including:

- The appropriate assay user manual supplied with the nCounter product(s) (link to manuals).
- Any relevant Tech Notes issued by NanoString Technologies or your Regional Account Manager or Field Application Scientist (link to tech notes).

Please contact NanoString Support (support@nanostring.com) to resolve any issues or queries.

1. Unpack and Install the Instrument

Chapter 1, Introduction, provides useful safety information to avoid personal injury as well as to prevent damage to the nCounter SPRINT Profiler. Review this section before selecting an appropriate location for installing the instrument.

Chapter 2, Installation, provides additional information for installing the instrument.

2. Network the Instrument

Chapter 2, Section C, Product Use Limitations, provides detailed instructions on networking the instrument. Although networking is not required, it is recommended. Without networking or a direct connection, users will only have access to a default run profile and will not be able to customize or comment on samples.

Networking is the ideal configuration for the *SPRINT* Profiler and enables users to create and monitor runs and download data from a remote computer located on the same internal network. Networking instructions can be found via the instrument touchscreen interface at **Administration > Support > System Information**.

Direct connection via Ethernet is an alternative solution and may work well for first-time users during instrument setup. Connect a personal laptop or desktop computer to the nCounter *SPRINT* Profiler using an Ethernet cable. Additional instructions can be found on the System Information screen. Click on the question mark in the upper right corner.

3. Hybridize Samples

Typically, samples are combined with nCounter probes on Day 1, and the hybridization process occurs overnight before a run is initiated on Day 2. Follow the guidelines in the appropriate assay manual for more instructions on sample hybridization as well as any special processing steps (e.g., DNA fragmentation and denaturation).



4. Create a Run (Optional)

If the instrument is networked or connected to a personal computer, *Chapter 4, Operating the Control Center*, provides information on how to create a new run and enter information about an assay using the Control Center. These details may include the CodeSet being used and the names for each sample.

5. Load Samples

Chapter 5, Loading a Cartridge, provides information on how to load samples into the nCounter Cartridge. It is important to review this chapter to avoid sample loss. A one-page guide in this chapter summarizes sample loading instructions and can be printed and stored near the instrument for future reference.

6. Initiate a Run

Chapter 6, Operating the Instrument, provides instructions to operate the nCounter SPRINT Profiler. Video demonstrations are available on the instrument touchscreen by pressing the ricon in the upper right corner. Load samples immediately before initiating a run. If a run was previously created using the Control Center, it will be available as a selection. If this is a new run, select New.

7. Analyze Data

Once sample processing and data collection are complete, the data will be available to download from the instrument as a comma-separated value (.csv) file. Analyze the data with NanoString's free nSolver™ Analysis Software or a spreadsheet application.



1 Introduction

The nCounter SPRINT Profiler is a benchtop instrument designed to analyze RNA, DNA, and proteins from challenging sample types such as unpurified cell lysates or Formulin-fixed Paraffin-embedded (FFPE) extracts. This manual provides information on installation, maintenance, and operation of the instrument.

This chapter provides guidelines for safe and successful operation of the instrument. It is important that you read and comprehend the provided information. Please contact support@nanostring.com to address any questions or concerns.

A. Overview

This manual is concerned exclusively with the operation of the nCounter *SPRINT* Profiler. Separate manuals are provided for sample hybridization, data analysis, and the operation of other instrument models.

The instrument can be operated without a network connection. However, the touchscreen interface provides limited opportunity to customize run details, and it may require more time to create new runs or download data from completed runs. When the instrument is networked and properly configured, you may use the Control Center web application to manage to the Profiler.

Samples are loaded into an nCounter cartridge after sample hybridization has been completed according to the assay or reagent instructions. This cartridge is placed in the instrument for sample purification and data collection. Only one cartridge can be loaded into the instrument at a time, and each cartridge has room for up to 12 samples.

The nCounter SPRINT Profiler operates in two phases. The first phase uses a multichannel microfluidics system to purify target:probe complexes while removing unbound probes. These complexes are immobilized and aligned on the imaging surface before a second phase of digital microscopy within the same instrument. Images are automatically interpreted to provide the user with counts for each reporter barcode.

While the instrument is processing samples, email alerts may be available to track run status if the system has been networked and properly configured.

After the run has completed, results are available for download. Data files contain the number of counts for each target and may be accessed through the web application, remotely transferred with SFTP (Secure File Transfer Protocol), or downloaded onto a USB flash drive. These are open-format, comma-separated value (.csv) files. Data can be analyzed using NanoString's nSolver software and most spreadsheet applications (e.g., Microsoft Excel).



B. Instrument Specifications

The nCounter *SPRINT* Profiler is a single instrument. A power cable and waste bottle are included with each instrument. Never dispose of the waste bottle. All other consumables and reagents may be purchased separately.

Lanes per Run	1–12
Weight	210 lbs./95.25 kg
Dimensions (W x D x H)	42 x 28 x 32 in. /107 x 72 x 82 cm
Power Requirements	100-240 VAC, 50/60 Hz, 600 VA
Fuse	110 VAC: 250 VAC, 6A
	220 VAC: 250 VAC, 3A

Environmental Conditions

Temperature	18-28°C
Humidity	30-80% relative humidity (non-condensity)
Installation Site	Indoor use only. Avoid locating in direct sunlight.
	Place the instrument on a flat, stable surface with access to a dedicated power supply.
	Ensure adequate clearance to access power controls in the rear, to open the reagents and waste compartment on the left side, and to open the cartridge drawer in the front.
	Avoid locating near other equipment that may cause vibration, or near large electrical equipment that may cause interference from noise and/or voltage fluctuation.

C. Product Use Limitations

The nCounter SPRINT Profiler is intended for research use only when used in conjunction with NanoString nCounter® assays and reagents within their Intended Use. See the instructions for use that accompany the reagents for information regarding performance and sample type limitations.

D. Disposal of Electronic Equipment



IMPORTANT: Review and follow all laws regarding the safe and proper disposal of electrical instrumentation. The symbol of a crossed out, wheeled bin on the product is required in accordance with the Waste Electrical and Electronic Equipment (WEEE) Directive of the European Union. The presence of this marking on the product indicates that:

- The device was put on the European Market after August 13, 2005.
- · The device is not to be disposed via the municipal waste collection system of any member state of the European Union.

For products under the requirement of WEEE directive, please contact your dealer or local NanoString office for the proper decontamination information and take-back program, which will facilitate the proper collection, treatment, recovery, recycling, and safe disposal of the device.



E. Conventions

The following conventions are used throughout this manual and are described for your reference.

Text Style	Meaning
Bold	Bold text is typically used to highlight a specific button, keystroke, or menu option. Bold text may appear elsewhere to highlight important text or terms.
Italic	Italic text is typically used to highlight references to another section or chapter of the manual. Italic text may also be used to highlight references to other manuals or instructional material.
Green	Green text is typically used to highlight references to specific figures or tables. Green text may also be used to indicate online content or email addresses.



NOTE: This symbol indicates general information that may be useful for improving assay performance. These notes may clarify other instructions or provide guidance to improve the efficiency of the assay workflow.



IMPORTANT: This symbol indicates important information that is critical to ensure a successful assay. Following these instructions may help improve the quality of your data.



CAUTION: This symbol indicates the potential for bodily injury or damage to the instrument if the instructions are not followed correctly. Always carefully read and follow the instructions accompanied by this symbol to avoid potential hazards.



F. Safety Information (English)

The nCounter SPRINT Profiler may only be operated by appropriately trained, professional users. NanoString recommends that all users read and understand this manual prior to attempting to operate the system. Keep this manual in close proximity to the instrument for easy access to instructions and safety information. Failure to comply with the instructions in this manual may pose a dangerous risk to the operator and void the manufacturer's warranty.



CAUTION: Do not attempt to install, move, or perform maintenance on the instrument while in use. Always contact NanoString for instruction before installing or moving the instrument.



CAUTION: Do not use this device in close proximity to sources of strong electromagnetic radiation or vibration, as these may interfere with proper operation.



CAUTION: Operate the system using only NanoString reagents in accordance with their indications for use. Ensure that all consumables are properly inserted prior to starting a run.



CAUTION: Connect the instrument power supply to a properly grounded receptacle with adequate voltage and current (see instrument specifications). Unplug the instrument when not in use for extended periods of time.

Caution Symbols



ELECTRICAL HAZARD: Do not attempt to disassemble the instrument at any time. An electric shock can occur if the instrument is operated without its outer case. Disconnect the instrument from the power source before attempting to replace the fan filter.



G. Consignes de Sécurité (Français)

Le nCounter *SPRINT* Profiler ne peut être utilisé que par une personne ayant reçu une formation professionnelle appropriée. NanoString recommande que tous les utilisateurs lisent et comprennent ce manuel avant de tenter de faire fonctionner le système. Conservez ce manuel à proximité de l'instrument pour faciliter l'accès aux instructions et consignes de sécurité. Le non-respect des instructions de ce manuel peut présenter un risque dangereux pour l'opérateur et peut annuler la garantie du fabricant.



ATTENTION: Ne pas tenter d'installer, de déplacer ou d'effectuer l'entretien de l'instrument en cours d'utilisation. Toujours contacter NanoString pour instruction avant d'installer ou de déplacer l'instrument.



ATTENTION: Ne pas utiliser cet appareil à proximité de sources à fortes radiations électromagnétiques ou vibrations, car elles peuvent interférer avec le bon fonctionnement de l'appareil.



ATTENTION: Faire fonctionner le système en utilisant uniquement des réactifs NanoString conformément à leurs guides d'utilisation. Assurez-vous que tous les consommables soient correctement insérés avant de commencer une opération.



ATTENTION: Branchez l'alimentation de l'appareil électrique à une prise correctement mise à la terre avec une tension et un courant adéquat (voir les spécifications de l'instrument). Débranchez l'appareil lorsque vous ne l'utilisez pas pendant des périodes de temps prolongées.

Définitions des Étiquettes de Sécurité



RISQUE ÉLECTRIQUE: Ne jamais tenter de démonter l'appareil. Un choc électrique peut se produire si l'appareil est utilisé sans son enveloppe extérieure. Débranchez l'appareil de la source d'alimentation avant de remplacer le filtre du ventilateur.



2 Installation

This chapter describes the installation of the nCounter SPRINT Profiler. All customers—including those who utilize a delivery service to unpack and place the instrument—should familiarize themselves with the size of the instrument as well the relevant safety precautions described in Chapter 1. Additional setup by the user may be required to connect the instrument to a network and enable certain functions.

A. Placement

Use a tape measure to determine the available space for the nCounter SPRINT Profiler. Confirm that there is a minium space of $42 \times 28 \times 32$ inches or $107 \times 72 \times 82$ centimeters (width x depth x height). Do not install the instrument unless this minimum space is available. Additional space is required behind the instrument (to access the power outlets and data ports), in front of the instrument (clearance is required for the cartridge drawer to open), and to the left and above the instrument (clearance is required for the reagents compartment door to open).



IMPORTANT: Never attempt to move the instrument any distance without first contacting NanoString Support (support@nanostring.com). NanoString may be able to provide a service technician to move the instrument or provide advice on moving it without assistance. Individual situations vary, and there is a risk of damage to the instrument whenever it is moved.



B. Networking

The nCounter SPRINT Profiler Control Center is a web application that is located on an internal server and can only be accessed through a network or personal computer. Many tasks can be performed when there is no network connection, and files can be transferred with a USB flash drive; however, it is strongly recommended to connect the instrument to your network. When placing the instrument, ensure that a network connection is available. Two Ethernet ports and two USB ports are available for network connections. They are located at the back of the instrument above the power switch (FIGURE 2.1).



FIGURE 2.1 Ethernet (top two) and USB (side-by-side) ports.

Access the networking instructions on the instrument touchscreen, **Main Menu > Administration > Support > System Information**. Press the **?** button on the System Information screen (**FIGURE 2.2**).



FIGURE 2.2 The System Information screen.

USER MANUAL nCounter* SPRINT Profiler

Instructions are provided to physically connect the instrument to a desktop or laptop personal computer using an Ethernet cable (FIGURE 2.3).

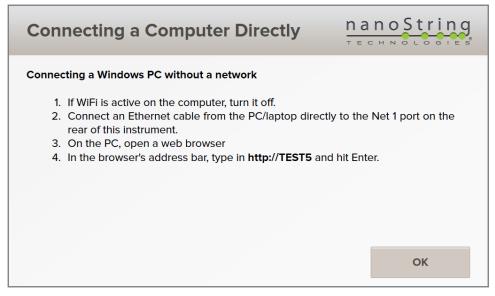


FIGURE 2.3 The instrument networking instructions.

To perform various configuration tasks, such as changing the instrument name or IP address, it is necessary to access the instrument via the Control Center after the instrument and personal computer are connected.

Open an Internet web browser on the computer and navigate to the Control Center using the URL provided on the instrument touchscreen (**FIGURE 2.2**) or by the system administrator. The Control Center has been designed and tested to be compatible with a variety of web browsers including Internet Explorer 11, Chrome 41.0, Firefox 36.0, and Safari 8.

Once the Control Center loads, several settings can be configured:

- **Network Settings**—Configure optional settings such as IP address. The Control Center will function without changing these settings, but access may be limited if attempting to use another computer that is not physically connected.
- Mail Server—Configure the instrument to send notification emails and log files to users when a run is complete or encounters an error.
- Date/Time Settings—Change the date and time used by the instrument to record events.

These settings can only be configured using the web application, though read-only information may be available on the instrument. For more information, review the Administration instructions in *Chapter 4, Operating the Control Center*.

Software Updates

NanoString recommends updating the software before using the nCounter *SPRINT* Profiler. See the software update instructions provided in *Chapter 8, "Technical Support and Maintenance"*.

3 Hardware Overview

Both new and existing customers should familiarize themselves with the design of the nCounter SPRINT Profiler (FIGURE 3.1). This system represents a significant advancement in the nCounter platform, incorporating sample processing and data collection in a single instrument. A new microfluidics system requires new formats for nCounter cartridges and reagents, and an online web application called the Control Center makes this instrument easier to use when creating new runs, monitoring instrument progress, and accessing data.



FIGURE 3.1 The nCounter SPRINT Profiler.

A. General Information

The instrument serial number and compliance information can be found on the rating plate on the back panel of each instrument. Please refer to the rating plate and declaration of conformity (EU only) for compliance information.



NOTE: The nCounter *SPRINT* Profiler can be operated as a stand-alone instrument by transferring files with a USB flash drive and initiating new runs with default settings. NanoString strongly recommends connecting the instrument to an external computer or network for access to additional features, including the ability to define individual sample information, set email notifications, and manage data delivery options.

When the nCounter SPRINT Profiler is networked, the Control Center web application allows you to manage the instrument. In addition, the instrument contains a touchscreen display to initiate runs and perform maintenance tasks. There are several buttons that appear throughout the user interface. These include:

- Next—Proceed to the next screen.
- Back—Return to the previous screen.
- Cancel—Return to beginning of the current workflow or the Main Menu.



B. Sample Purification

Prior to using the nCounter *SPRINT* Profiler, samples must be hybridized according to the protocol outlined in the appropriate assay user manual (link to manuals). Samples are then injected into a cartridge, which is placed in the instrument. During sample processing, the instrument performs a number of tasks including liquid transfers, magnetic bead separation, and immobilization of molecular labels on the sample surface. This is followed by data collection with an automated fluorescence microscope. Only one cartridge can be loaded into the instrument at once, with capacity for up to 12 assays.

All consumable reagents required to process hybridized samples are provided in the nCounter SPRINT Profiler Reagent Pack (FIGURE 3.2 and FIGURE 3.3). The reagents include:

- · Hybridization buffer
- Reagent A (green)
- · Reagent B (blue)
- · Reagent C (red)

Reagent bottles must be loaded on the instrument prior to use. A fourth bottle, which is round, is included with the instrument to collect liquid waste. Give close attention to the instructions when removing or replacing bottles to ensure they are correctly placed and detected by the level sensors (see *Chapter 7, Reagents and Waste*).



FIGURE 3.2 The nCounter SPRINT Reagent Pack and nCounter SPRINT Cartridge.



FIGURE 3.3 The nCounter SPRINT reagent bottles and nCounter hybridization buffer.

No reagent preparations or dilutions are required. The nCounter *SPRINT* Profiler can process 12 samples per run and 16 runs per Reagent Pack. Each run requires approximately 6 hours depending on the assay type.

Cartridges are required for all runs and are sold separately. Users may also be required to purchase other reagents that vary by application. More information about these reagents can be found with the instructions for each assay.



C. Data Collection

The second phase of the nCounter *SPRINT* Profiler's operation consists of a multi-channel epifluoresence scanner and digital analysis system. This imaging system is specifically designed to image samples processed within nCounter cartridges.

The digital analysis system collects data by taking images of the immobilized fluorescent reporters in the cartridge with a CCD camera through a microscope objective lens. Multiple fields of view (FOVs) are imaged within each sample lane.

Fields of View (FOVs) are processed internally, counting the number of each fluorescent reporter tag, and the results are exported as a comma separated values (CSV) file, called an RCC (Reporter Code Count) file, that can be downloaded to a USB flash drive or emailed to the user. This file can be opened by most commonly used spreadsheet applications, including Microsoft* Excel, and can be analyzed using NanoString's nSolver Analysis Software or other data analysis and visualization software packages.

D. Overview of File Structure

There are two types of files used by the nCounter Analysis System: the Reporter Library File (RLF) and the Reporter Code Count (RCC) file.

- Reporter Library File—Generated by NanoString and unique to each custom CodeSet. It contains the information used during image
 processing to assign target identities to the barcodes.
- Reporter Code Count file—Generated by the instrument. Each RCC file contains the data for one of the twelve flow cells (assays) in a cartridge, detailing the number of counts for each target in an assay.

Each custom CodeSet ships with a USB flash drive containing the following files for use on the instrument:

- · Two read-only folders for Reporter Library Files (RLFData) and Reporter Code Count files (RCCData)
- A PDF with links to product-specific assay manuals, product inserts, data analysis guides, and Safety Data Sheet(s)



NOTE: The USB flash drive and the instrument have been designed specifically to enable quick and accurate file transfers. Users may create sub-folders for other file types to facilitate organization. The instrument will scan the entire drive to locate and upload new RLFs. However, it may create its own sub-folders when downloading files from the instrument.



NOTE: nCounter Elements™ and PlexSet TagSets do not ship with a USB flash drive; the RLF is provided by Bioinformatics at the time of the TagSet creation.



4 Operating the Control Center

The Control Center web application resides on an internal server of the nCounter SPRINT Profiler. When the system is connected to a network or personal computer, the Control Center communicates with the instrument through a web browser. Basic functions can also be performed using the instrument's user interface without connecting it to a network. However, many customization features are available only through the Control Center. Its primary functions are to:

- · Create and edit runs
- · View the status of runs
- · Download data and other files
- Perform administrative and maintenance functions

A. Access

You can access the web application by navigating to the URL from a networked personal computer. The URL can be found on the System Information screen, FIGURE 2.2). Click on the URL to view the Control Center (FIGURE 4.1).



FIGURE 4.1 The Control Center home page while the instrument is idle.



B. Application Layout and Navigation

Menu

The Control Center home page icons allow you to create a new run, view/edit an existing run, download data, and administer the system.

Tables

Tables can become large after frequent use of the Control Center or instrument. Filtering is available in tables to allow users to search for and view data of interest.

By default, filtering is turned off for most status pages. To turn filtering on, click the **filter [off]** button. The header will expand and display available filter settings. Define the filter and click Apply to refresh the displayed information (**FIGURE 4.2**). The **filter [off]** button will change to read **filter [on]**.



FIGURE 4.2 Filter settings are available when viewing most data

Sort

All table columns allow users to sort the displayed rows in ascending or descending order. Click on the column heading containing the data to sort the rows (FIGURE 4.3). To re-sort the data in the reverse order, click again on the same column heading. A small arrow will appear next the column heading text when sorted.

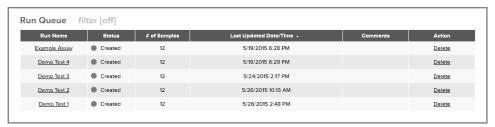


FIGURE 4.3 An example of sorting runs by the last updated date/time.



NOTE: The default display setting for most tables is chronological order with the newest items at the top.



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C. Create and Edit Runs

Home

The home page is the first screen displayed after accessing the Control Center and is accessible from other pages by clicking on the **Home** button in the navigation menu or the NanoString logo in the upper right corner (**FIGURE 4.4**).



FIGURE 4.4 The Control Center home page while a run is in progress.

The home page lets you quickly create new runs, edit runs, and download data. In addition, it displays information about the current run, including percent complete, estimated time remaining. and green progress bars on the cartridge illustration.



Create a New Run

Use the Control Center Users to create a new run and associate sample IDs with each lane. (A separate Default Run option is also available on the instrument; see *Chapter 6, Operating the Instrument*.) Click the **New Run** icon on the Home page to open the Run Definition page (**FIGURE 4.5**).

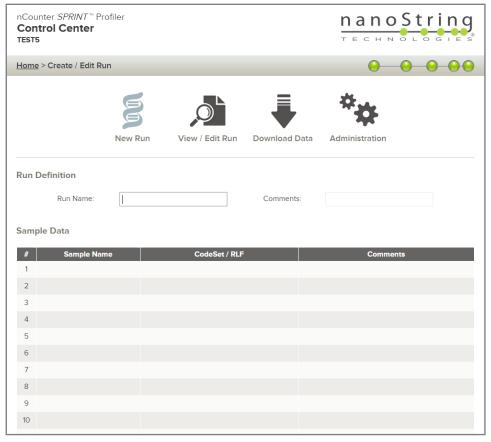


FIGURE 4.5 The Run Definition page.

- 1. Enter the following information to define a new run:
 - Run Name—Choose a unique name to describe each run on the instrument.
 - Comments—Enter any comments describing this run.
 - Sample Data—Enter information for all 12 lanes; empty lanes can be labeled "empty," but the sample data must still be entered. (Click in the appropriate field in each row to enter sample data.)
 - Sample Name—Sample names can be entered manually to more easily track the data from each sample. Choose unique sample names or number them as 1, 2, 3, etc. There is a maximum limit of 64 characters.
 - CodeSet/RLF—Choose an existing Reporter Library File, or select Upload to create a new one (see the next section; the specific assay type will be associated with the RLF during the upload process).
 - Comments (optional)—Any notes about the sample can be entered here. There is a maximum limit of 32 characters.

After entering the information for one lane, click the **Fill** button for that column if you want to copy it to all lanes below that (**FIGURE 4.6**; the lanes above will *not* be auto-filled). Delete the entered information from all fields in an individual lane by clicking the **Clear** button (**FIGURE 4.7**).

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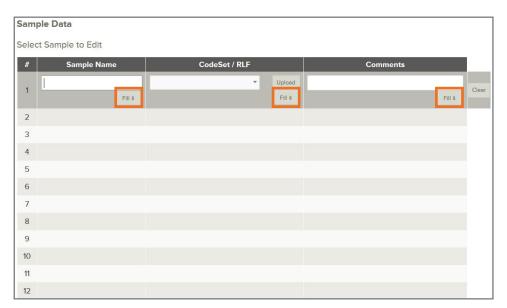


FIGURE 4.6 Click Fill to copy information to the lanes below the highlighed cell for the rest of the column.

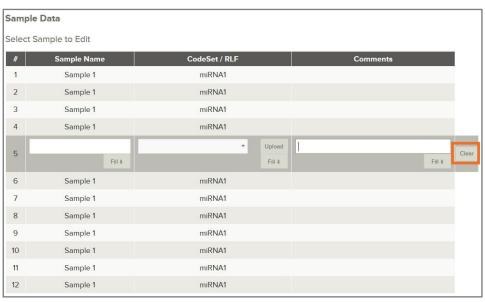


FIGURE 4.7 Click Clear to remove information from specific lanes.

- Select Email Recipients—If desired, choose email recipients to receive status reports by selecting users from the contact list on
 the left side and clicking the → button (FIGURE 4.8). Remove recipients by selecting the users from the list on the right side and
 clicking the ← button. Press the Ctrl key (or the Command key if using an Apple computer) on the keyboard while selecting multiple
 addresses to add or remove multiple recipients at once.
 - If necessary, add email addresses by typing the address in the text field and clicking **Add Recipient**.
 - A checkbox will be automatically selected when a new email recipient is added. This checkbox indicates that this email address will receive both a status notification and data files. Uncheck the box to deliver a status notification only.
 - If a user is added from the existing contact list, the checkbox will not be selected and only a status notification will be delivered. Check the box to deliver both a status notification and data files.





FIGURE 4.8 Email recipients can be added or removed.

3. Choose **Save Run** when complete, or click **Cancel** to exit. The Control Center will display a confirmation message with instructions to proceed to the instrument to begin the run. Press **Return to Run Queue**.



NOTE: If any fields are incomplete, the run cannot be saved. An error message will appear asking you to review the information provided.

Upload Reporter Library Files (RLFs)

Upload your CodeSets/RLFs at any time, including at the creation of a new run:

- 1. During "Create a New Run", click the Upload button in the CodeSet/RLF column of the Sample Data table.
- 2. When the Upload File dialog box opens, navigate to the desired file, select it, and click Open.
- 3. The uploaded RLF populates the selected cell and appears on the CodeSet/RLF dropdown menu for future runs (FIGURE 4.9).

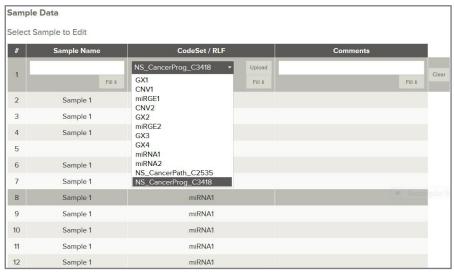


FIGURE 4.9 The new RLF is now available on the CodeSet/RLF dropdown menu for future runs.

- If you upload an invalid file format, you will see an error message.
- If the RLF is already uploaded, you will see a notification message.

Create a Run without a Network Connection

If the network connection is disabled, a run can still be created using a direct connection between the instrument and a personal computer.

Use the instrument touchscreen to navigate to **Main Menu > Administration > Support > System Information** and press the **?** button on the System Information screen, as described in the networking instructions in *Chapter 2, Installation* (see **FIGURE 2.2**). Follow the instructions provided for connecting the instrument to a personal computer with an Ethernet cable. While the cable is still connected, open a web browser and enter the URL provided to access the instrument.

Default Run Option

Some users may not need to enter the detailed information requested when creating a new run, particularly if they are satisfied with the default settings of the instrument. Detailed instructions are provided in *Chapter 6, Operating the Instrument*. Even if the Control Center is not required to define a run, it can still be used to upload an RLF for use in a default run.

Edit a Run

To edit a run, select the **View/Edit Run** icon on the home page (**FIGURE 4.10**). A list of saved runs will be displayed. Filtering options will be available to help find the desired run. Click on the link for the desired run to open it for editing.

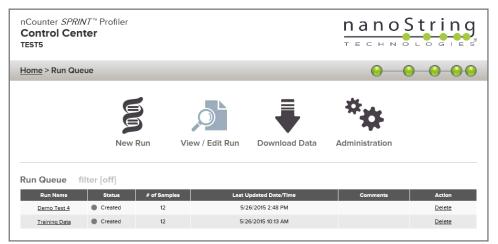


FIGURE 4.10 The Run Queue with the list of saved runs available to edit.

Verify that the correct run has been opened and then edit the run as needed. Save changes by clicking the **Save Run** button at the bottom or choose **Cancel** to discard any changes.

Delete a Run

Runs can be deleted if necessary. To delete a run, choose **View/Edit Run**, select the appropriate run from the Run Queue, and click the **Delete** button.



D. Download Data

Download Reporter Code Count (RCC) Files

After a run is partially or fully completed, you can download the RCC files for analysis. These files contain the number of counts for each reporter imaged by the instrument.

1. Click the **Download Data** icon on the Home Page.

A list of completed runs will appear (FIGURE 4.11).

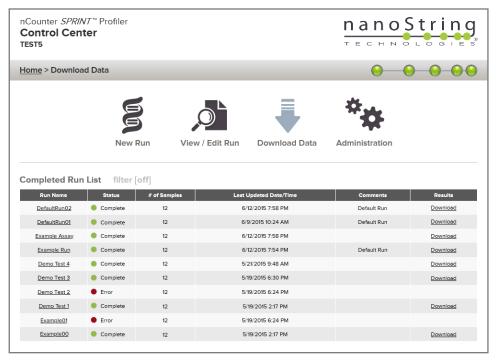


FIGURE 4.11 A list of runs and the status of each.

- 2. Click the **Download** link in the Results column to download the results for that run.
- 3. Alternatively, click on the **Run Name** link to view the Results and Run Information (**FIGURE 4.12**). This page allows you to download the results (RCC files), download or fix the CodeSets (RLFs) associated with the run, and download or email the log files.
- 4. Click the **Download Results** icon. Click **Save**, then click **OK** to download.

USER MANUAL nCounter* SPRINT Profiler

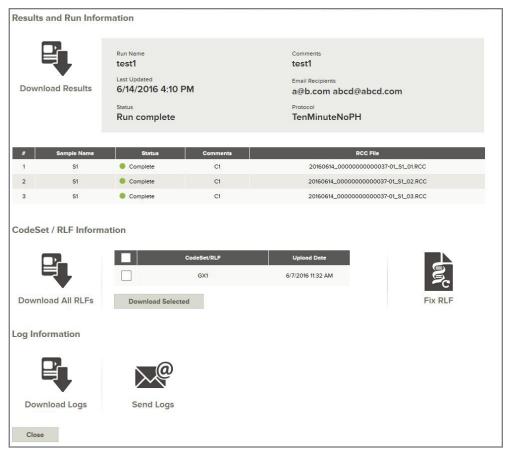


FIGURE 4.12 The Results and Run Information page lets you download results (RCC files), download or fix CodeSets (RLFs), and download or email logs.

Download Reporter Library Files (RLFs)

Download RLFs from the instrument for subsequent analysis with the nSolver™ Analysis Software.

- Click the **Download All RLFs** icon to download all of the RLFs associated with the run.
- Alternatively, use the checkboxes to select the desired RLFs and click Download Selected.

Fix Reporter Library Files (RLFs)

In the event that a sample is associated with an incorrect RLF, you may redefine the associated RLF for completed runs. You may also edit the Sample Name and Comments for the modified lanes. The email recipient list is *not* editable; the same recipients will receive the updated results.



NOTE: When you click Fix RLF on the RLF Definitions page, the results are recalculated for the lanes with a modified RLF.

- 1. Click the Fix RLFs icon.
- 2. In the RLF Redefinition table, click in the desired CodeSet/RLF cell. Use the dropdown menu to select an available RLF, or use the Upload button to upload a new RLF (FIGURE 4.13). Update any corresponding sample names and/or comments.
- 3. When finished, click Fix RLF at the bottom of the page. You will be asked to confirm the update.
- 4. Once you confirm the update, you will see the number of samples modified. Click \mathbf{OK} .
- 5. You will return to the Results and Run Information page, where you can download the updated results file.



Select Sample to Edit GX1 S1 C1 2 S2 GX2 C2 S3 Upload C3 4 5 6 8 10 12 **Email Recipients** a@b.com abcd@abcd.com Cancel FIX RLF

FIGURE 4.13 The RLF Redefinition page lets you re-associate an RLF. Click in the CodeSet/RLF cell and use the dropdown to select an existing RLF or click Upload to upload a new RLF. The email list is not editable; the same recipients will receive the updated results.

E. Administration

Click the **Administration** icon to view the Administration page, which contains Configuration Settings and Other (including maintenance functions) settings (**FIGURE 4.14**).



NOTE: Administrative and maintenance functions are unavailable while a run is in progress.



FIGURE 4.14 The Administration page.

Configuration Settings

Configuration settings include:

- Email Server
- Network
- Date/Time
- SSL
- Remote Access
- Manage Users



Email Configuration

Email settings (FIGURE 4.15) must be configured in order for the instrument to send automated emails that include notifications of a completed run or assay data.

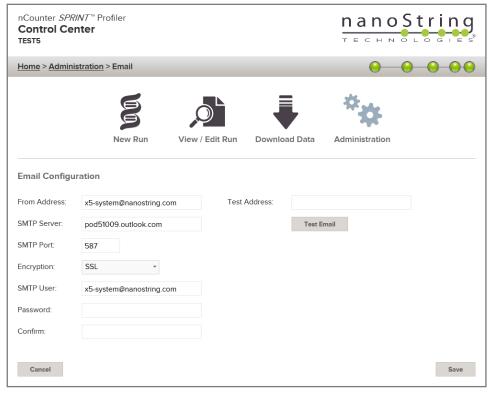


FIGURE 4.15 The Email Configuration page.

The following fields are available:

- · From Address—Address from which automated emails are sent
- SMTP Server—Simple Mail Transfer Protocol email server name
- SMTP Port—Port used for email server
- **Encryption—**This drop-down menu provides options to encrypt email data sent over the Internet between the client and email server. These are required if **Enable SSL** is checked. Two options are available:
 - SSL (Secure Socket Layer)—Widely-used encryption protocol
 - TLS (Transport Layer Security)—More secure encryption protocol
- SMTP User—Account name for secure email server (required if Enable SSL is checked)
- Password—Password for SMTP username (required if Enable SSL is checked)
- Confirm Password—Same password as above (required if Enable SSL is checked)
- **Test Address (optional)**—This can be used to test if the email settings are configured correctly. Enter an email address and click **Test**. Check that email account to confirm that the test email was received.

Network Configuration

Configuring the SPRINT Profiler on your network allows you to access the instrument more easily while controlling access for users on the network. The IP address function is an essential feature to enable networking of the instrument with a personal computer. The instrument and Control Center are in frequent communication, and the IP address is the basis for establishing this communication. Use caution when making changes to the TCP/IP network configuration settings (FIGURE 4.16).



NOTE: The nCounter SPRINT Profiler supports user profiles and password protection. See "Manage Users" for further information.

The instrument can be connected directly to a personal computer using an Ethernet cable without networking the instrument. However, the Control Center will only be accessible from that personal computer.



FIGURE 4.16 The TCP/IP Network Configuration page.

The following fields are available:

- **Type**—The following options are available:
 - **Dynamic IP address** (default)—Known as DHCP, this option allows the local network to automatically find and assign an IP address. This address is subject to change.
 - Static IP address—This requires the network administrator to set an address that will not change.
- IP Address—IP address provided by the network administrator (for Static IP address).
- **Subnet**—Subnet provided by the network administrator (for Static IP address).
- Gateway—Gateway address provided by the network administrator (for Static IP address).
- DNS 1—Domain Name Server provided by the network administrator (for Static IP address)
- DNS 2—A second DNS is optionally provided by the network administrator and used as a backup (for Static IP address).

Date/Time Settings

Date and time settings are initially set when NanoString personnel set up the instrument. Change these settings only when necessary (FIGURE 4.17). Click **Save** to preserve changes or **Cancel** to discard changes.



FIGURE 4.17 The Date/Time Configuration page.



NOTE: Users cannot adjust the Daylight Saving Time setting. Contact NanoString if this setting needs to be adjusted.



NOTE: Changing the date and/or time in the web application adjusts these values on the instrument as well. Take care when making changes to these settings.

USER MANUAL nCounter* SPRINT Profiler

SSL Settings

SSL (Secure Sockets Layer) provides security for your network connections. Set a password and upload an SSL certificate (PFX file) (FIGURE 4.18) or disable and remove the certificate.

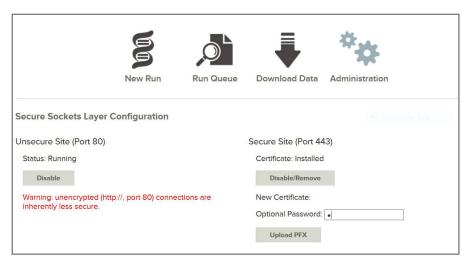


FIGURE 4.18 SSL Settings page

To upload and install an SSL certificate:

- 1. Enter a password in the Optional Password field.
- 2. Click the **Upload PFX** button.
- 3. Navigate to the desired PFX file, select it, and click **Open**.

Under Secure Site, the Certificate is now listed as Installed (FIGURE 4.19). Click the **Disable/Remove** button to remove SSL access on Port 443 and delete the uploaded certificate.

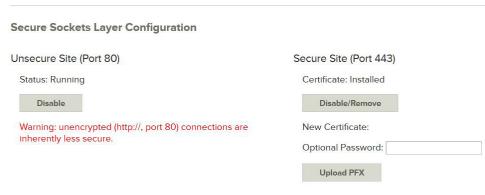


FIGURE 4.19 SSL Settings page

Remote Access Configuration

Remote Access Configuration lets you activate an SFTP (Secure File Transfer Protocol) server for your data transfers (FIGURE 4.20). SFTP is a network protocol that that provides file access, transfer, and management over any reliable network and helps to ensure the security of your data.

To configure an SFTP server:

- 1. Click the **Enable** button.
- 2. The SFTP Server and SFTP User are not editable (contact your system administrator for assistance)
- 3. Select a password and reenter it to confirm.
- 4. Click the **Save** button to save your settings.

To disable the SFTP server, click the **Disable** button.



FIGURE 4.20 Remote Access Configuration with the SFTP server enabled.

An SFTP client will be needed to access the server. Any SFTP client may be used. Popular clients include WinSCP (Windows), PSFTP (Windows), Transmit (MacOS), and Interarchy (MacOS). The following steps are specific to FileZilla, a cross-platform SFTP client available for both Mac OS and Windows.

1. If no SFTP client is installed, navigate to https://filezilla-project.org/ and select **Download FileZilla Client**. Follow the prompts to download FileZilla, agree to the terms of the License Agreement, and Install.

If already installed, run the FileZilla program (or your preferred SFTP client) from its saved location.

USER MANUAL nCounter* SPRINT Profiler

Enter host, username, password, and port. Select Quickconnect.

An error message may appear (FIGURE 4.21), warning against connecting to an unknown server. Ensure that this is a trusted host, then select **OK**.



FIGURE 4.21 Unknown host key warning message

- 3. The resulting window (FIGURE 4.22) will feature Local site file hierarchy and details on the left and Remote site file hierarchy and details on the right. Expand the file tree in each upper window to reveal the associated files in the lower window.
- Open DataFiles under the Remote site window. Click on any files of interest and drag them to the desired location in the Local site window.

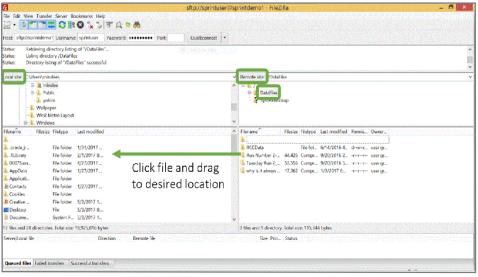


FIGURE 4.22 Filezilla orientation screen

Manage Users

The Manage Users page lets you administer system users and their access to the system (FIGURE 4.23). On this page, create new users, edit existing user information, enable user logon requirement, and set session timeouts.



NOTE: You must log in as Adminstrator to make changes on this page. The first time you log in as Adminstrator, you will need to set and confirm a password.

- Select Require User Logons to require all users to log on to access the Control Center. Requiring users to log on helps prevent
 unauthorized access.
- Set the **Session Timeout** (minutes), the length of time before the user must log in again. Enter a value and click the **Update** button. Setting a session timout helps secure the system and prevent unauthorized access if a user leaves their computer unsecured.

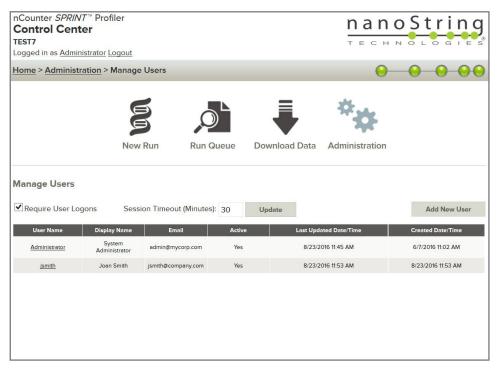


FIGURE 4.23 The Manage Users page allows you to create/edit users, require user logons, and set session timeout intervals.

- · Add a new user or edit an existing one.
 - If you are adding a new user, click Add New User. The fields on the Create/Edit User page will be blank (FIGURE 4.24). Enter the user information and click Save.



FIGURE 4.24 For new users, fill in the text fields on the Create/Edit User page

If you are editing an existing user, click on the user name. The Create/Edit User page
will be populated with the user's information (FIGURE 4.25). Update as needed and
click Save.



FIGURE 4.25 For existing users, edit the text fields on the Create/Edit User page

Other Administration Tasks

Some tasks concerned with instrument maintenance involve uploading or downloading files to the instrument. Some of these functions can also be performed using the instrument touchscreen (see *Chapter 8, Technical Support and Maintenance*).

Download or Send Logs

When selecting logs, use the date filter to limit the size of the download (FIGURE 4.26). After choosing an appropriate date range, select the Log Type based on what the support team has requested: User Run, System or Service (the most commonly requested logs are the User Run logs). Then, save the log files to the personal computer's local drive or email them to NanoString (it is not possible to select individual log files on the Download Logs page; see Section D: Download Data for this feature). Refer to the Downloading Sprint Logs Quick Guide.

- Click Download to save the files; this option may be preferable if there are many files or it is unclear which files are needed.
- Click Send Email to email the files to NanoString; the files will be delivered as a single attachment. A new window will open (FIGURE
 4.27) with the attachment size and a short form to enter contact information, a brief description of the problem (the email subject
 line), and further details (the email content).



FIGURE 4.26 The Download or Send Logs page



FIGURE 4.27 Complete this form before emailing log files to NanoString Support.



NOTE: Some email systems do not allow attachments, or place a cap on attachment file sizes. Selecting a large number of log files may prevent email delivery. If you have not received a response from NanoString within 24 hours of sending log files, email again without any attachments to verify your logs were received. Always include your case number in any communication.



Manage CodeSets/RLFs

New Reporter Library Files (RLFs) can be uploaded during the creation of a new run or during a separate workflow from the Administration screen. The RLF will be provided by NanoString either as a USB drive that comes with the CodeSet or as an email attachment from the NanoString Support team.

Click on the Manage CodeSets/RLFs icon (FIGURE 4.28) to view the page.

- To download RLFs, select the desired files, click the **Download Selected** button, and save the files.
- To upload new RLFs, click the Add RLF Files button, select the desired files from the File Upload dialog box, and click OK.

CodeSet/RLF	Upload Date	Active
GX1	6/7/2016 11:32 AM	Yes
CNV1	6/7/2016 12:52 PM	Yes
miRGE1	6/7/2016 12:57 PM	Yes
CNV2	6/7/2016 1:56 PM	Yes
GX2	6/7/2016 1:57 PM	Yes
miRGE2	6/7/2016 1:57 PM	Yes
GX3	6/8/2016 2:12 PM	Yes
GX4	6/8/2016 2:12 PM	Yes
miRNA1	6/8/2016 2:12 PM	Yes
miRNA2	6/8/2016 2:12 PM	Yes

FIGURE 4.28 The Manage CodeSets/RLFs page.



5 Loading a Cartridge

Proper sample loading is important because the microfluidic system can process samples incorrectly if there are missing samples or unusual air gaps in the channels. In addition, there is a small risk of sample loss if the pipette and the cartridge form an incomplete seal.

Please review this chapter in detail before loading an nCounter cartridge to ensure successful sample loading.

A. nCounter® Cartridge Overview

Samples should be loaded into the cartridge after creating a run on the Control Center and immediately before using the instrument. (Runs may be created and saved well in advance if desired.) A diagram of the cartridge and its key features is provided in **FIGURE 5.1**.

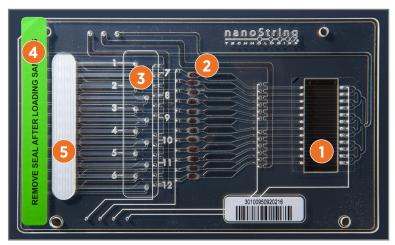


FIGURE 5.1 Diagram of the nCounter SPRINT cartridge.

- 1. Avoid touching the imaging surface.
- **2.** Orient the cartridge so that the NanoString logo is at the top.
- 3. Load samples 1-6 on the left and samples 7-12 on the right. Wipe the surface of the sample input area until dry and then cover the loading ports with the provided seal.
- **4.** Remove the green seal before loading into the instrument
- **5.** Do not write on the white bubble vent or the imaging surface.

The protocol for initiating a new run begins by inserting the loaded cartridge into the instrument drawer. Leaving samples at room temperature for an extended period may increase background signal during data collection.



IMPORTANT: Do not let the hybridized samples sit at room temperature for longer than 15 minutes. If there is a delay before starting the run, store hybridizations with nCounter CodeSets at 65°C until they can be processed. Store hybridizations with nCounter Elements TagSets at 4°C. Do not exceed 24 hours between hybridization and sample processing.



IMPORTANT: NanoString recommends using a standard pipette and filtered pipette tips with a volume of 100–200 μ L when loading samples. Examples are the Rainin® L-100 Pipet-Lite XLS and RT-L200F pipette tips. The sample loading protocol requires depressing the pipette plunger to the second stop, which adds an air gap after the sample. Using larger or smaller pipettes may affect the volume of this air gap and disrupt sample processing. Therefore, it is important to follow the instructions detailed on the next page for introducing a defined size air gap for each sample.



B. Cartridge Loading Instructions



IMPORTANT: Inject 30-35 μ L of each hybridized product into the nCounter cartridge. Inject the same volume into each lane. If the hybridization reaction results in a total volume less than 30 μ L, dilute under-volume samples with RNase-free water before loading the cartridge. Do not load a volume less than 30 μ L.

- 1. Remove the sample cartridge from its packaging. Orient it with the barcode on the bottom and the NanoString logo at the top. Avoid touching the top of the imaging surface on the right side of the cartridge.
- 2. Use a 100 or 200 μ L pipette with filtered tips to inject 30-35 μ L of each hybridized product, one at a time, into the sample loading ports on the cartridge. Discard each pipette tip after using it to load a single sample. See note above for products less than 30 μ L.
 - a. Hold the pipette perpendicular to the cartridge and press the pipette tip against the sample loading port. Do not press hard enough to force the tip into the loading port. See FIGURE 5.2 for an example.

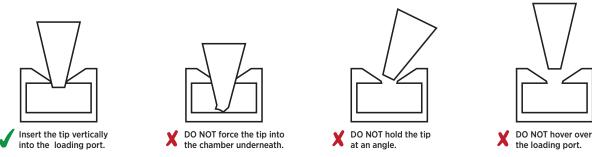


FIGURE 5.2 Proper and improper sample loading techniques. Gently insert the tip into the sample loading port to create a seal, but do not force it into the chamber underneath the port.

b. Introduce an air gap of defined size. For samples 1-6, introduce an air gap to the lane numbers 1-6 molded on the cartridge. For samples 7-12, the ports are offset so an air gap should extend only to the the vertical line. See FIGURE 5.3 for an example.

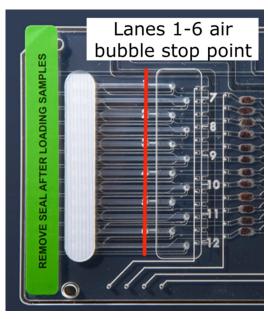




FIGURE 5.3 Introduce an air gap of defined size, as illustrated by the red lines.

- c. Remove the pipette tip from the cartridge without releasing the plunger. Releasing the plunger early will pull the sample back into the tip.
- d. Release the plunger and dispose of the used pipette tip.



IMPORTANT: When processing fewer than 12 samples, it is necessary to load 30 µL of water into each unused fluidics lane. Empty lanes will result in cartridge failure and the loss of all other samples during fluidics processing.



- 3. Remove all excess liquid from the surface of the cartridge using a lint-free wipe (e.g., KimWipe). Avoid the imaging surface.
- **4. Apply the provided seal over the sample loading ports**, ensuring that all ports are completely covered. The seal should completely cover the area within the outline on the cartridge. Use moderate thumb pressure to remove any air bubbles. Avoid covering the ports along the margin of the plate.
- **5.** Remove and dispose of the green seal that covers the reagent ports.
- **6.** Labeling the cartridge is optional. Use a felt-tip pen to write on the plastic cartridge. Do not write on the white bubble vent, on the barcode, or on the imaging surface.

C. Recommendations for Proper Cartridge Loading

This section describes proper cartridge loading techniques in order to ensure successful assays.

- Some users are accustomed to holding a pipette at an angle. However, always hold the pipette in a vertical orientation when loading the nCounter cartridge to create a seal with the loading port (FIGURE 5.3). After loading samples into the cartridge, the sample will pass through a channel and into a second chamber underneath. This is normal, and it may be difficult to see the sample.
- Some users are accustomed to depressing a pipette to the first stop, just enough to expel the liquid. However, always depress the pipette plunger to the second stop when loading the nCounter cartridge in order to create an air gap behind the sample. These air gaps need to be the same size to facilitate sample processing.
- Always pull the pipette away from the sample loading port before releasing the plunger. The microfluidic channels of the nCounter cartridge are a contained system, and releasing the plunger too soon will withdraw the sample back into the tip.
- The instrument utilizes pressure to move samples and reagents through small channels within the nCounter cartridge. An improper seal or a missing sample can cause pressure to drop unexpectedly. Always load water into unused lanes if there are fewer than 12 samples. Place the provided seal so that it covers every sample loading port.
- The nCounter cartridge surface must be dry, otherwise the adhesive on the provided seal will fail. Wipe the cartridge carefully to remove any moisture.
- A clean imaging surface is necessary for accurate data collection. Do not touch the imaging surface on the nCounter cartridge or allow
 other materials to come in contact with it. If the imaging surface becomes dirty, it may be cleaned with a small amount of ethanol



FIGURE 5.3 Demonstration of proper cartridge loading technique.

• During initialization, the instrument will ensure that the green sticker has been removed by applying pressure to a lane and checking for resistance. If any resistance is detected, the error, "System detected a protective seal on the cartridge. Please Open the Drawer to address this issue" will be displayed. If this error occurs for a cartridge which has had its green sticker removed, use a P200 pipette set to 100 µL to push air into the left-most well to clear any debris that may be blocking (**FIGURE 5.4**).

Using a 200 μ L pipette set to 100 μ L, push air into this port to clear any potential blockages

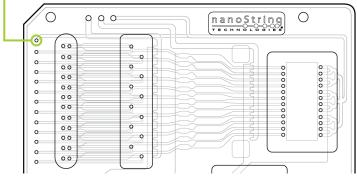


FIGURE 5.4 Clearing cartridge blockages.



6 Operating the Instrument

The nCounter SPRINT Profiler was designed to be simple and intuitive to operate; in many cases there is only one function available on each screen. After loading hybridized samples into a cartridge, that cartridge is placed into the instrument and a run is selected. When a run is complete, data can be downloaded directly using a USB flash drive or through the Control Center.

A. Prior to Initiating a Run

Consumables

There are several consumable items that may be purchased through NanoString Technologies as part of the nCounter *SPRINT* Profiler Reagent Pack (**FIGURE 6.1**). This kit contains sufficient reagent volumes to process 16 runs. No reagent preparations or dilutions are required.



FIGURE 6.1 Reagent bottles and hybridization buffer are included in the Reagent Pack.

The nCounter SPRINT Profiler Reagent Pack includes:

- Reagent A (green)
- Reagent B (blue)
- Reagent C (red)
- nCounter Hybridization Buffer

These materials must be purchased separately:

- nCounter SPRINT Cartridge (one cartridge per 12 lanes)
- nCounter CodeSet or nCounter Elements[™] TagSet



NOTE: Each cartridge can be used to process up to 12 samples (96 for PlexSet) at any one time.



NOTE: The SPRINT Profiler requires that you replace reagents after their expiration, or "best by," date, or when they have been on board the instrument for 180 days, whichever comes first.



Reagents and Waste

Reagent and waste levels are always visible on the Main Menu in the lower left corner. A yellow alert icon will appear when the reagents are empty or only one run remains, when the waste bottle is full or has capacity for only one run, or when the reagents have expired (FIGURE 6.2). These issues may be resolved between runs or when initiating a new run. NanoString recommends checking the reagent and waste levels before loading samples onto a cartridge in order to avoid delays that may diminish data quality. Detailed instructions are available in *Chapter 7, Reagents and Waste*.

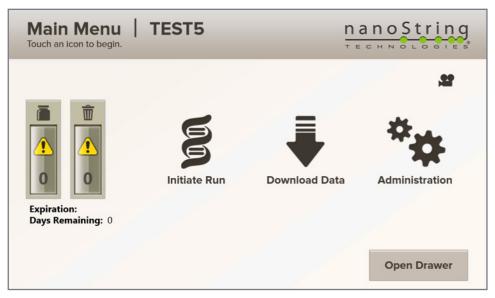


FIGURE 6.2 Reagents and Waste alert icons on the Main Menu.



CAUTION: Dispose of waste according to the rules and regulations of your institution. Return the waste bottle to the instrument and reattach the tubing before starting a run.

ATTENTION: Éliminez les déchets selon les règles et réglementations de votre institution. Remettre en place le récipient à déchets dans l'instrument et rattachez le tuyau avant de commencer une opération.

B. Initiating a Run

The following workflow begins on the Main Menu, after loading samples into the nCounter cartridge (see Chapter 5, Loading a Cartridge).



IMPORTANT: Process hybridized samples as soon as possible. Do not exceed 24 hours between ramping the hybridization to 4°C and sample processing, as extended storage may increase non-specific background counts.

1. To begin a new run, select Initiate Run on the Main Menu (FIGURE 6.3).

The cartridge drawer will open.

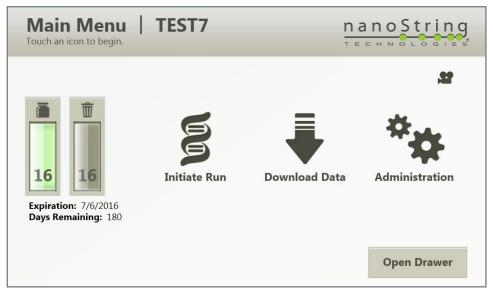


FIGURE 6.3 The Main Menu.



NOTE: The instrument will determine if there are enough reagents to complete the run and if the waste bottle is full. If necessary, the instrument will guide the user through the reagent changing or waste removal procedures before opening the drawer (see Section A).

2. After the drawer has fully opened, review the reminders on the Insert Cartridge screen (FIGURE 6.4). Ensure that the samples were properly loaded, that the clear tape was placed over the sample loading ports, and that the green protective seal was removed from the reagent ports.

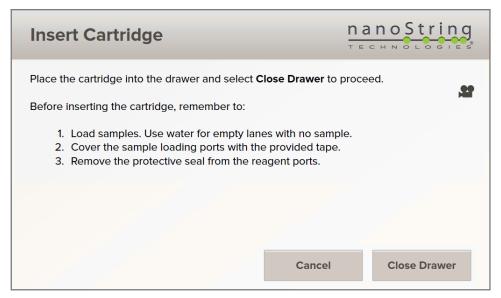


FIGURE 6.4 The Insert Cartridge screen.

3. Insert the cartridge in the drawer, aligning the notch in the upper left corner (FIGURE 6.5). Ensure the cartridge is flush with the drawer.



FIGURE 6.5 Place the nCounter cartridge in the drawer. After the drawer closes, the instrument will scan the barcode.

4. Press Close Drawer.

The Closing Drawer screen will appear, and the instrument will read the barcode on the cartridge.



CAUTION: Do not place your fingers near the drawer while it is moving. Always allow the drawer to open and close automatically without touching it.

ATTENTION: Ne placer pas vos doigts près du tiroir pendant que celui-ci soit en mouvement. Permettez toujours au tiroir de s'ouvrir et de se fermer automatiquement sans le toucher.

If the cartridge was previously used for another run, or if the barcode cannot be read, an error message will appear. It is not possible to override the error if the cartridge was previously used. If the barcode cannot be read, the user may override the error and a new barcode will be automatically generated. Press **Replace Cartridge** to try again or **Cancel** to return to the Main Menu.

After the cartridge is accepted, any pre-defined runs are displayed if they have not yet been initiated.
 The Run Selection screen will appear (FIGURE 6.6).

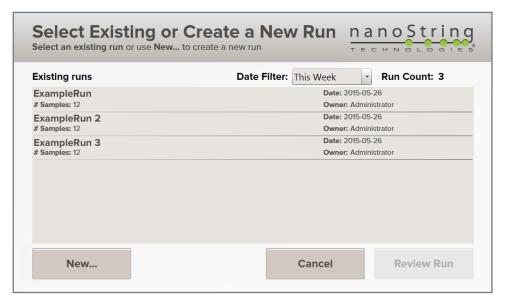


FIGURE 6.6 The Run Selection screen.

6. Runs will be displayed if previously created and saved using the Control Center web application. Select a run and press **Review Run** to confirm sample and processing information. Alternatively, select **New**... to initiate a run using default settings and without specifying detailed information for each sample. (This workflow is explained in the next section.)

The Review Run screen will appear (FIGURE 6.7).

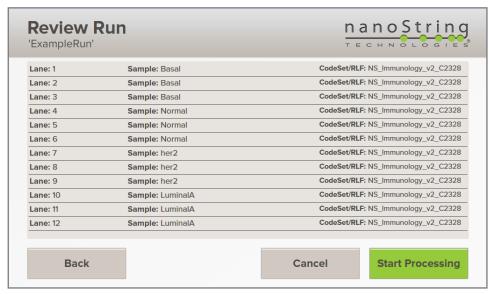


FIGURE 6.7 The Review Run screen.

7. Confirm the run information. If necessary, press **Back** to choose a different run or press **Cancel** to abort the workflow and return to the Main Menu. Sample information cannot be edited from the instrument display panel; if changes must be made, abort the workflow by pressing **Cancel** and use the Control Center to edit the run.



IMPORTANT: Once a run begins, it cannot be paused. Any action affecting the instrument after fluidics processing commences such as attempting to change reagent bottles or pressing **Abort** will result in sample loss.

8. When satisfied with the run information, press the green **Start Processing** button to begin the run. A status page will appear and count down toward the end of the run.

The Run Status screen will appear (FIGURE 6.8).



FIGURE 6.8 The Run Status screen.

The instrument status will also be displayed on the Control Center home page (FIGURE 6.9).

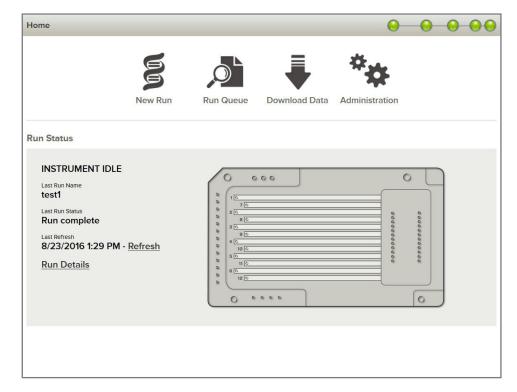


FIGURE 6.9 The Control Center home page while a run is in progress. If logons are enabled, you will see a <u>Refresh</u> link above the <u>Run Details</u> link.



Creating a Default Run

To define specific sample information (e.g., specific sample names or different CodeSets for each sample) it is necessary to use the Control Center to create and save a new run. However, many experimental designs do not require this level of detail. A "default" run option is available that uses many default options to create and begin a run using only the instrument touchscreen.

The following workflow begins on the Main Menu, after loading samples into the nCounter cartridge (see Chapter 5, Loading a Cartridge).



IMPORTANT: Process hybridized samples as soon as possible. Do not exceed 24 hours between ramping the hybridization to 4°C and sample processing, as extended storage may increase non-specific background counts.

1. After the cartridge is accepted, any pre-defined runs are displayed if they have not yet been initiated.

The Run Selection screen will appear (FIGURE 6.10).

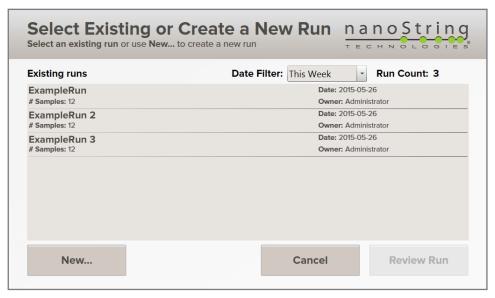


FIGURE 6.10 The Run Selection screen.

2. Select **New...** to define a new run with default settings.

The Create a New Run screen will appear (FIGURE 6.11).



FIGURE 6.11 The Create a New Run screen.

- **3.** Choose the desired assay type, the desired protocol, and the CodeSet/RLF that associates each fluorescent barcode with a unique target. The same RLF must be used for all samples, and samples will be assigned generic identification numbers (e.g., "SampleO1, SampleO2, SampleO3, ...") that correspond to each cartridge lane.
- **4.** If the desired CodeSet is not displayed, insert a USB drive containing the RLF into the port on the side of the touchscreen display and then press the **Upload CodeSet/RLF** icon.
- 5. Select the desired file and press Upload.
- 6. The Create a New Run screen will re-appear and the new RLF should be available. Select the desired CodeSet/RLF.

7. Select Review Run.

The Review Run screen will appear (FIGURE 6.12). Sample details will appear with the same default settings for every lane. The run will be named 'DefaultRunXX' where 'XX' is a unique number.

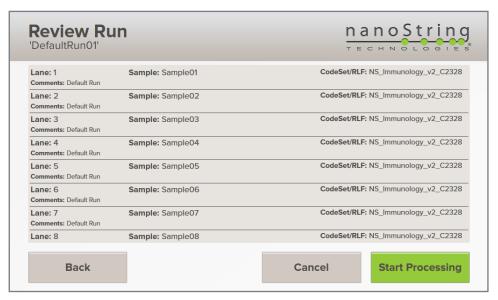


FIGURE 6.12 The Review Run screen.



IMPORTANT: Once a run begins, it cannot be paused. Any action affecting the instrument after fluidics processing commences such as attempting to change reagent bottles or aborting the run will result in sample loss.

8. Press Start Processing to begin the run. A status page will appear and count down toward the end of the run.



Aborting a Run

To abort a run in progress, press the **Abort** button on the Run Status screen (FIGURE 6.13).
 A confirmation screen will appear (FIGURE 6.14).

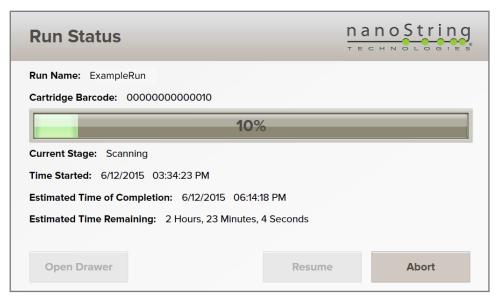


FIGURE 6.13 The Run Status screen.

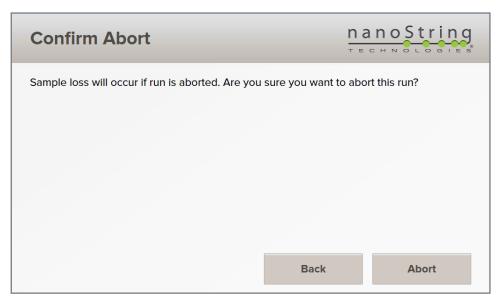


FIGURE 6.14 The Abort Confirmation screen

2. Press **Abort** again only if certain that the run should be aborted. If not press **Back** to continue processing the run.



IMPORTANT: When a run is aborted, it cannot be restarted. It may be possible to image samples at a later date if fluidic processing is complete, and the instrument will warn the user if fluidic processing is ongoing. Samples will be lost if any fluidic processing has already commenced and is not complete.



C. Completing a Run

When a run ends, the Run Complete screen will appear and a timer will start to count up (FIGURE 6.15). An email notification and associated log files will be sent to all email addresses listed when the run was setup as described in *Chapter 4, Operating the Control Center*.

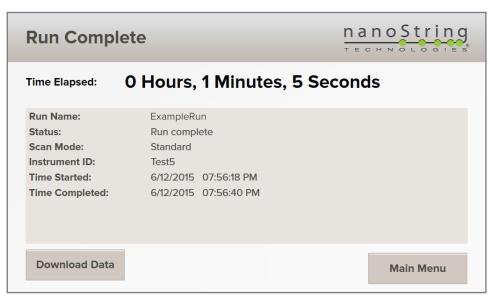


FIGURE 6.15 The Run Complete screen.

- 1. Press **Download Data** to download RCC data and log files (see the next section for detailed instructions.) Alternatively, press **Main Menu** to return to the Main Menu. Data files can be accessed at a later time from the instrument or the Control Center.
- 2. After returning to the Main Menu, press **Open Drawer** to remove the cartridge. Alternatively, wait until beginning a new run; the old cartridge can be removed when a new one is inserted.



Download Data and Log Files via USB Flash Drive

The RCC data and log files created after each run can be accessed from the instrument or, if the instrument is properly networked, from the Control Center web application. These instructions describe how to transfer files to a PC from the instrument utilizing a USB flash drive. See Chapter 4, Operating the Control Center for instructions on downloading files from the Control Center.

1. Press Download Data on the Run Complete screen if at the end of a run or on the Main Menu if between runs.

The Download Data screen will appear with two options: Results and Logs (FIGURE 6.16).

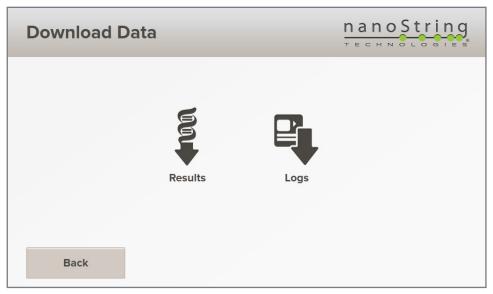


FIGURE 6.16 The Download Data screen.

2. To download RCC data files, click on the Results icon.

A list of available results will appear (FIGURE 6.17).

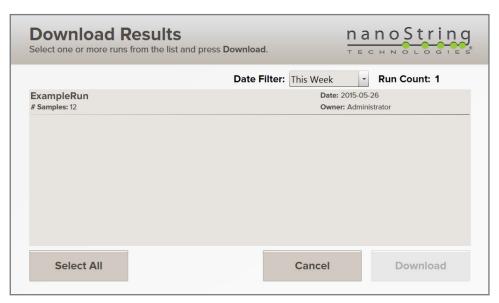


FIGURE 6.17 The Download Results screen.

3. All data that have been processed and are available will be displayed on this screen. To select a run for download, touch the run name on the screen. One or more runs may be selected by touching each name. To deselect a run, touch it again. Use the **Date Filter** to select a different period if trying to access older runs (**FIGURE 6.18**).



FIGURE 6.18 Results can be filtered by date.

- 4. Insert a USB drive into the USB port on the side of the display.
- **5.** After selecting the file(s) to download and inserting a USB drive, press Download.
- 6. Repeat Steps 1-5 to access maintenance log files on the instrument. Press the Logs icon in Step 2 and the Download Logs screen will appear (FIGURE 6.19). The selected log files will be saved to a separate folder on the USB drive. Only user and service logs are available on-board the instrument. For user, service, and system logs, use the web app. Refer to the Downloading Sprint Logs Quick Guide.

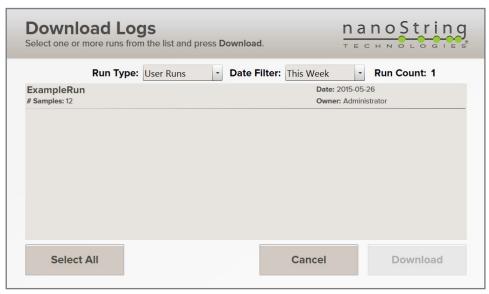


FIGURE 6.19 The Download Logs screen.



Transfer Data and Log Files via Email

If instrument networking and email have been configured and a valid email address was entered during the creation of a new run, then RCC data from that cartridge can be delivered by email. (See *Chapter 4, Operating the Control Center* for detailed instructions.) Be aware that not all institutions allow ZIP files to be emailed. If you are having trouble receiving data, contact your IT department. Some institutions prohibit email delivery of ZIP files, or there may be a limit on the maximum file size permitted.



7 Reagents and Waste

Reagents and waste bottles for the nCounter *SPRINT* Profiler have sufficient volume for multiple runs. However, users must periodically replace reagents, empty the waste bottle, and confirm that reagents have not expired if there is a significant period between runs. The instrument tracks container levels and expiration dates and will provide a reminder if necessary.

A. Overview

Reagent bottles have color-coded labels and matching color-coded tubing. Reagent B and the waste bottle are also equipped with unique fittings to reduce the risk of connecting bottles to the wrong tubing (FIGURE 7.1).



FIGURE 7.1 The reagent bottles and waste bottle.

When replacing bottles, ensure that the bottle label faces the front of the instrument (with the cartridge loading drawer and touchscreen). Bottle labels that face the wrong direction may interfere with the level sensor. Bottles should also be arranged in a specific order within their compartment, from rear to front (FIGURE 7.2):

- Reagent A (green, square)
- Reagent B (blue, square)
- Waste Bottle (white, round)
- Reagent C (red, square)





FIGURE 7.2 The reagent bottles and waste bottle inside their compartment. Bottle labels should face the front of the instrument (not the side).

To disconnect the tubing, press the grey button on the side of the fitting. To connect the tubing, match the bottle with the correct tubing and press the fittings together. It may be necessary to pull back on the ring surrounding one of the fittings.

- 1
- **IMPORTANT:** Do not attempt to unscrew the fitting from the cap assembly or the tubing. Only disconnect the bottle from the tubing by pressing on the release button. Lift the bottle by holding the cap or bottle; never pull on the fitting or tubing.
- A
- **IMPORTANT:** When reconnecting the fitting using the quick connect system, there should be an audible "click" when fully engaged. A partial connection will introduce bubbles to the fluidics system and may cause assay failure.
- 1
- **IMPORTANT:** Do not attempt to unscrew the cap on a bottle while it is inside the instrument. Always detach the tubing while the cap remains securely attached to the bottle. Then remove the bottle before unscrewing the cap.

Check Reagents and Waste Status

Three reagents are required for processing hybridized samples. All reagent bottles have a unique barcode used to track reagents and their expiration, and the instrument has sensors that track their volume. This system ensures that sufficient quantities of reagents are available to complete a run. The waste bottle is also equipped with a sensor that will warn when the container has inadequate capacity for another run.



NOTE: The waste bottle may not appear full even if the capacity reaches zero. However, some empty space is required for the instrument to function.

The status of the reagents and waste are always visible on the Main Menu (FIGURE 7.3). To view additional information or to change these bottles, press the reagents and waste status bars on the Main Menu. Alternatively, press the Administration icon and then choose Reagents & Waste.

All three reagent bottles must be changed together to maintain the same number of available runs across all reagents. The Change Reagents protocol should be performed when the **Expiration** listed on the screen is reached, or the **Days Remaining** has reached 0, whichever comes

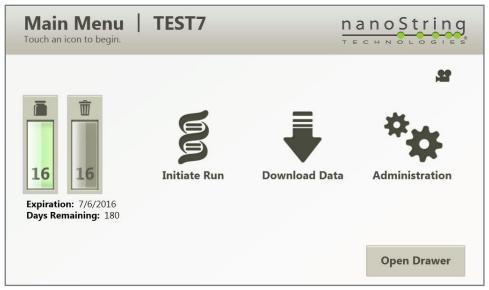


FIGURE 7.3 The Main Menu when reagents are full and waste is empty.

Two icons will appear, representing reagents and waste, respectively (FIGURE 7.4), as well as other details including the remaining volume, the expiration date of the reagents, and the remaining capacity of the waste bottle.



FIGURE 7.4 The Reagents & Waste screen when reagents are full and waste is empty.



B. Change Reagents



IMPORTANT: At no time should reagent bottles be refilled or combined with the contents of other bottles. Bottles are loaded in a precise order to reduce the risk that Reagent C comes in contact with Reagents A and B. Contamination may cause assay failure. If contamination occurs, contact NanoString Support at **support@nanostring.com**. A service call may be required to flush the system or replace tubing.



NOTE: Each bottle contains 16 runs worth of reagent, so it is critical to change all reagents together as the Change Reagents workflow indicates. While the reagents can be purchased individually, this is for instances of expiration or damage before the bottle is loaded onto the instrument.

When reagent bottles are expired, or the contents are low or empty, an alert will appear on the Main menu and the Reagents & Waste screen. An error message will also appear when attempting to initiate a run (see *Chapter 6, Operating the Instrument*).

The user may override this error message, but NanoString recommends replacing the reagents. Poor quality data may result from running on expired reagents. The entire workflow requires approximately 10 minutes as the instrument will check the connection with the tube assembly after replacing each bottle.

1. If an error message appears at the beginning of a run, press **Change Reagents** to begin the workflow or **Cancel** to return to the Main Menu. To access the workflow when a run is not in progress, press **Reagents & Waste** on the Administration Menu, and then press the **Change Reagents** icon.

The Change Reagent screen appears with instructions to replace Reagent A (green bottle) (FIGURE 7.5).

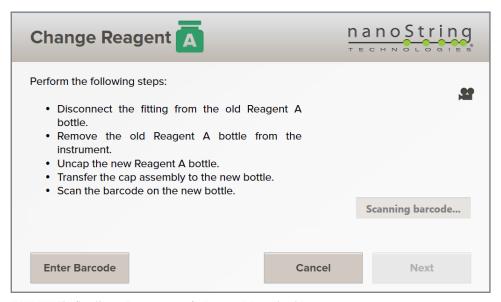


FIGURE 7.5 The first Change Reagent screen for Reagent A (green bottle).

- 2. Lift the door to the reagents and waste compartment. The door will remain open on its own.
- **3.** Disconnect the fitting between the bottle and its tubing by pressing on the grey button.
- 4. Remove the bottle from the instrument.

5. Unscrew the caps from both the new and old reagent bottles for Reagent A. Switch caps so that the coupling is attached to the new bottle (FIGURE 7.5). Use the other cap to seal the old bottle and dispose of it according to the rules and regulations of the local institution.



FIGURE 7.6 Switch caps between reagent bottles.

6. Scan the barcode on the new bottle by holding it under the instrument touchscreen (**FIGURE 7.7**), or enter the barcode manually by pressing **Enter Barcode**. Hold the bottle 1 to 4 inches (2.5 to 10 cm) away from the reader.



FIGURE 7.7 Scan the bottle using the barcode reader under the instrument touchscreen.

7. If the barcode indicates the reagent has expired, then an error message will appear. The user may override this message. An error message will also appear if the barcode indicates the reagent was previously used, or is the wrong reagent. Replace the reagent and try again.

Once the barcode for a new bottle is successfully entered, the Change Reagent screen will refresh with instructions to replace the reagent bottle (FIGURE 7.8).



FIGURE 7.8 The Change Reagent screen for Reagent A.

- **8.** Place the new reagent bottle in the reagents and waste compartment, orienting the bottle so that the label faces the front of the instrument. Reattach the tubing to the reagent bottle; tubing is color coded to match the bottle's label and cap. Each bottle/tubing pair has a unique set of fittings to prevent mix-ups.
 - a. Reagent A (green) belongs in the rear of the compartment.
 - b. Reagent B (blue) belongs in front of Reagent A.
 - c. Waste (white) belongs in front of Reagent B.
 - **d.** Reagent C (red) belongs in the front of the compartment.

9. When the bottle is placed in the instrument and the fitting is reconnected, press **Next**. As each reagent bottle is replaced, a level sensor will confirm that the bottle is full. If the level sensor produces an error message, the user may visually confirm the bottle is full and override the instrument.

After pressing Next, a status bar indicates the progress while the instrument checks the connection (FIGURE 7.9).



FIGURE 7.9 The instrument will provide a status bar while it checks the connection with the tube assembly after replacing each bottle. Additional time is required to flush the tube assembly after replacing Reagent C.

10. The Change Reagent screen for Reagent B (blue bottle) will appear next (FIGURE 7.10). Repeat Steps 3-9 to change Reagent B.

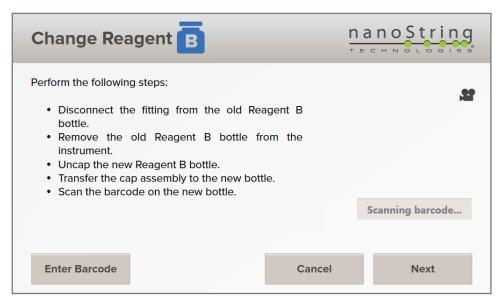


FIGURE 7.10 The Change Reagent screen for Reagent B.

11. The Empty Waste screen will appear next with instructions to empty the waste bottle (FIGURE 7.11).



FIGURE 7.11 The Empty Waste screen.

12. The waste bottle must be emptied before replacing Reagent C. Dispose of the contents according to the rules and regulations of the local institution. Reattach the cap to the bottle and return it to the instrument.



NOTE: Carefully remove the waste bottle from the instrument. This bottle may contain a large amount of liquid.

13. Press **Next** to reset the waste bottle level and check the connection. When it is finished, the Change Reagents screen for Reagent C (red bottle) will appear (**FIGURE 7.12**).

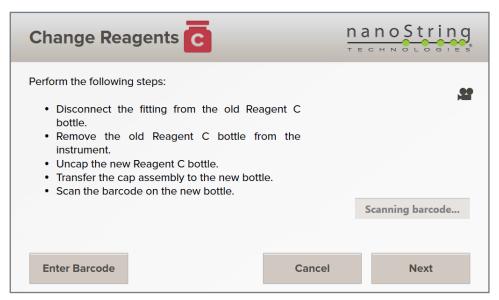


FIGURE 7.12 The Change Reagent screen for Reagent C (red bottle).

- 14. Repeat Steps 3-9 for Reagent C (red bottle). Additional time is required after changing Reagent C while the tube assembly is flushed.
- **15.** After all reagents are replaced and the waste bottle is emptied, a completion screen will appear. Press **Finish** to complete the Change Reagents workflow and reset the reagent indicator levels to full.

At the end of the workflow, any notifications will be removed and the timer that tracks the useful life of the immobilization buffer will be reset. Be sure to close the door to the reagents and waste compartment.



NOTE: The waste bottle may not be fully empty at the end of this workflow because it collects some waste as the tube assembly is checked and flushed.

C. Empty Waste



NOTE: Emptying the waste bottle is included in the Replace Reagents workflow. However, the waste bottle may fill sooner than the reagents are consumed, making it necessary to empty the waste bottle separately.

When the waste bottle is near capacity, an alert will appear on the Main Menu and the Reagents and Waste screen. An error message will also appear when attempting to initiate a run (see *Chapter 6, Operating the Instrument*). It is not possible to override the instrument if the waste bottle is full.

If an error message appears at the beginning of a run, press Empty Waste to begin the workflow or Cancel to return to the Main Menu.
 To access the workflow when a run is not in progress, press Reagents & Waste on the Administration Menu, and then press the Empty Waste icon.

The Empty Waste screen will appear with instructions to remove and empty the waste bottle (FIGURE 7.13).



FIGURE 7.13 The Empty Waste screen.

- 2. Lift the door to the reagents and waste compartment. The door will remain open on its own.
- **3.** Press the plastic button on the coupling between the white waste bottle and its tubing.
- 4. Remove the bottle.
- 5. Unscrew the cap. Dispose of the waste bottle's contents according to the rules and regulations of the local institution.



NOTE: Remove the waste bottle carefully. It may contain liquid and could become tangled in the tubing connected to the reagent bottles.

- **6.** Attach the cap and replace the bottle in the reagents and waste compartment. Recouple the tubing to the waste bottle; the waste bottle has a bundle of three tubes that lead to one coupling. Press **Next**.
- 7. When the confirmation screen appears, press **Finish** to reset the waste bottle level. This will end the Empty Waste workflow and return to the Reagents & Waste screen.

At the end of the workflow, any notifications will be removed. Be sure to close the door to the reagents and waste compartment.



Technical Support and Maintenance

A. Technical Support

- Technical support is available by phone, mail, and email.
- When reporting a technical issue, please email User and System log files to support@nanostring.com to expedite troubleshooting.
- Be sure to include the product number and serial number in all communications.



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B. Administration

Press the Administration icon on the Main menu (FIGURE 8.1) to access the Administration menu icons (FIGURE 8.2):

- Reagents & Waste—Check the levels of the reagent and waste bottles, monitor the expiration of reagents, replace reagents, and empty the waste bottle.
- Upload CodeSet/RLF—Upload new RLFs to the instrument for use when initiating a run.
- Support—Additional maintenance functions:
 - System Information—View the System Clock, Instrument Name, Serial Number, IP Address, and Control Center URL.
 - Logs—Download log files for remote analysis by NanoString Support.
 - Service Tool—Run maintenance functions provided by NanoString Support.
 - Software Update—Update the instrument's system software when a new release is available.
- Power Off—Power off or restart the system when necessary. Select this icon before reaching around the back of the instrument and turning off the main power switch.

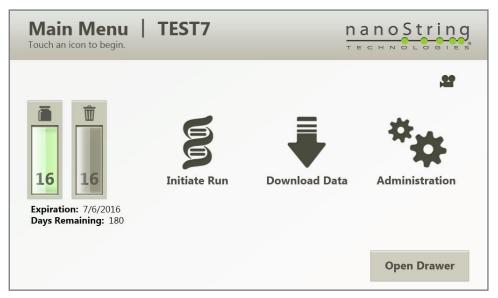


FIGURE 8.1 The Main menu.

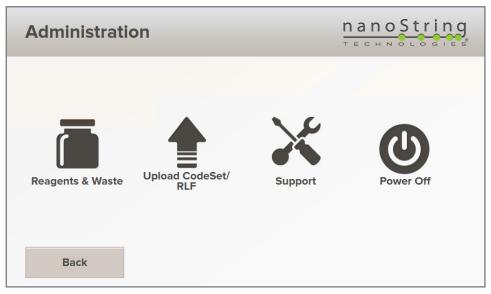


FIGURE 8.2 The Administration menu.



Reagents and Waste

Refer to Chapter 7, Reagents and Waste for more information on this feature.

Upload CodeSet/RLF

Users only need to upload Reporter Library Files (RLFs) when they first receive a new CodeSet. Once an RLF has been uploaded, it will always be available until it is manually deleted from the instrument (see *Manage CodeSets/RLFs* in *Chapter 4, Operating the Control Center*).

New RLFs can be uploaded during the run creation process, either using the Control Center or on the instrument (see *Chapter 6, Operating the Instrument*). This section describes how to upload a new RLF to the instrument between runs.

- 1. Insert the USB drive into the USB port on the right side of the display.
- 2. From the Administration Menu, press Upload CodeSet/RLF.

The Upload Files screen will appear.

3. Select Upload CodeSet/RLF.

The Select RLF Source screen will appear.

4. Those folders and RLFs found on the memory stick that have not already been uploaded will be displayed. Select one or more files and press Next to upload the file(s).

The RLF will be saved on the instrument's local drive.

Support

Additional system features are located in a separate section. To access the System Information, Logs, Service Tool, or Software Update features, press the **Support** icon on the Administration Menu.

The Support Menu will appear (FIGURE 8.3).



FIGURE 8.3 The Support menu.

System Information

The System Information screen (FIGURE 8.4) allows users to view the system clock, instrument name, ID (serial number), IP address, MAC address, and Control Center URL. To access the System Information screen, press the System Information icon on the Support Menu.



FIGURE 8.4 The System Information screen.

If the instrument was not originally connected to a network during installation, it is possible to complete this task at a later date. See *Chapter 2, Installation* for more information on networking the instrument.



NOTE: System settings may be viewed by selecting System Information, but the Control Center must be used to adjust any of these settings.

Logs

Log files are created for each run and may be useful for troubleshooting in the event of an error or other problem. NanoString Support may ask for the instrument log files when providing assistance. Log files can be accessed through the Control Center (see Chapter 4, Operating the Control Center) and on the Download Data screen through the instrument Main Menu (see Chapter 6, Operating the Instrument). The Administration functions provide a third option. Refer to the Downloading Sprint Logs Quick Guide.

1. Press the **Logs** icon on the Support Menu.

A list of available log files will appear (FIGURE 8.5).

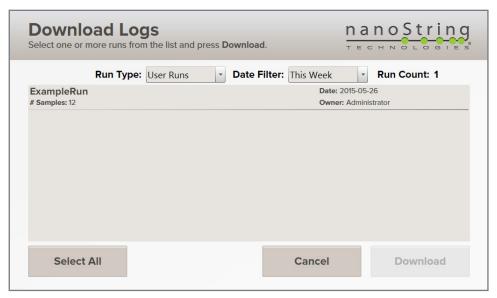


FIGURE 8.5 The Download Logs screen.

2. To select a log for download, touch the run name on the screen. One or more runs may be selected by touching each name. To deselect a run, touch it again. If you are trying to access older runs, use the Date Filter to select a different period (FIGURE 8.6).

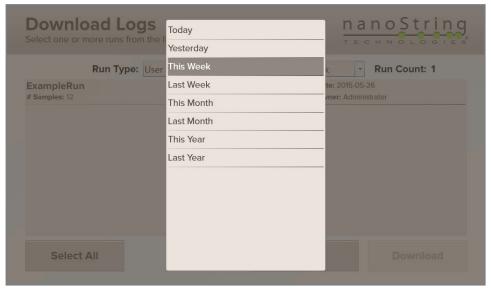


FIGURE 8.6 Files can be filtered by date.

- 3. Insert a USB drive into the USB port on the side of the instrument touchscreen.
- 4. After selecting the file(s) to download, press Download.



Service Tool

NanoString support may ask the user to upload and run a script during some troubleshooting scenarios if a particular test is not already installed on the instrument. These functions are performed using the Service Tool (FIGURE 8.7).

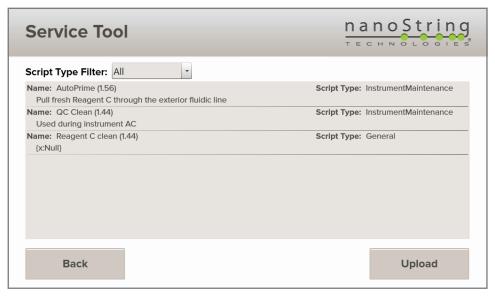


FIGURE 8.7 The Service Tool screen.

Insert a USB drive containing the script into the USB port on the side of the instrument touchscreen. Press the **Service Tool** icon on the Support Menu and select the file to upload. The system will install and run the script automatically, followed by a message with test results or further instructions.

On systems running software v2.2.0.36 or higher, the Service Tool window will contain an Auto Prime script. When the instrument is powered on, this script will automatically run in the middle of the night when it has been 2 or more weeks since last being used for fluidic processing. NanoString recommends leaving the instrument on when not in use, so Auto Prime can continue to operate on this schedule and keep the lines clear. To run Auto Prime on command, first ensure that the instrument is in working order, all reagents are plugged in, and the waste bottle is in place. Then, access this window and run the Auto Prime scr

Software Update

NanoString will periodically release new versions of software for instruments. Press the **Software Update** icon on the Support Menu for the Update Software screen (**FIGURE 8.8**). If the instrument is networked and has Internet access, it will automatically check for updates and notify you on the instrument touchscreen. Press **Install Updates** to update the SPRINT software.



FIGURE 8.8 The Update Software screen

Without a network connection and Internet access, you can update to the latest version of the software with a USB drive. Use the NanoString-provided pre-loaded USB drive or download the installation files from the NanoString website to a blank USB drive. Do not unzip the installation file; the instrument will expect to see a .zip file. To perform the update, insert the USB drive into the USB port on the side of the instrument touchscreen, and then press the **Software Update** icon on the Support Menu.



C. Storing or Moving the Instrument

The instrument should remain powered-on unless moving it to a new location, this will allow the auto prime function to maintain the health of the lines when the instrument is idle for more than two weeks. A re-start once a month is recommended to reset the on-board computer.

If moving the instrument, before turning it off, ensure that the cartridge drawer is empty and closed and that a run is not in progress. Press the **Power Off** icon located on the Administration Menu (**FIGURE 8.2**). Locate the main power switch at the right rear of the instrument and turn it off. Unplug the power supply cable from the wall and the instrument. Ensure there is enough space in the new location (see *Chapter 1, Introduction*). Clear space around the instrument to provide space for two individuals to grasp and move it to the new location. It may be necessary to clean or replace parts of the system, particularly the fluidics tubing, if there is microbial growth after prolonged storage. See



IMPORTANT: Always contact NanoString Support at support@nanostring.com for additional information before moving the instrument.



CAUTION: Never attempt to move the instrument alone. At least two individuals are required to safely lift the instrument. Lift carefully, holding the bottom of the instrument with both hands.

ATTENTION: Ne jamais tenter de déplacer l'instrument seul. Au moins deux personnes sont nécessaires pour soulever en toute sécurité l'instrument. Soulevez prudemment, tenant le bas de l'appareil avec les deux mains.

D. Instrument Maintenance

Additional maintenance tasks may need to be performed as necessary to ensure proper operation of the instrument. These include minor cleaning and repair tasks that do not require the presence of a service technician. Review all tasks in this section on a regular basis and after removing the instrument from prolonged storage.

General Cleaning

Follow all safety and operating instructions provided in this manual. Use safe laboratory operating precautions, including personal protective equipment such as safety glasses and gloves.

- Always ensure the waste bottle has available capacity before starting a new run. The touchscreen will display an indicator if the
 waste bottle is full.
- If a spill occurs, clean the affected area by wiping with a disinfectant followed by wiping with water or 70% ethanol. A disinfectant such as DNAZap from Invitrogen is recommended. Use a damp towel rather than spraying directly on the instrument.
- Clean the exterior of the instrument using a diluted neutral soap, followed by water. Use a damp towel rather than spraying directly
 on the instrument.

Replace Air Fllter

The air filter located on the back of the instrument will need occasional replacement, typically every 12–18 months. If the filter appears to be visibly obstructed by debris, contact NanoString Support (support@nanostring.com) to request a replacement.

- 1. Lift up on the filter chassis to remove it from the instrument (FIGURE 8.9).
- 2. Unscrew the cover by hand (do not use tools).
- 3. Remove the metal cover.
- **4.** Remove and dispose of the mesh filter. Do not attempt to clean and re-use the filter.
- 5. Insert the new filter supplied by NanoString.
- 6. Replace the metal cover and affix the screw by hand (do not use tools).
- **7.** Return the filter chassis by lowering it into the instrument.



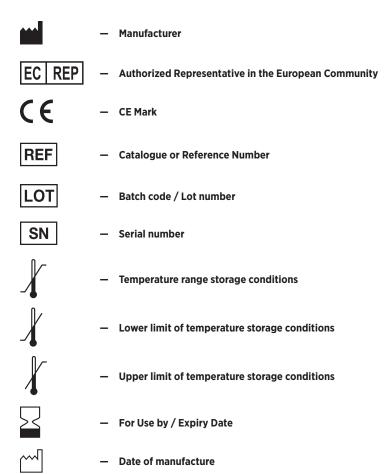
FIGURE 8.9 The air filter chassis

Replace Fluidics Tube Assembly

If the fluidics tubing or filter becomes damaged, the entire tube assembly will need to be replaced. This includes the cap with its specialized fitting, the filter that rests inside the bottle, and the tubing that attaches the assembly to the instrument. Contact NanoString Support (support@nanostring.com) to request a replacement. Follow the instructions below to remove the damaged assembly and install a new one.

- 1. Detach the reagent bottle from the tube assembly by pressing on the fitting (see Chapter 7, Reagents and Waste for more information).
- 2. Unscrew the tubing from the instrument.
- 3. Remove the reagent bottle and unscrew the cap and filter.
- 4. Dispose of the entire tube assembly, including the cap and filter.
- 5. Separate the new tube assembly by pressing on the fitting.
- **6.** Replace the cap and filter on the reagent bottle.
- **7.** Replace the tubing by screwing it into the empty port on the instrument.
- 8. Return the reagent bottle to the instrument and re-attach the tubing.
- 9. NanoString support may provide automated scripts to run using the instrument Service Tool (FIGURE 8.7). Follow the instructions provided to install and run the scripts.

Symbols and Definitions





nCounter® SPRINT Profiler USER MANUAL



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