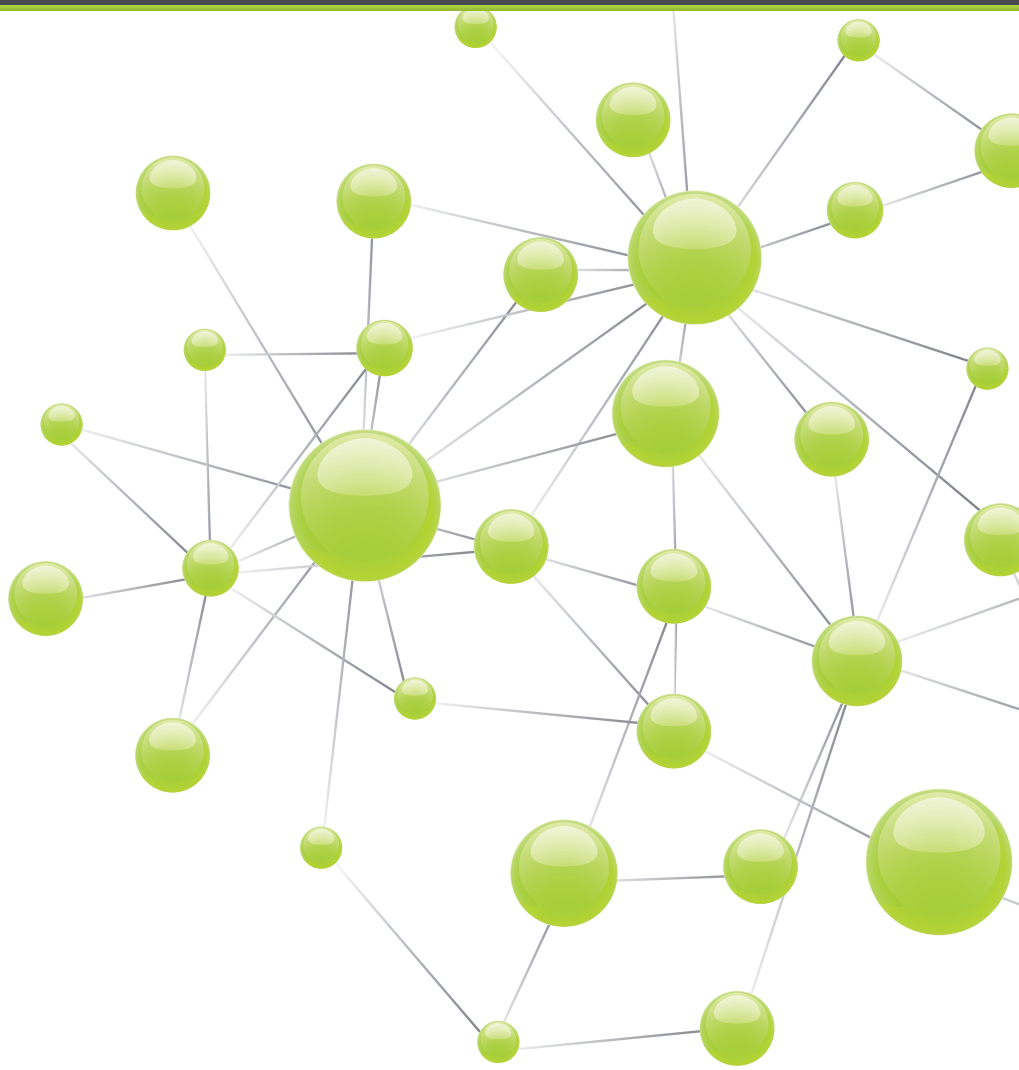
nanoString®
TECHNOLOGIES



nCounter® Tech

nCounter®

GEN2 Prep Station User Manual

NanoString® Technologies, Inc.

530 Fairview Ave N
Suite 2000
Seattle, Washington 98109

www.nanostring.com

Tel: 206.378.6266
888.358.6266

E-mail: info@nanostring.com

Molecules That Count®

Translational Research • Gene Expression • miRNA Expression • Copy Number Variation • Single Cell Expression

MAN-C0020-04

FOR RESEARCH USE ONLY. Not for use in diagnostic procedures.

Intellectual Property Rights

This nCounter® Analysis System manual and its contents are the property of NanoString Technologies, Inc. ("NanoString"), and is intended solely for the use of NanoString customers, for the purpose of operating the nCounter Analysis System. The nCounter Analysis System (including both its software and hardware components) and this User Guide and any other documentation provided to you by NanoString in connection therewith, are subject to patents, copyright, trade secret rights and other intellectual property rights owned by, or licensed to, NanoString. No part of the software or hardware, may be reproduced, transmitted, transcribed, stored in a retrieval system, or translated into other languages without the prior written consent of NanoString.

Limited License

Subject to the terms and conditions of the nCounter Analysis System contained in the product quotation, NanoString grants you a limited, non-exclusive, non-transferable, non-sublicensable, research use only license to use the proprietary nCounter Analysis System only in accordance with the manual and other written instructions provided by NanoString. Except as expressly set forth in the terms and conditions, no right or license, whether express, implied or statutory, is granted by NanoString under any intellectual property right owned by, or licensed to, NanoString by virtue of the supply of the proprietary nCounter Analysis System. Without limiting the foregoing, no right or license, whether express, implied or statutory, is granted by NanoString, to use the nCounter Analysis System with any third party product not supplied or licensed to you by NanoString, or recommended for use by NanoString in a manual or other written instruction provided by NanoString.

Trademarks

NanoString®, NanoString Technologies®, nCounter®, Molecules That Count®, nSolver™, Plex2™, ChIP-String™, miRGE™, and nDesign™ are registered trademarks or trademarks of NanoString Technologies, Inc., ("NanoString") in the United States and/or other countries. All other trademarks and or service marks not owned by NanoString that appear in this document are the property of their respective owners. The manufacture, use and or sale of NanoString product(s) may be subject to one or more patents or pending patent applications owned by NanoString or licensed to NanoString from Life Technologies Corporation and other third parties.

Copyright

Original Instructions

This manual contains the original instructions for the nCounter® Digital Analyzer and is the native language version of the manual.

nanoString TECHNOLOGIES

CONTENTS

nanoString TECHNOLOGIES

This page intentionally left blank.

PREFACE

Purpose

This user manual details specific information pertaining to safe and proper handling, operation and maintenance of the nCounter® Digital Analyzer.

Conventions Used

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

Note Types

Special font formatting is used in this manual. Such formatting conventions are used in specific instances as described below:

TIP Information contained in a Tip may offer helpful suggestions, alternative procedures, methods and/or shortcuts.

NOTE This note type emphasizes general information.

IMPORTANT  This note type presents essential content indicating that the potential exists for assay failure, diminished data quality, and/or a loss of data if the information presented is ignored.

WARNING  This note type indicates that a potential hazard to your personal safety, or the potential for equipment damage exists.

BOLD When appearing in text or in a procedure, the bold text serves to highlight a specific button, key stroke, or menu option available.

- **Bold** text may appear elsewhere to highlight important text or terms.
- **Green** text is used to help the reader identify active hyperlinks.

ITALICS Used to emphasize an important word or expression within the text.

- Formatting of a book title, journal, or other documentation.
- Used to indicate the special or unusual meaning of a word or phrase.

Procedures

Numbered procedures appear frequently providing step-by-step instruction for accomplishing a task. Typically, a numbered step provides direction for a specific action and may be followed by the expected response. Additional information may be presented in the form of a specific note type, bullets, screen capture or other image important to facilitate clarity and understanding. For example:

In the (next) screen, the active data entry field is indicated by a green box around it. Simply move from one field to the next, simply press the desired field on the touchscreen with your finger.

1. To add an email address, press **ADD**.

   >>> The email address keyboard screen appears.

2. Enter a valid email address and press **ENTER**. The email address gets saved.

Contact Information

NanoString® Technologies, Inc.

530 Fairview Ave N
Suite 2000
Seattle, Washington 98109 USA

Tel: 206.378.6266
888.358.NANO (6266)

Fax: 206.378.6288

Email: support@nanostring.com

1 General Information

This chapter provides a general introduction to the instrumentation of the nCounter System as well as specific information pertaining to the nCounter Prep Station. Specific information provided in this chapter includes:

- Serial Number Location
- Safety Considerations
- Specifications
- Installation Information and Environmental Conditions
- Maintenance, Service and Support

nCounter® Analysis System (GEN2)

The GEN2 nCounter Analysis System is comprised of two instruments, the Prep Station 5s used for post-hybridization processing, and the Digital Analyzer 5s used for data collection.

NOTE: The GEN2 Prep Stations and Digital Analyzers began shipping in November 2011, and can be identified by the "5s" designation on the name plate located on the back of the instruments.

NOTE: The Prep Station and the Digital Analyzer are stand alone units. These instruments do not require connection to an external PC and are not networked to one another. They may be set up and installed separately. Connecting the Digital Analyzer to a network is optional, and this enables data delivery to the user via a network connection. Likewise, if the Prep Station is networked, then notifications can optionally be sent to users.

The GEN2 nCounter Analysis System uses Setup and Process wizards on an embedded touchscreen user interface to guide you through the sample processing and data collection steps of the assay. Following the instructions will walk you through setting up runs step-by-step on the Prep Station and Digital Analyzer.

nCounter® Prep Station 5s Overview

The nCounter Prep Station 5s is the automated fluidic handling component of the GEN2 nCounter System and processes samples post-hybridization to prepare them for data collection on the nCounter Digital Analyzer 5s.

FIGURE 1.1: nCounter Prep Station

Prior to placing samples in the Prep Station, samples need to be hybridized according to the protocol outlined in The nCounter® Gene Expression Assay Manual, The nCounter miRNA Assay Manual and the nCounter CNV Assay Manual. On the deck of the Prep Station, hybridized samples are purified and immobilized in a Sample Cartridge for data collection.

FIGURE 1.2: nCounter Cartridge

All consumable components and reagents required for sample processing on the Prep Station are provided in the nCounter Master Kit and are ready to load onto the deck of the robot. No reagent preparation or dilutions are required. The Prep Station can process up to 12 samples per run in less than two and a half hours.

nCounter® Digital Analyzer 5s Overview

The nCounter Digital Analyzer 5s collects data by taking images of the immobilized fluorescent reporters in the sample cartridge with a CCD camera through a microscope objective lens. At the highest standard data resolution, 280 fields of view (FOV) are collected per flow cell (sample) yielding data of hundreds of thousands of target molecule counts. The number of images taken corresponds to the number of reporters counted and this, in part, determines the dynamic range and level of sensitivity in the system.

Images are processed internally and the results are exported as a comma separated values format file that can be downloaded via memory stick. The file can be opened by most commonly used spreadsheet packages, including Microsoft® Excel and can be analyzed using NanoString's nSolver Analysis Software or other data analysis and visualization software packages.

FIGURE 1.3: nCounter Digital Analyzer

Touchscreen Overview

The nCounter System's touchscreen is a pressure-sensitive method of controlling the Prep Station 5s and Digital Analyzer 5s which enables the user to interact with the system by touching a selection on the screen. There are several standard option 'buttons' that appear in the touchscreen user interface (**TABLE 1.1**).

TABLE 1.1: nCounter system's touchscreen interface buttons

Button	Action
Next	Proceeds to the next screen.
Back	Moves back to the previous screen.
Cancel	Returns to beginning of the current workflow or the main menu.
Save	Saves entered data, and allows you to continue. The Save button is primarily used during file creation, upload, and download.
Done	Allows you to skip instructional steps and jumps to the next screen that requires input. The Done button is also used to indicate that data entry is complete and that you are ready to initiate processing.

nCounter Prep Station Information

For proper operation and testing results, the instructions in this manual must be followed by experienced personnel. It is important to read and understand the contents of this manual prior to operating the GEN2 nCounter Analysis System.

For service and support information, please refer to **General Maintenance, Service & Support on page 14**.

NanoString Technologies® does not assume any liability arising from your use of the nCounter Analysis System, this manual or other documentation provided by NanoString.

Serial Number

Each nCounter Prep Station has a model and serial number located on the back panel of the unit. Please include your serial number in communications with NanoString pertaining to the Prep Station.

For your reference, please record the model and serial number of your nCounter Prep Station in the space provided below.

Serial Number _____

Date Received _____

Specifications

Description	Specifications
Power supply	• 100–120 VAC • 200–240 VAC
Size (W x D x H)	35.0 x 26.4 x 24.6 inches; 89 x 67 x 63 cm
Pollution degree	2
Weight	265 lbs; 120 kg

nanoString TECHNOLOGIES

Important Safety Information

Please read through this manual and the following guidelines carefully to ensure safe operation of the NanoString nCounter™ Prep Station. Always keep this manual near 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 will void the manufacturer's warranty.

In the event of an Emergency, immediately turn the power switch OFF and unplug the Prep Station.

WARNING: Ensure that water or chemicals do not come in contact with the electrode fixture or touchscreen.

WARNING: Do not touch the heat block surface. Surface temperature can reach up to 45°C during a run.

NOTE: Unplug the equipment when not in use for extended periods of time.

NOTE: When using the equipment, follow generally accepted procedures for quality control.

WARNING: Do not attempt to disassemble the equipment. The nCounter Prep Station contains no user-serviceable parts. Service personnel trained by the manufacturer must perform repairs. Do not modify any part of the equipment as this may cause fire, malfunction, and will void the manufacturer's warranty.

NOTE: Disposal of Electronic Equipment

It is important to understand 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.

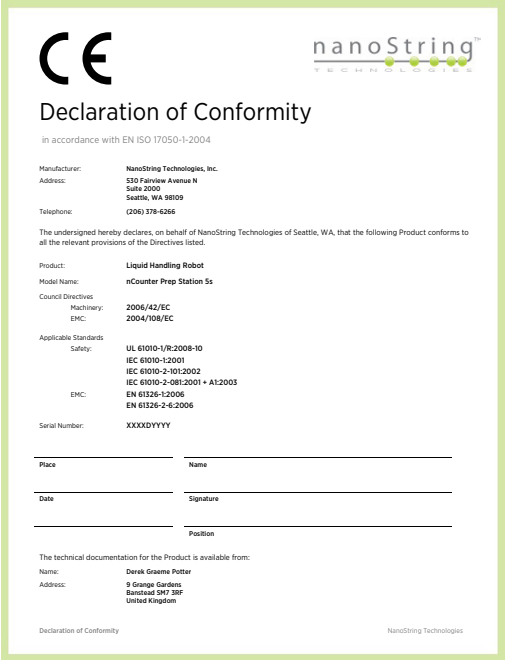
Safety Compliance

Declarations and Marks of Conformity

The nCounter™ Prep Station has been manufactured according to EN61010-1: 2001.

2nd Edition (Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements) and has been checked in accordance with all relevant safety standards prior to leaving the factory.

FIGURE 1.4: Declaration of Conformity



CE

nanoString™ TECHNOLOGIES

Declaration of Conformity

in accordance with EN ISO 17050-1-2004

Manufacturer: NanoString Technologies, Inc.
Address: 530 Fairview Avenue N
Suite 2000
Seattle, WA 98109
Telephone: (206) 378-6266

The undersigned hereby declares, on behalf of NanoString Technologies of Seattle, WA, that the following Product conforms to all the relevant provisions of the Directives listed.

Product: Liquid Handling Robot
Model Name: nCounter Prep Station 5s
Council Directives
Machinery: 2006/42/EC
EMC: 2004/108/EC
Applicable Standards
Safety: UL 61010-1/R:2008-10
IEC 61010-1:2001
IEC 61010-2-101:2002
IEC 61010-2-081:2001 + A1:2003
EMC: EN 61326-1:2006
EN 61326-2-6:2006
Serial Number: XXXXDYYYY

Place | Name
Date | Signature
Position

The technical documentation for the Product is available from:
Name: Derek Graeme Potter
Address: 9 Grange Gardens
Banstead SM7 3RF
United Kingdom

Declaration of Conformity NanoString Technologies

The nCounter Prep Station has been approved for use by the following testing institutions, confirmed by their respective test/conformity symbols:

TABLE 1.2: Description of nCounter Prep Station Safety Compliance

Acronym	Test Symbol	Testing Institution
CE	CE	The instrument meets the protection requirements laid down in the European Council directive 89/336/EEC on electromagnetic compatibility.
CB Scheme		This instrument has been tested for safety of its electrical, electronic, and mechanical components according to IEC61010-1, 2nd Edition and IEC61010-2-101, 1st Edition. A CB Scheme test reports available upon request.

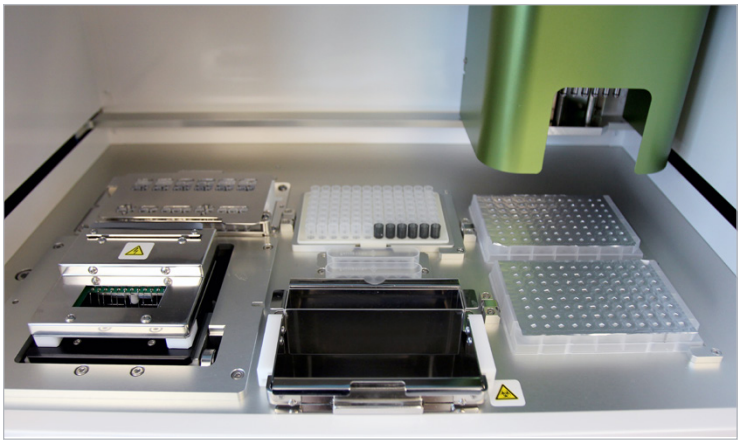
Safety Labels

TABLE 1.3: Description of Caution Labels used on the nCounter Prep Station

Icon	Meaning
	Caution Hot Surface. The surface temperature of the heat block can reach 45°C while running a protocol.
	Potential hazard from a biological source exists. If you choose to use biohazardous materials on your Prep Station the area of the Waste Tip may become contaminated with biohazardous materials. Please affix the proper warning labeling to your Prep Station if you use biohazardous inputs. Be careful not touch this area without gloves or other biohazard protection.

nanoString TECHNOLOGIES

FIGURE 1.5: nCounter Prep Station Deck Layout



Installation and Environmental Conditions

The following table provides information on the environmental requirements necessary for the safe operation of the nCounter Prep Station.

TABLE 1.4: Environmental conditions required for the nCounter Prep Station

Installation site	• Indoor use only. • The Prep Station should be placed on flat, stable surface with access to an electrical power source (see Power Source requirements, below). • Avoid locating the Prep Station near large electrical equipment to prevent possible interference from noise and/or voltage fluctuation. • Avoid locating in direct sunlight. • Avoid locating where the potential for vibration from other sources exists. • Do not position the equipment so that it is difficult to operate the disconnecting device.
Power source	• An independent power outlet used only for the nCounter Prep Station is required. • The Prep Station requires an electrical power source at 100-120VAC / 200-240VAC, 610W and 50/60Hz*. • Always use a grounded power outlet and use the power cable provided by NanoString in order to ensure proper grounding.
Fuse	• 100-120VAC: 250V/8AT • 200-240VAC: 250V4AT
Temperature	• The room temperature required for operation is 18 to 28°C.
Humidity	• 30-80% relative humidity (non-condensing)

*The nCounter Prep Station 5s can be modified from US to EU power by inverting a fuse found on the back of the instrument. Please check with your Technical Support representative for detailed instructions.

General Maintenance, Service and Support

Technical Support

Technical support is available by phone, fax, mail, or email. Please be sure to include your product number and serial number in communications. Please refer to **Serial Number on page 10** for information on locating the model and serial number of your product.

NanoString Technologies, Inc.

530 Fairview Ave N
Suite 2000
Seattle, Washington 98109 USA

Tel: 206.378.6266
888.358.NANO (6266)

Fax: 206.378.6288

Email: support@nanostring.com

General Cleaning

When using the nCounter Prep Station it is important to follow all safety and operating instructions provided in this manual. Always use safe laboratory operating precautions, including wearing a mask, safety glasses and gloves.

As human and/or biohazardous materials may be used, always follow the appropriate precautions outlined by your lab.

Instructions and Guidelines

- Keep the waste boxes clean.
- Wear gloves, safety glasses and a mask when handle specimens and reagents to avoid contamination and infection.
- Clean the Prep Station after each run.
  - After a completed run, remove all consumables and waste.
  - Clean the stage surface of the Prep Station by wiping with a disinfectant followed by wiping with water or 70% ethanol. Avoid the electrode fixture. A disinfectant such as DNA Zap® from Ambion is recommended.
  - Clean the exterior using a diluted neutral soap, followed by water.

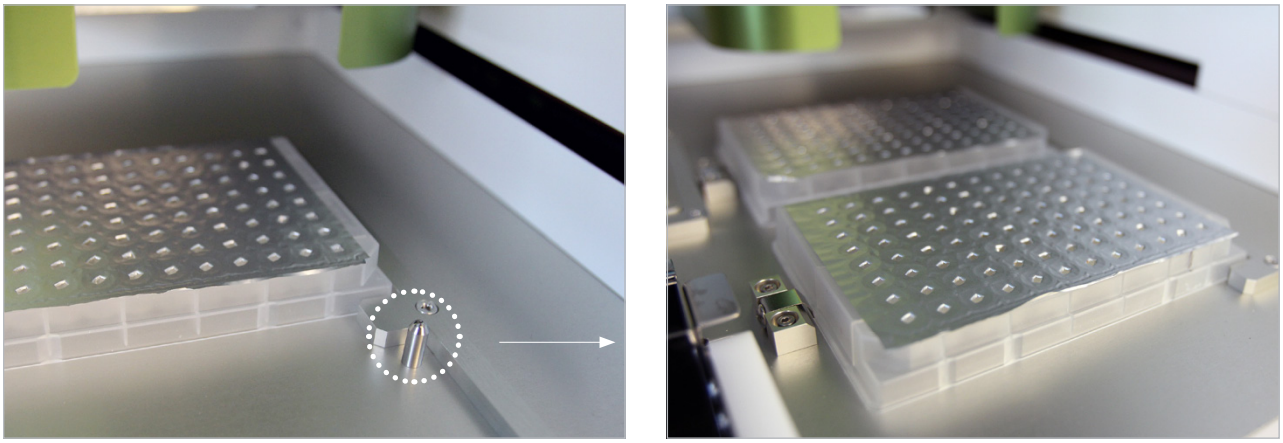
Tips for Using the nCounter Prep Station

The following section provides helpful information for the proper use of the Prep Station.

Reagent Plates

Reagent plates are properly placed (on the deck) using the positioning pins. Incorrectly placed plates will interfere with the positioning pin and will not sit flat upon the deck. Use FIGURE 1.6 to confirm the correct placement of reagent plates.

FIGURE 1.6: Use the positioning pins to ensure correct placement of reagent plates

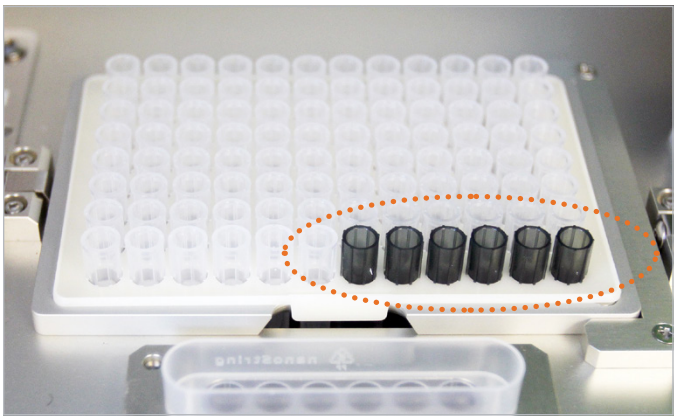


Tips and Piercers

The nCounter Prep Pack, a sub-component of the nCounter™ Master Kit, contains the Racked Tips and Foil Piercers. Each rack contains 96 positions, with the six front-right positions reserved for the piercers and the remaining 90 positions containing the tips.

The location of the six Foil Piercers is shown in FIGURE 1.7, below.

FIGURE 1.7: Location of the six Foil Piercers on the rack



Purchasing

All reagents and consumables required to process 48 or 192 nCounter Gene Expression Assays are supplied in the nCounter Master Kit (individual kit components may be purchased separately if required). For further information, please refer to **Consumables Required on page 19**.

Accessories and consumables may be purchased from NanoString Technologies. Please contact your representative directly, or email **sales@nanostring.com** to have a representative contact you.

2 Running the nCounter Prep Station

Introduction

nCounter® Assays are designed to provide an ultra-sensitive, reproducible and highly multiplexed method for detecting nucleic acid species across all levels of biological expression. This assay provides a method for direct detection of specific nucleic acid sequences with molecular barcodes called nCounter Reporter Probes without the use of reverse transcription or amplification.

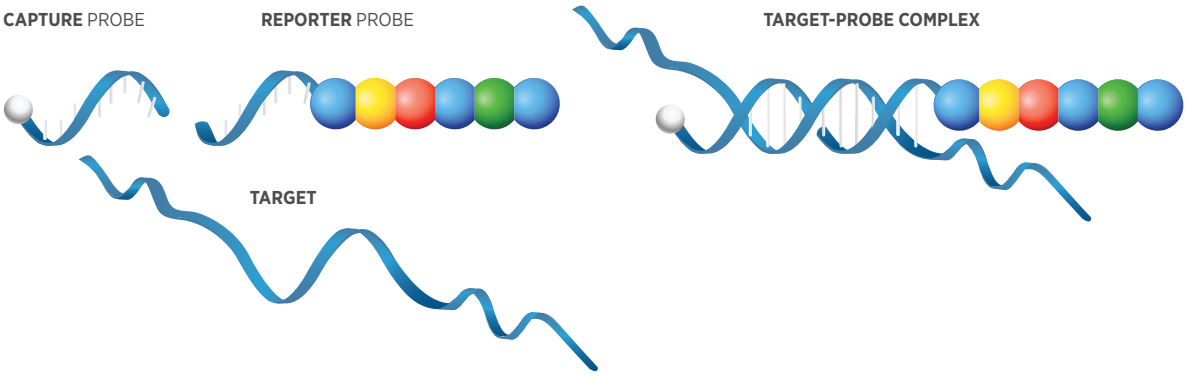
This manual describes the process for post-hybridization processing using your nCounter Prep Station. Please refer to the nCounter® Gene Expression Assay Manual, the nCounter® miRNA Assay Manual, the nCounter® CNV Assay Manual, the nCounter® Digital Analyzer User Manual, the nCounter® Expression Data Analysis Guide, and the nCounter® CNV Data Analysis Guidelines for information on setting up your assay and post-hybridization processing. Please refer to the nSolver Analysis Software Manual and assay specific data analysis guides for information on data analysis.

NanoString Technology

Principles and Procedures

NanoString's technology is based on digital detection and direct molecular barcoding of target molecules through the use of a color coded probe pair. The probe pair consists of a Reporter Probe, which carries the signal on its 5' end, and a Capture Probe which carries a biotin on the 3' end. The color codes carry six positions and each position can be one of four colors, thus allowing for a large diversity of tags that can be mixed together in a single well for direct hybridization to target and yet still be individually resolved and identified during data collection.

FIGURE 2.1: Capture and Reporter Probes (left) and, Probe pair bound to an mRNA (right)



Probe pairs are placed into a reaction in massive excess to target RNA or DNA species to ensure that each target finds a probe pair. After hybridization, excess probes are washed away using a two step magnetic bead-based purification on the nCounter Prep Station.

Magnetic beads derivatized with short nucleic acid sequences that are complementary to the Capture Probe and the Reporter Probes are used sequentially. First, the hybridization mixture is allowed to bind to the magnetic beads by the Capture Probe. Wash steps are performed and excess Reporter Probes and non-target cellular transcripts are removed during wash steps. After washing, the Capture Probes and Target/Probe complexes are eluted off of the beads and are hybridized to magnetic beads complementary to the Reporter Probe. Wash steps are performed and excess Capture Probes are washed away. Finally, the purified Target/Probe complexes are eluted off and are immobilized in the cartridge for data collection.

The Prep Station includes two protocol options for binding to the cartridge. The difference between the two protocols is the amount of time the purified nucleic acids are incubated with the derivatized cartridge surface. Using the Standard protocol, the assay is complete in 2.5 hours. If the High Sensitivity protocol is chosen, the purified probes are passed over the cartridge surface several additional times, increasing the number of molecules detected and the assay time is increased slightly to just around 3 hours. This increases all counts recovered by up to two-fold.

NOTE: The increase in number of probes binding to he slide could result in higher binding densities. If the binding density gets too high, the quality of the data could be compromised. For more information, please contact support@nanostring.com or your Field Application Scientist. The High Sensitivity protocol takes an additional thirty minutes, so the total time to process using the High Sensitivity option will be three hours.

Data Collection is carried out in the nCounter Digital Analyzer. At the highest standard data resolution, 280 fields of view (FOV) are collected per flow cell (sample) using a microscope objective and a CCD camera yielding data of hundreds of thousands of target molecule counts. Digital images are processed on the nCounter Digital Analyzer and the barcode counts are tabulated in a comma separated value (CSV) format.

The nCounter Analysis System was created by NanoString Technologies. The nCounter system is an easy-to-use integrated system that includes a Prep Station (robot) and a Digital Analyzer (analyzer). The Prep Station and the Digital Analyzer together make lab work and sample analysis a simpler process by limiting the variables in experiments for lab technicians. The end result is a very precise and accurate measurement, enabling you to gather data on your targets of interest rapidly with minimal intervention.

nCounter® Expression Assay Overview

The nCounter Expression Assay is run on the nCounter System. The system is comprised of two instruments, the nCounter Prep Station used for post-hybridization processing, and the Digital Analyzer used for data collection. Follow the instructions on the touchscreen to guide you step-by-step through setting up runs on the Prep Station and Digital Analyzer. For further details on the nCounter System instrumentation, please refer to **The nCounter® Analysis System on page 7**.

TABLE 2.1: Suggested workflow for the nCounter Expression Assay

	Manual Processing	Hands-on Time
Day 1	Set Up Hybridization	5 minutes

	Automated Processing	Hands-on Time
Day 2	Set Up Prep Station Run	5 minutes
	Set Up Data Collection	5 minutes

Before You Begin

Waste Removal

Prior to starting a new run you must ensure that the waste containers have been emptied. Empty waste containers are required for every run.

WARNING: If waste containers are not emptied before a run, tips could come into contact with waste liquids and contaminate samples, or excess tips could pile up and cause a system malfunction.

1. Remove the combined waste receptacle by lifting it straight up and out of the Prep Station.
2. Remove the liquid waste container from the combined receptacle by using the latch on the front and dispose of the liquid appropriately.
   - Tips should be discarded into the appropriate waste stream as outlined in your laboratory's procedures.
   - If you are not handling biohazardous samples on your system, you may dispose of the liquid waste in the drain.
3. Verify that the plastic rack holding the used piercers, tip sheaths, prep plates and strip tubes from the previous run have all been removed from the deck.

Consumables Required

There are several consumable items that must be kept in stock. These consumables may be purchased through NanoString Technologies.

The consumables required for each run are available as part of the nCounter™ Master Kit or can be purchased separately. The kit contains the reagents and consumables required to process 48 nCounter Gene Expression Assays. No reagent preparation or dilutions are required.

WARNING: Used plasticware, such as reagents cartridges and pipetting tips, may contain hazardous chemicals or infectious agents. Such wastes must be collected and disposed of properly in accordance with the local safety regulations and laboratory procedures.

The components of the nCounter Master Kit include:

- **nCounter Cartridges**
- **nCounter Prep Plates (foil sealed 96-well plates used by the Prep Station)**
- **nCounter Prep Pack**
  - Racked Tips & Foil Piercers
  - 12-Tube Strips
  - Strip Tube Caps
  - Tip Sheaths (for storing tips to prevent cross contamination and unnecessary tip consumption)
  - Cartridge Well Seals
  - Hybridization Buffer

NOTE: If you are processing fewer than 12 samples at a time, you will need to purchase more nCounter Cartridges to process all 48 assays..

Setting Up a Prep Station Run

Hybridized samples should be processed immediately using the nCounter™ Prep Station. Hybridizations can stay at 65°C for up to 24 hours. The following steps outlined are from the Main Menu on the Prep Station touchscreen.

IMPORTANT: Storing hybridizations at 4°C is NOT recommended as it increases non- specific background.

1. To set up a new run select start processing from the Main Menu (**FIGURE 2.2**).

   >>> The Select Plate Type screen appears (**FIGURE 2.3**).

FIGURE 2.2: Main Menu screen

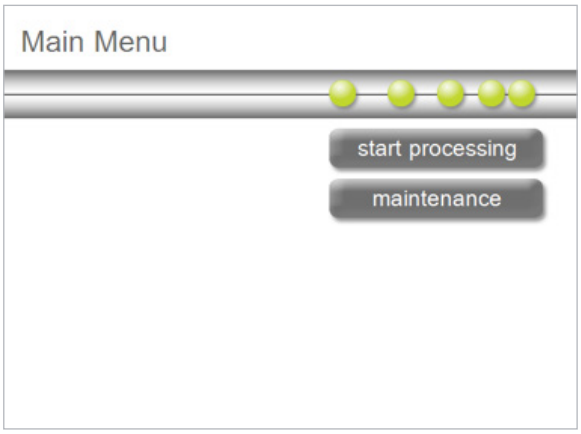
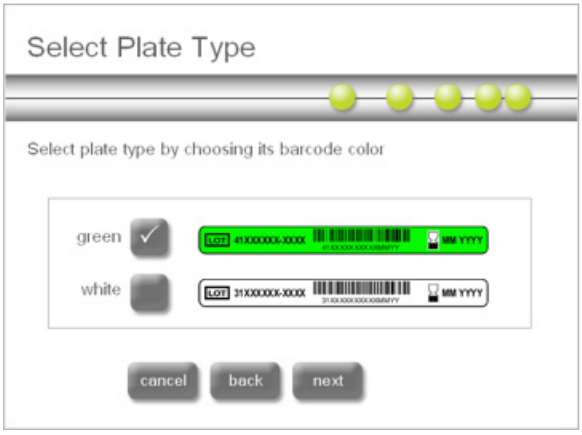


FIGURE 2.3: Select Plate Type screen

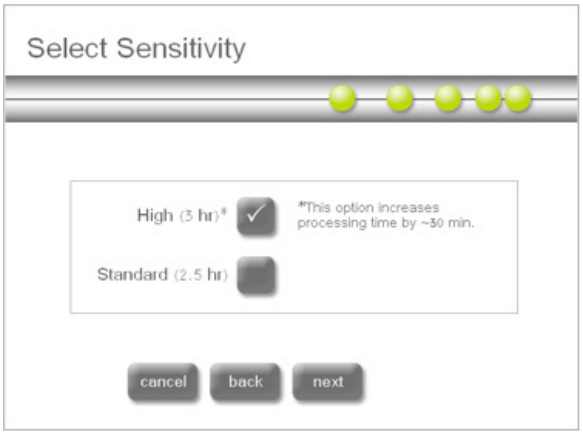


2. Choose the type of plate that will be used for the assay. Touch “next”.

   >>> The “Select Sensitivity” screen appears (**Figure 2.4**).

WARNING: Running the protocol with white plates after choosing the green plate option will cause the assay to fail.

FIGURE 2.4: Select Sensitivity screen



3. Select the desired sensitivity of the processing steps. Touch “next”.

NOTE: The High Sensitivity protocol increases the binding of all molecules to the slide by about 2-fold vs. prior protocols.* It adds an extra 30 minutes to the processing time.

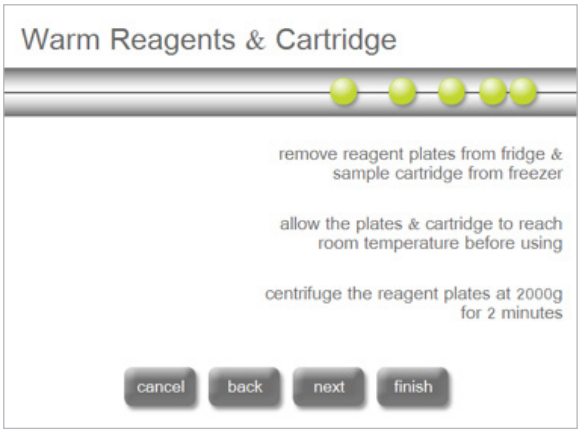
NOTE: Both the Standard and High Sensitivity protocols contain improvements in the Prep Station process. The Standard option has similar binding properties to the version of the protocol installed on Prep Stations v4.0.1.1. If you want to run the v4.0.1.1 protocol with green plates, choose the “white plates” option and place the green plates on the deck. The protocol and reagents used will be identical to v4.0.1.1 or previous.

IMPORTANT: The Select Sensitivity screen does not appear if white plates have been chosen. If white plates are chosen the program will launch one of the prior software versions v3.1.1.2 Gen1/v4.0.11 Gen2.

*Ongoing improvements to the performance of our platform include software, reagents and consumables. Specific performance improvements may vary depending on prior configurations.

>>> The Warm Reagents & Cartridge screen appears (**FIGURE 2.5**).

FIGURE 2.5: Warm Reagents & Cartridge screen

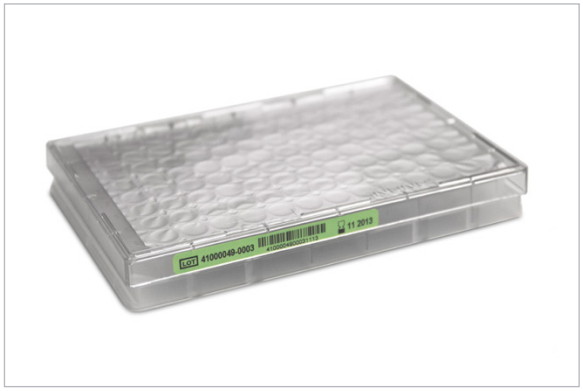


4. Cartridges and Prep Plates must be at room temperature prior to processing.

   a. Set out the sealed Sample Cartridge and Prep Plates for at least 10 minutes to warm to room temperature.

NOTE: If the cartridges and Prep Plates are not at room temperature prior to processing them, you may experience increased assay variability. Do not open the cartridge pouch until it has reached room temperature to prevent condensation on cartridge.

FIGURE 2.6: Sealed Sample Cartridge (left) and Prep Plate (right)

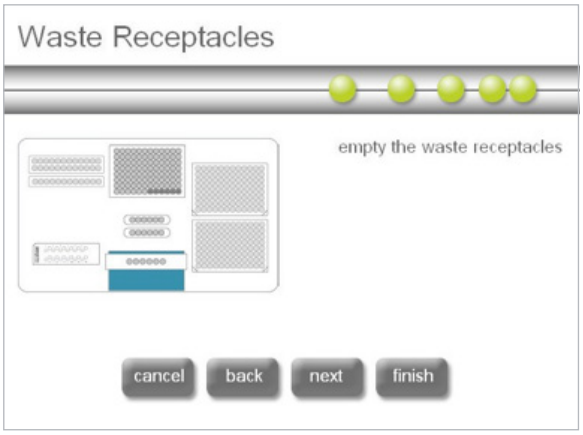


   b. Centrifuge the Prep Plates at 2000 x g for 2 minutes to collect all liquids in the bottom of the wells. A medium deceleration speed will guard against shaking which may produce splattering within the plate well.

c. Press **next**.

>>> The Waste Receptacles screen appears (**FIGURE 2.7**).

FIGURE 2.7: Waste Receptacles screen

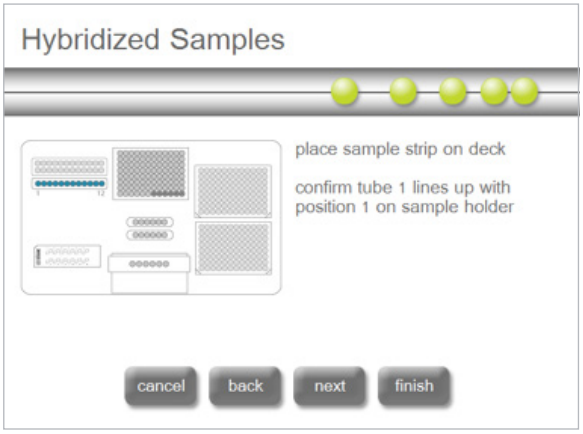


5. Remove the liquid waste container from the combined receptacle and dispose of the liquid appropriately. See **Waste Removal on page 19** for further information.

- Tips should be discarded into the appropriate waste stream as outlined in your laboratory's procedures.
- If you are not handling biohazardous samples on your system, you may dispose of the liquid waste in the drain.
- Press **next**.

>>> The Hybridized Samples screen appears (**FIGURE 2.8**).

FIGURE 2.8: Hybridized Samples screen options



6. The Hybridize Samples screen:

   a. Place your hybridized sample tubes on the deck of the Prep Station, ensuring that tube 1 aligns with position 1 (see FIGURE 2.9).

FIGURE 2.9: Hybridization sample tubes and number orientation guides.

IMPORTANT: The numbering on the deck of the robot corresponds to the numbers on the hybridized sample tubes. If the sample tubes are inverted, your sample specific information will be assigned to the incorrect data file.

IMPORTANT: Ensure that all tube caps are removed from the hybridized samples prior to placing tubes on the deck. Leaving the caps on will result in a pause in the protocol that requires user intervention.

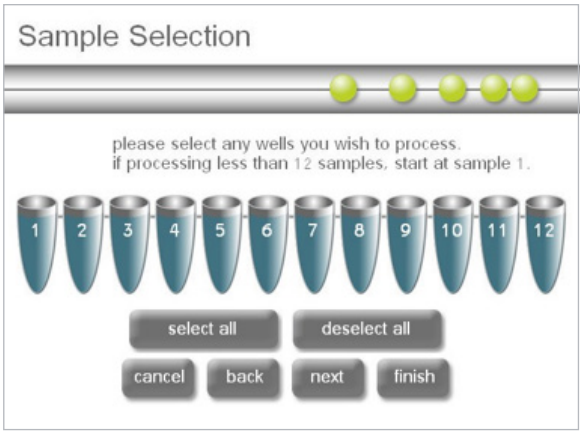
IMPORTANT: All tubes should be seated fully and evenly in the rack to ensure proper processing.

IMPORTANT: Only use NanoString supplied strip tubes. Other tubes may have different dimensions and cause system failure.

   b. In the Hybridize Samples screen press **next.**

   >>> The screen appears (FIGURE 2.10).

FIGURE 2.10: Sample Selection Screen



7. On the Sample Selection screen:

   a. Select the number of samples that are to be processed.

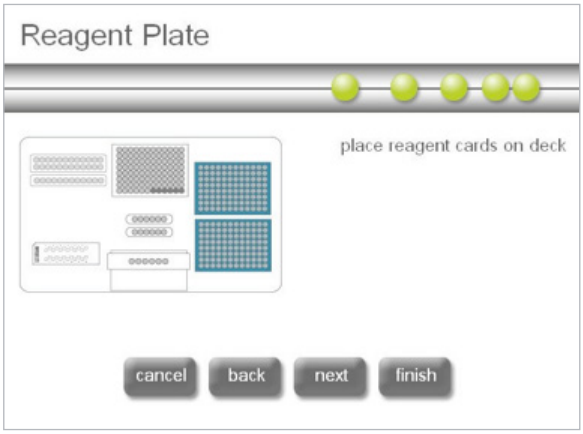
   - You can run fewer than 12 samples, however, it is important to note that one Prep Plate is for lanes 1 – 6, and the other is for lanes 7 – 12. You can run wells 1 – 6, or all 12.
   - If processing less than 6 samples use tubes 1 through 6. For processing 6 samples or less please place a prep plate, tip sheaths, and a strip tube with at least 6 tubes attached to keep reagents from being dispensed on the deck.
   - Reagents for all 12 samples will be used if more than 6 samples are entered.
   - Note that blue wells indicate that they will be processed, the gray wells indicate that they will not be processed.

   NOTE: There are automated protocols for either 6 or 12 samples; the protocol for 6 samples uses only one reagent plate but still consumes a whole cartridge.

   b. Press **next.**

>>> The Reagent Plate screen appears (**FIGURE 2.11**).

FIGURE 2.11: Sample Selection Screen

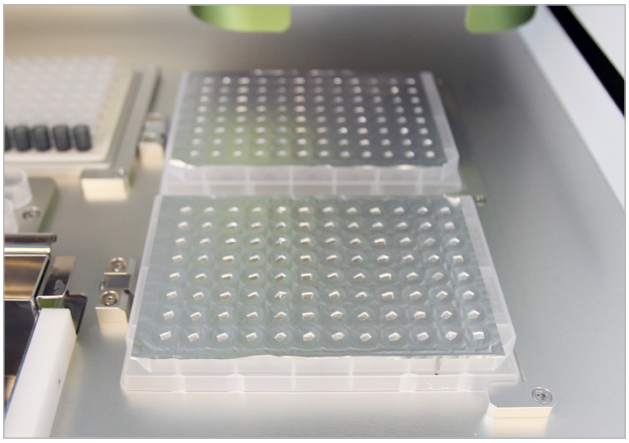


8. Remove the clear plastic lids and place the reagent plates on deck (**FIGURE 2.12**).

IMPORTANT: Do not remove the foil or pierce the wells on the reagent well. The Prep Station pierces the wells during processing.

The deck has alignment pins that will only allow the plates to sit flat if they are in the correct orientation. The Prep Plate should be oriented with the label facing the user. If the plate is placed in the wrong direction, the Prep Station will pause the protocol until the user intervenes.

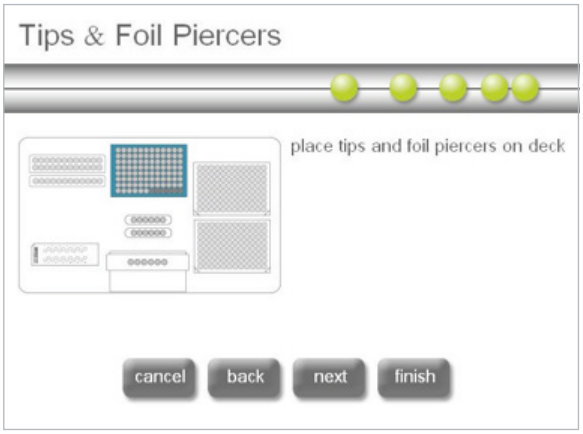
FIGURE 2.12: Reagent cards on deck



Press **next.**

>>> The Tips & Foil Piercers screen appears (**FIGURE 2.13**).

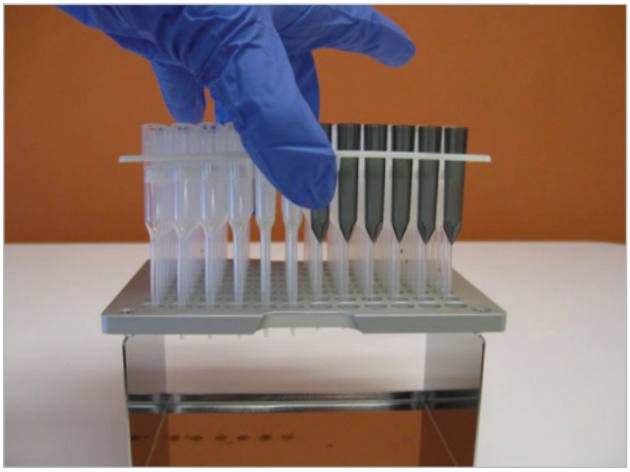
FIGURE 2.13: Tips & Foil Piercers screen



9. Remove the metal tip carrier from the Prep Station deck by lifting straight up. Place the tips and the foil piercers into the carrier. It is helpful to place the carrier at eye level to align the plastic tips in the carrier as shown in **FIGURE 2.14**.

IMPORTANT: There are two sets of tips nested in each box with each side of the box being the opening for one set of tips. Hold your hand firmly over the bottom of the box when opening to avoid accidentally spilling the second set of tips.

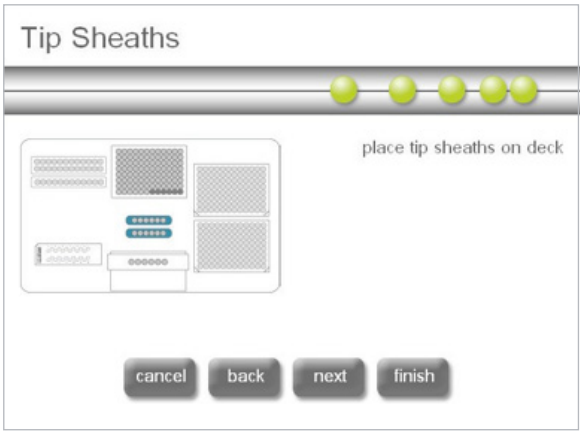
FIGURE 2.14: Placing the tips and foil piercers into metal tip carrier.



10. Replace the loaded tip carrier back onto the Prep Station deck with the foil piercers closest to the user. Press **next.**

>>> The Sample Cartridge screen appears (**FIGURE 2.15**).

FIGURE 2.15: Tip Sheaths screen

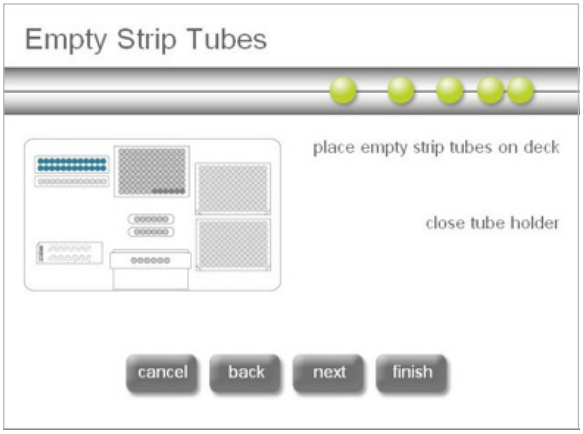


11. Place the tip sheaths on the deck and press firmly into place. Press **next.**

NOTE: Tip sheaths are used to reduce the amount of consumable waste. They allow the system to dedicate tips to a set of 6 samples and store them while other 6 samples are being processed.

>>> The Sample Cartridge screen appears (**FIGURE 2.16**).

FIGURE 2.16: Empty Strip Tubes screen



12. Place the empty tubes on the deck. Securely close the lid that flips down over the tubes (**FIGURE 2.17**). Press **next.**

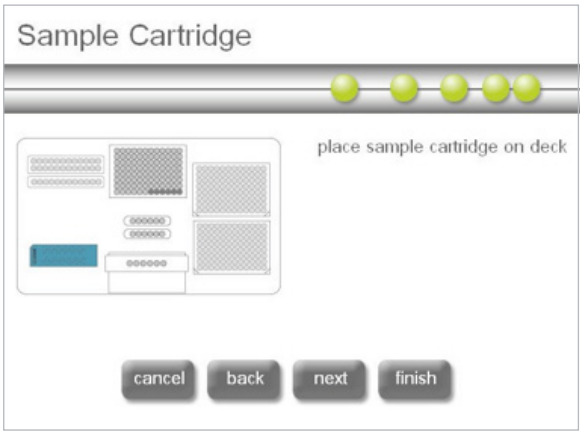
IMPORTANT: Failing to securely clip the lid over the tubes can result in a system malfunction. If the metal lid is not closed completely the sensor will return an error and the run will not be able to commence.

IMPORTANT: Ensure the same type of tube is inserted into both rows of the heater module. Only use NanoString supplied strip tubes. Other tubes may have different dimensions and cause system failure.

FIGURE 2.17: Lid closed over tubes

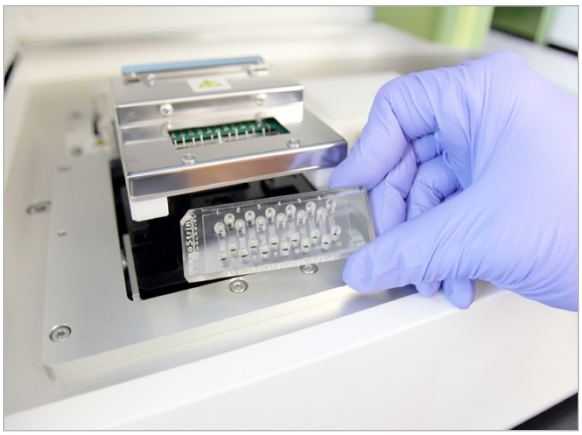
>>> The Sample Cartridge screen appears (**FIGURE 2.18**).

FIGURE 2.18: Sample Cartridge screen



13. Carefully place a Sample Cartridge under the electrode fixture in the orientation shown in **FIGURE 2.19**. Make sure that it is seated completely in the machined depression. If it is not seated properly, the electrodes may become bent.

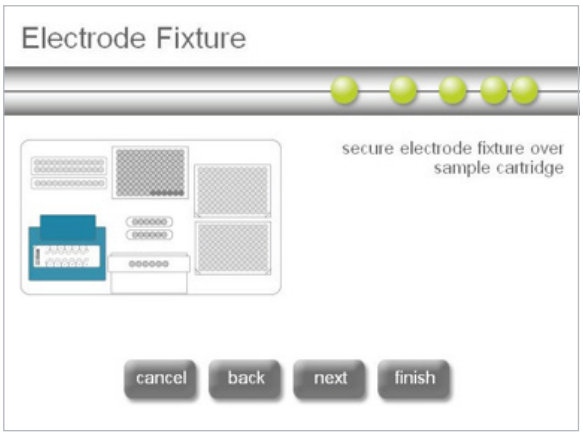
FIGURE 2.19: Sample Cartridge placed on deck



Press **next**.

>>> The Electrode Fixture screen appears (**FIGURE 2.20**).

FIGURE 2.20: Electrode Fixture screen

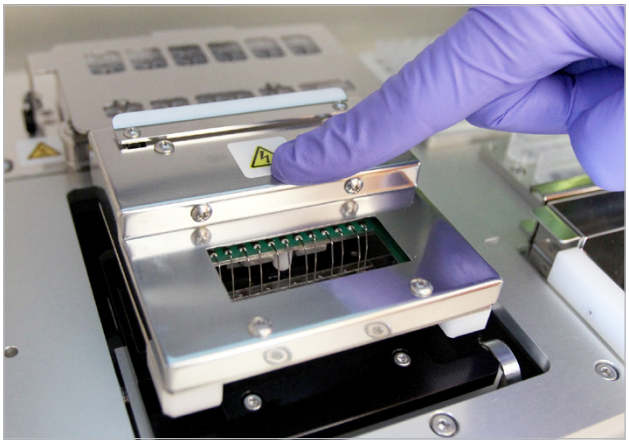


14. Carefully lower the electrode fixture in place over the cartridge. The 24 electrodes should insert into the 24 wells. If any resistance is felt while lowering the fixture, stop and make sure that the electrodes are correctly aligned.

IMPORTANT: Be careful not to press on the release handle while lowering the fixture. Doing so will prevent locking. Instead, press on the body of the fixture away from the release handle.

NOTE: Ensure that when you are placing the electrode fixture over the wells, you do not bend the electrodes. If the electrodes do get bent, bend them back to their correct position using the **align electrodes** function under the Maintenance menu. If there is no cartridge, the sensor will not allow the run to commence.

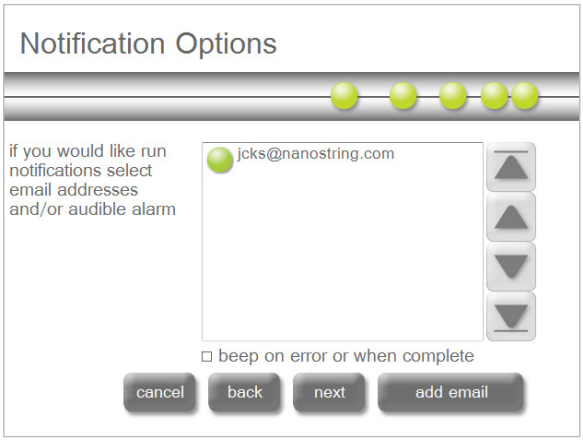
FIGURE 2.21: Lowering the electrode fixture in place over the cartridge.



Press **next**.

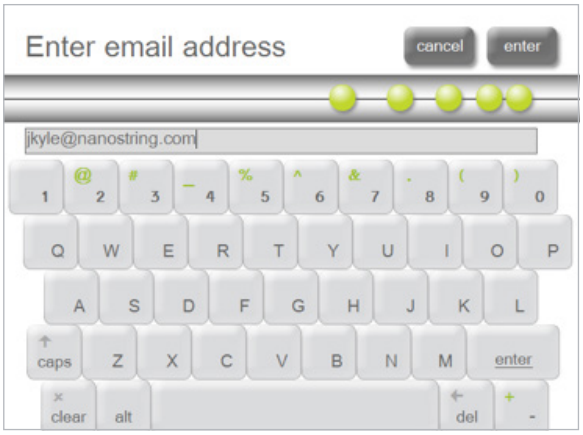
>>> The Notification Option screen appears (**FIGURE 2.22**).

FIGURE 2.22: Notification Option screen



15. In the Notification Option screen, select the email addresses for which you would like to send errors and completion confirmations emails, and select the **beep when** complete option if you would like an audible alarm to alert you that the run is complete. To add a new email address click the **add email** button and type in the correct email address followed by enter.

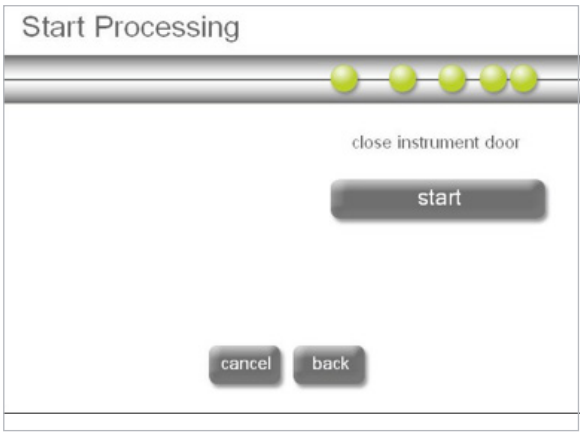
FIGURE 2.23: Enter email address screen



16. Press **next**.

>>> The Start Processing screen appears (**FIGURE 2.24**).

FIGURE 2.24: Start Processing screen



17. On the Start Processing screen, press start when you are ready to begin processing.

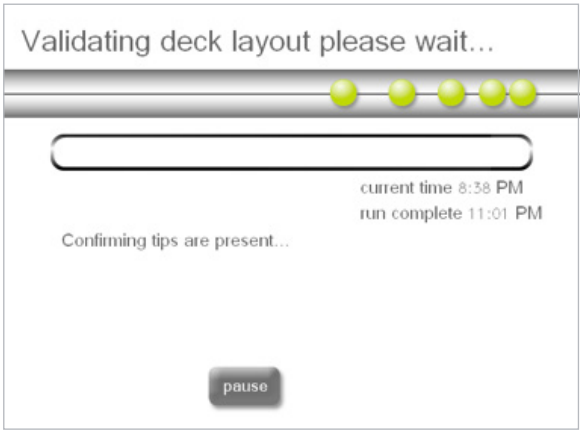
>>> The nCounter Prep Station first checks that all of the consumables and reagents have been placed properly on the deck. To do this, the Prep Station confirms that the sensors for the sample cartridge, electrode fixture, and heater lid are all in the correct state. The pipette head then checks that tips, tip sheaths, strip tubes and Prep Plates are all in place by touching them with a set of validation tips. Do not be alarmed that the Prep Station is touching the consumables; this is a part of normal operation. If the Prep Station determines that a consumable is misplaced, it will instruct you to fix the configuration.

IMPORTANT: It is advised to not abort and re-start the run when there is a deck layout failure, as this will result in sample loss.

NOTE: Please stand by the Prep Station during deck layout validation as any problems that may be encountered will require user intervention to proceed.

>>> The System Processing screen appears (**FIGURE 2.25**).

FIGURE 2.25: System Processing screen



The System Processing screen displays the current time of day and the estimated time of day that the run will complete. In this screen, you can also pause the run.

Pausing or Aborting a Run

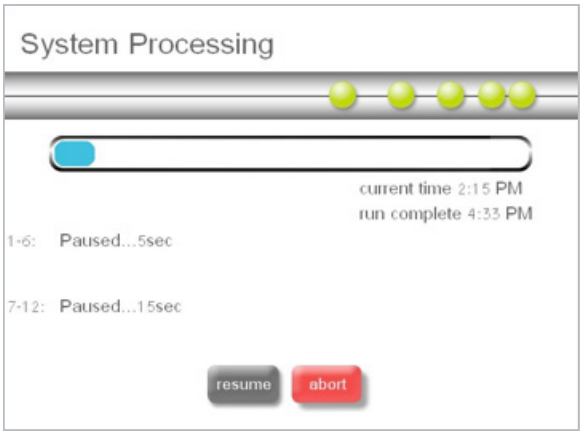
The following information is provided should you need to pause a run (optional). If you do not need to pause a run skip to **Step 18**, below.

IMPORTANT: When you pause a run, it is important that you resume it as quickly as possible. Incubation times are very important. Pausing the instrument can affect data quality and consistency. Pausing should only be used to avoid data loss due to operator error.

a. To pause a run, press **pause** on the System Processing screen. There may be a delay before pausing until the system reaches a "safe" state.

>>> The Paused screen appears (**FIGURE 2.26**).

FIGURE 2.26: Paused screen



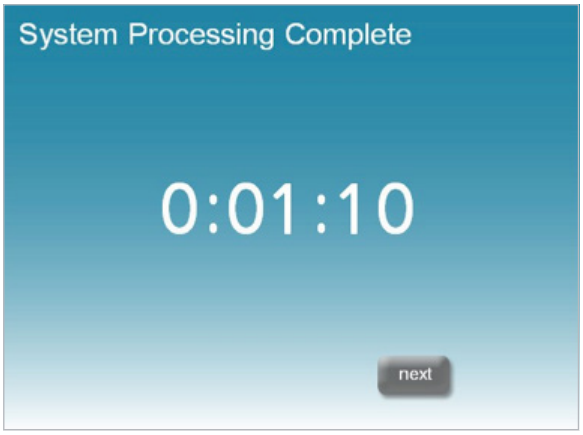
b. After you pause a run, you have the option of aborting a run. Once the hybridized samples have been moved to the Prep Plate for processing they cannot be recovered if the run is terminated.

c. Press **resume** to continue the run, or press **abort** to end the run.

WARNING: When you abort a run, you cannot restart the run and samples may be lost if fluidic processing had commenced.

18. When the run is complete, the blue System Processing Complete screen appears and the timer starts to count up from when the run has completed (**FIGURE 2.27**).

FIGURE 2.27: System Processing Complete screen



19. On the System Processing Complete screen press **next**.

>>> The Start Processing screen appears (**FIGURE 2.28**).

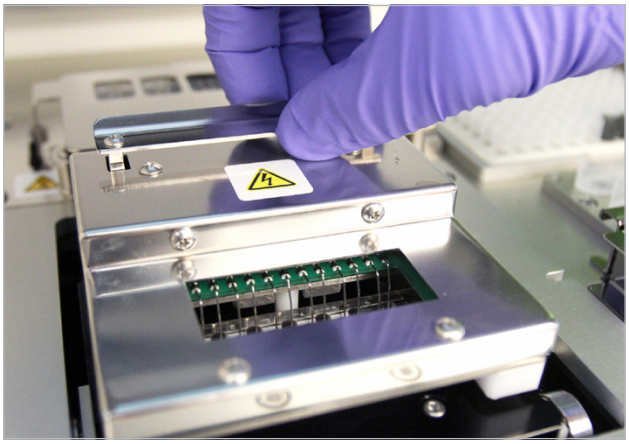
FIGURE 2.28: Run Successfully Completed screen

The Run Successfully Completed screen lists the steps to follow once the run is complete, including:

- Remove and discard empty reagent plates
- Remove and discard the empty tip racks and foil piercers
- Remove and discard the sample strips
- Remove the Sample Cartridge and seal the wells
- Emptying waste containers

a. To release the fixture after the run is complete, press the lever in the center top of the device towards yourself with your index finger as shown in **FIGURE 2.29**.

FIGURE 2.29: Releasing the fixture after a completed run



b. When the processing is complete, it is important to do the following:

- Seal the wells immediately with the sealing film provided to prevent evaporation.
- Do not leave the samples in direct sunlight or at a temperature greater than room temperature.
- If you will not be scanning the samples within an hour, store them in the refrigerator at 4°C. Once sealed, samples can be stored at 4°C for up to a week with minimal data loss.

20. Press **finish** to return to the Main Menu.

3 Maintenance

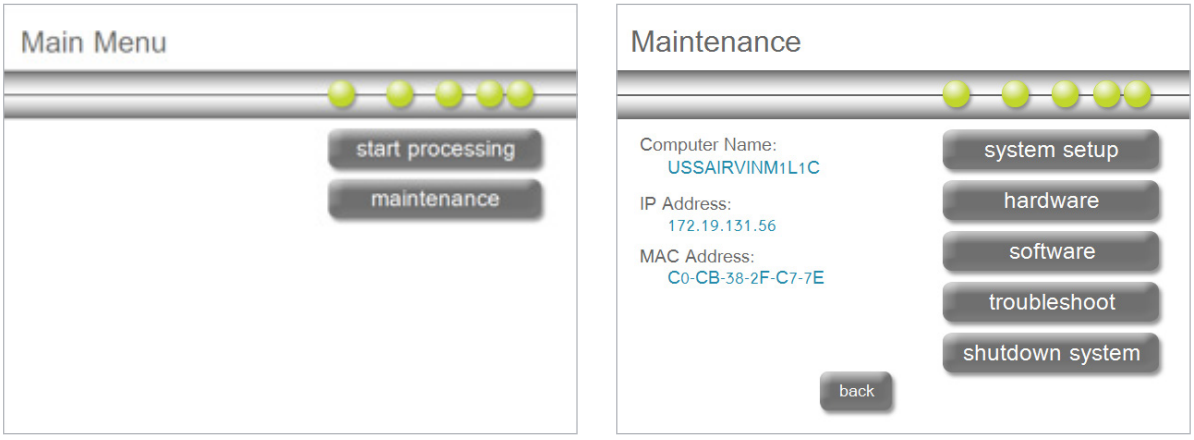
Maintenance Menu Tasks

There are several tasks that can be accomplished through the use of the Maintenance screens on the Prep Station:

- **system setup:** set the date and time on the system clock and configure email
- **hardware:** home robot, align electrodes, lubricate O-rings and calibration check
- **software:** update Prep Station's system software when a new release occurs and view the current software information (About)
- **troubleshoot:** download log files for remote analysis at NanoString
- **shutdown system:** system shutdown

To access the Maintenance Menu press maintenance on the Main Menu (**FIGURE 3.1**).

FIGURE 3.1: The Main Menu and Maintenance Menus



System Setup

The System Setup menu in the Maintenance section allows you to set the Prep Station's date and time, and configure email settings.

Setting the System Clock

Log files are created by the Prep Station and can be important for troubleshooting purposes. Setting the system clock allows for accurate time to be displayed on screen and in the log files. The system clock must be manually set during the initial system setup and for adjustments such as Daylight Saving Time.

FIGURE 3.2: System Setup screen

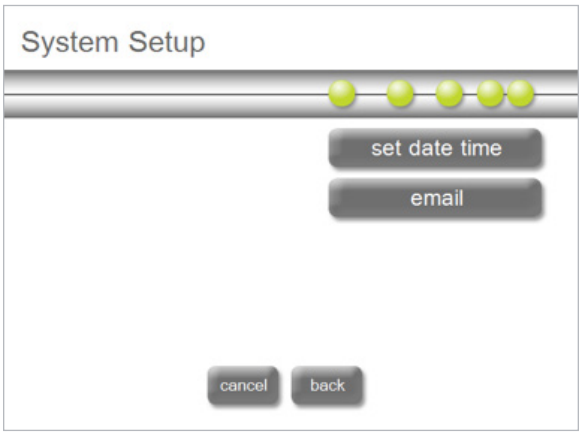
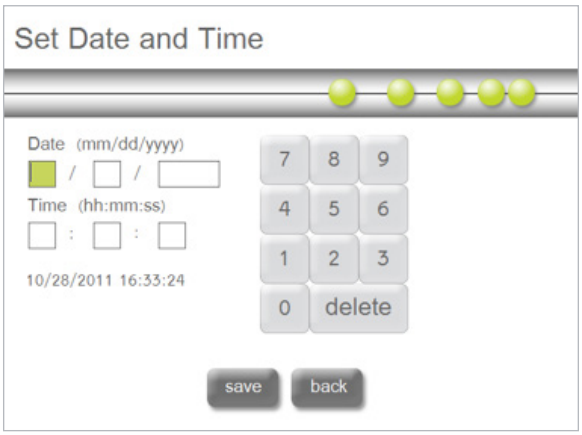


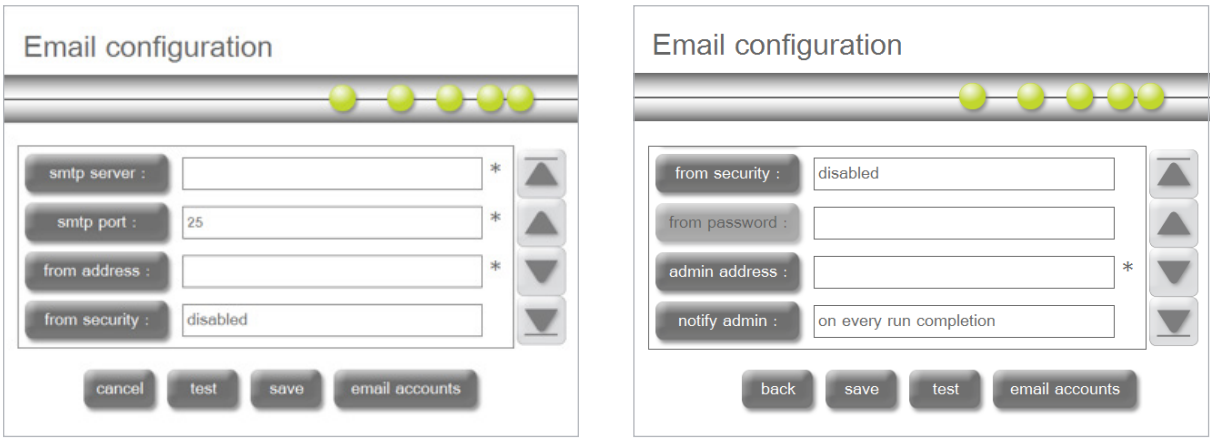
FIGURE 3.3: Set Date and Time screen



Email Address

Email addresses can be added, deleted, managed and saved on the Prep Station. This function allows users to receive notifications via email. Email must be configured for the notifications to work properly. To configure email, press the **email** button from the System Setup screen.

FIGURE 3.4: Email Setup screens



NOTE: Please contact your IT personnel for assistance in setting up email as an option on the Prep Station.

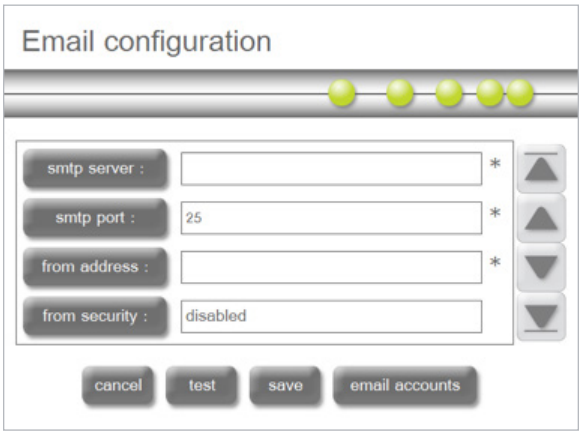
- **SMTP server:** Simple Mail Transport Protocol (SMTP) is the network protocol used to send email across the Internet. The SMTP server setting must be configured correctly or outgoing mail will not be sent. Enter the name of your SMTP server for outgoing mail.
- **SMTP port:** Enter the name of the port used by your SMTP server.
- **from address:** Enter a valid email address. This address will be seen in the "From" line of any outgoing emails sent from the system.
- **from security:** Security is disabled by default. To enable security on the Prep Station, press from security and then enter the **from password**.
- **from password:** If security is enabled, enter the appropriate password for the email address.
- **admin address:** In addition to user emails specified when processing a run, administrators can receive email notifications as well.
- **notify admin:** Designate whether the administrator will receive email notifications upon every run completion or disable it to not send email notifications to the administrator.

NOTE: It is recommended that you test the email settings prior to setting up a run by selecting the 'test' button and checking the inbox of the test email address.

Email Accounts

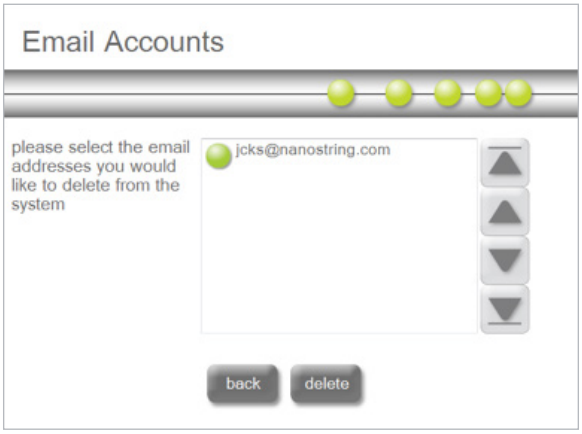
Email accounts can be deleted from the Prep Station as well. To delete an email address press the email accounts button from the Email configuration screen.

FIGURE 3.6: Email Configuration screen



To delete an email account from the Prep Station, select the appropriate email address and press the **delete** button.

FIGURE 3.7: Email Accounts screen

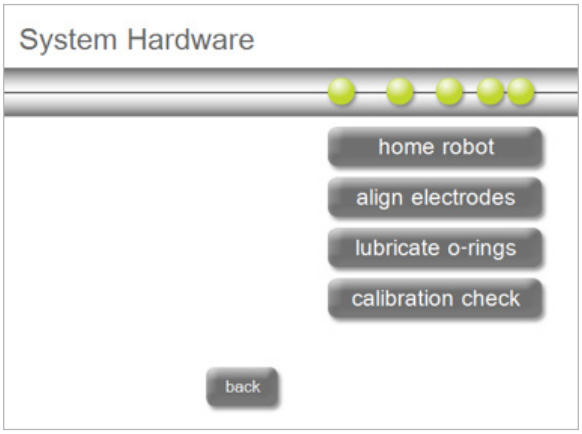


Hardware

The Hardware menu in the Maintenance section allows you to:

- Home Robot
- Align Electrodes
- Lubricate O-rings
- Calibration Check

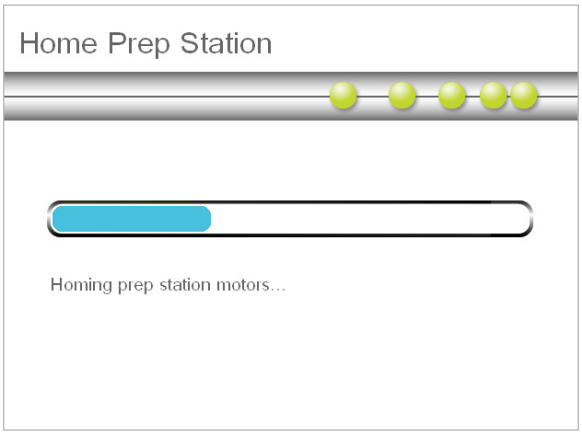
FIGURE 3.8: System Hardware menu screen



Home Robot

If the robot enters into an undesirable state, using the home robot button can home all the motors. This button should not be needed under normal operation.

FIGURE 3.9: Homing Robot message screens



Align Electrodes

From time to time, the electrodes used for stretching the reporters may become bent out of alignment and no longer fit into the cartridge. This usually occurs if the cartridge was not aligned properly when the electrode fixture was depressed causing the electrode to make contact with the cartridge and bend. The following describes a method for re-alignment of the electrodes using the system hardware interface. This method allows the electrode fixture to tilt at an angle that makes it much easier to view the electrodes during alignment.

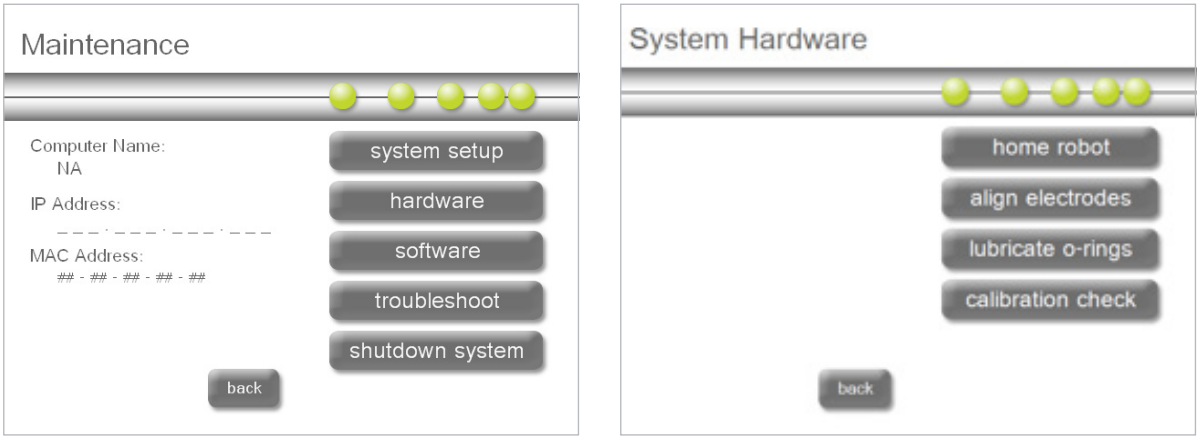
If the electrode is so severely bent that it cannot be inserted into a cartridge, the process outlined below may need to be repeated twice – once without a cartridge present to get the electrodes into roughly the right position so that they can be inserted into a cartridge and a second time with a cartridge present for fine tuning the placement of electrodes in the cartridge well.

NOTE: The Prep Station checks electrode function during the validation steps of the process, before any processing occurs to the samples. If it detects problems with an electrode, the processing will halt and wait for user intervention. The screens described below will then appear, allowing the user to fix the bent electrodes.

1. Select **hardware** on the Maintenance menu.

   >>> The System Hardware menu appears (**FIGURE 3.10**).

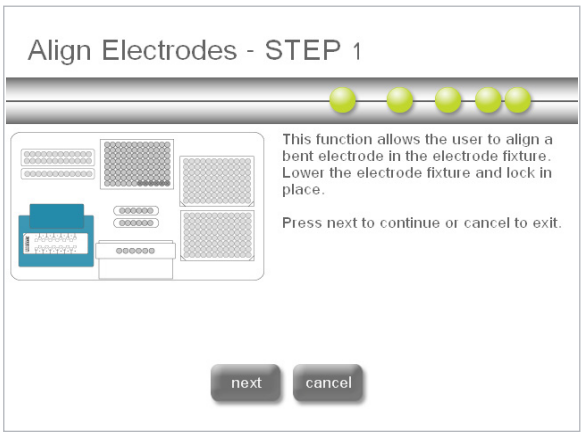
FIGURE 3.10: Maintenance Menu and System Hardware Menu



2. Select **align electrodes**.

   >>> The Align Electrodes screen appears. (**FIGURE 3.11**).

FIGURE 3.11: Align Electrodes Menu



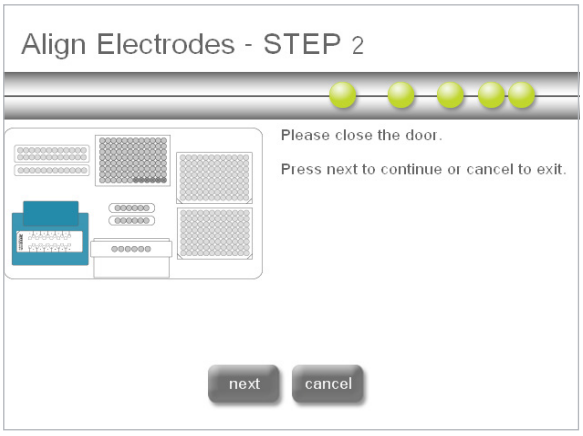
3. **If the electrode is severely bent to the point where a cartridge cannot be inserted:**

   Close the electrode fixture without a cartridge in it and adjust the position of the electrode such that it is positioned similarly to the other electrodes.

   If it is only slightly bent and a cartridge can be inserted by carefully maneuvering the bent electrode, then insert a cartridge before closing the electrode fixture. Press **next**.

   >>> **FIGURE 3.12** appears.

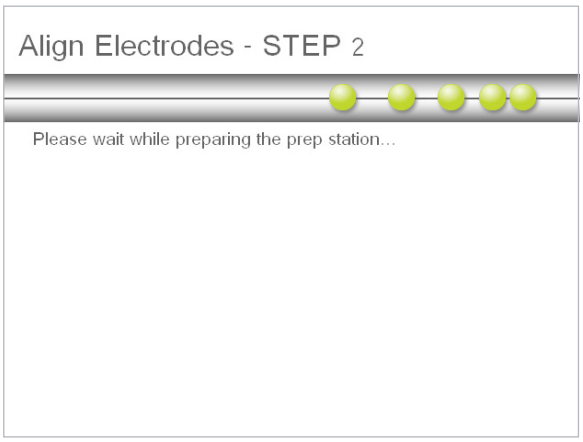
FIGURE 3.12: Align Electrodes Menu

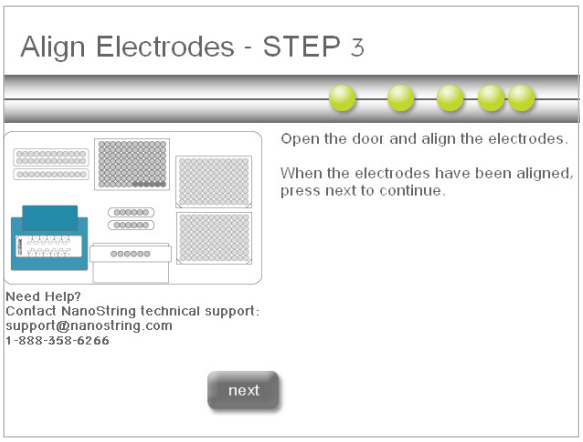


4. Close the door and select **next.**

   >>> While the door is closed, the electrode fixture tilts towards the operator for easier access to the electrodes. Two screens follow in succession (**FIGURE 3.13**).

FIGURE 3.13: Align Electrodes Menus

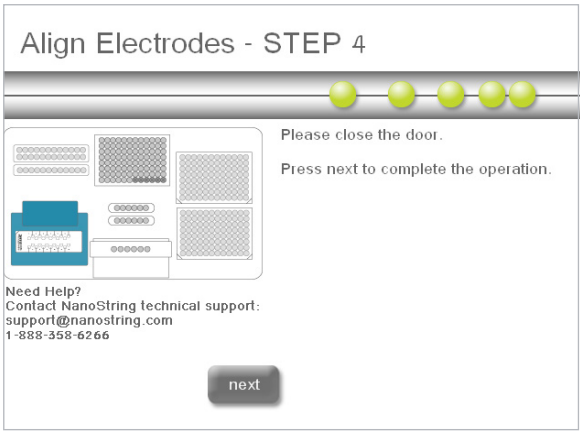




5. Using a pair of tweezers or fine tip pliers, bend the electrodes into alignment by trying to align the tip of the electrode to the center and bottom of the cartridge well. Press **next.**

   >>> **FIGURE 3.14** appears.

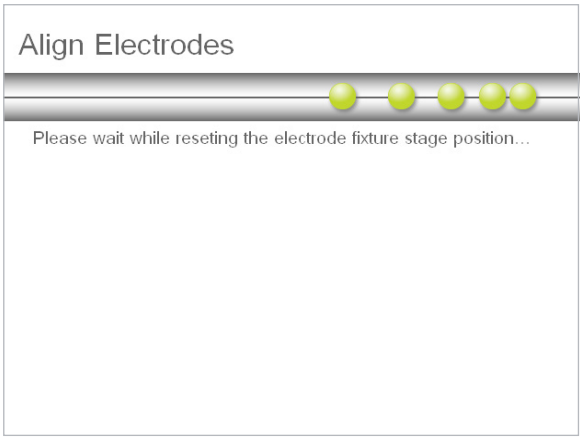
FIGURE 3.14: Align Electrodes Menu

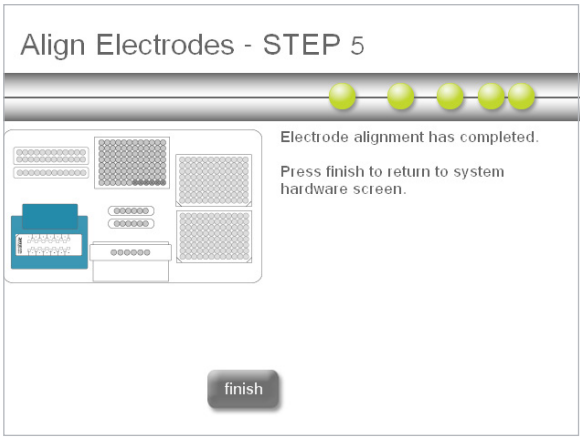


6. Close the door and the electrode fixture will tilt back to its home position. Press **next.**

   >>> Two screens appear in succession (**FIGURE 3.15**).

FIGURE 3.15: Align Electrodes Menus





7. Press **finish** to return to the Maintenance menu.

Lubricating the Pipetting Head O-rings

NOTE: When conducting maintenance activities always use appropriate safety precautions including wearing safety glasses and gloves.

The O-rings on the pipetting nozzles in the Prep Station are present to ensure a good seal with the pipette tips thus ensuring accurate liquid volume control. O-rings are the small black rings found on the lower end of the nozzle. These O-rings must be lubricated once a month in order to ensure a good seal.

The Prep Station Maintenance menu has an interface to make O-ring lubrication easier. Following the user interface brings the pipetting head to the front of the deck for easy access during lubrication.

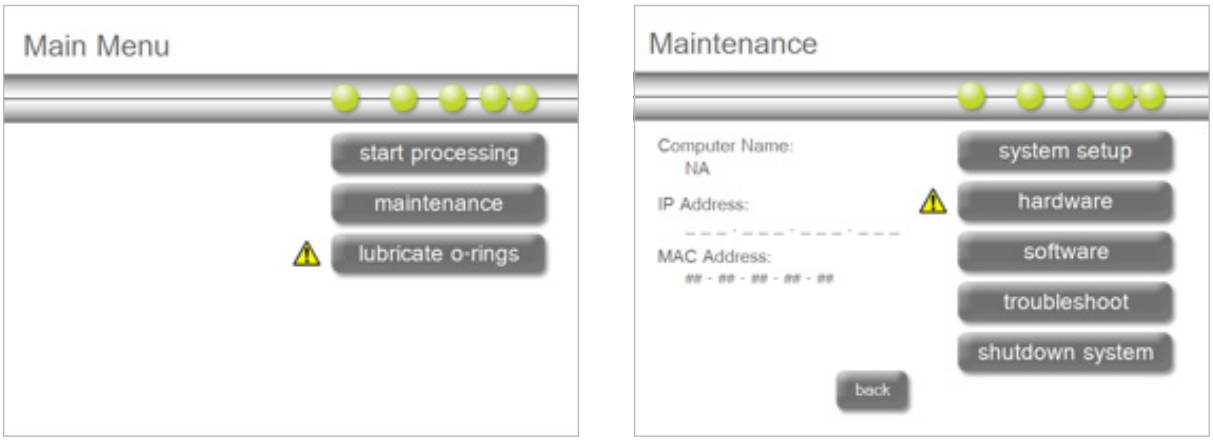
Materials required:

- Silicone Grease (light grey transparent grease in plastic container)
- Lint-free Paper (e.g. Kimwipe)
- Rubber Gloves
- Paper clip

A warning will appear on the main screen and maintenance screen when it is time to lubricate O-rings.

NOTE: The Prep Station will continue to function normally even though the yellow warning icon is present.

FIGURE 3.16: Lubricate O-rings maintenance warning screen



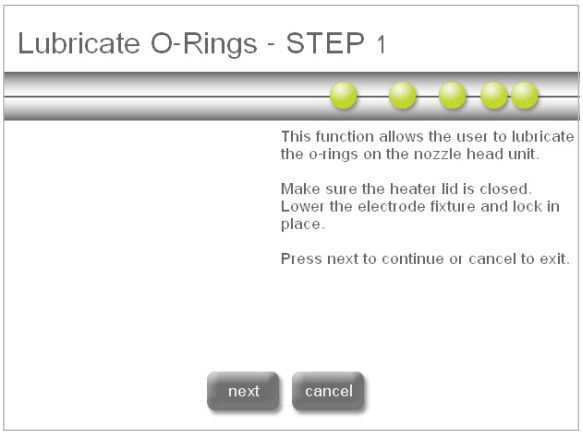
To Lubricate the O-rings

WARNING: DO NOT USE ANYTHING OTHER THAN SUPPLIED SILICONE GREASE ON THE O-RINGS.

1. Select **lubricate O-rings** on the main screen or from **hardware** on the Maintenance menu.

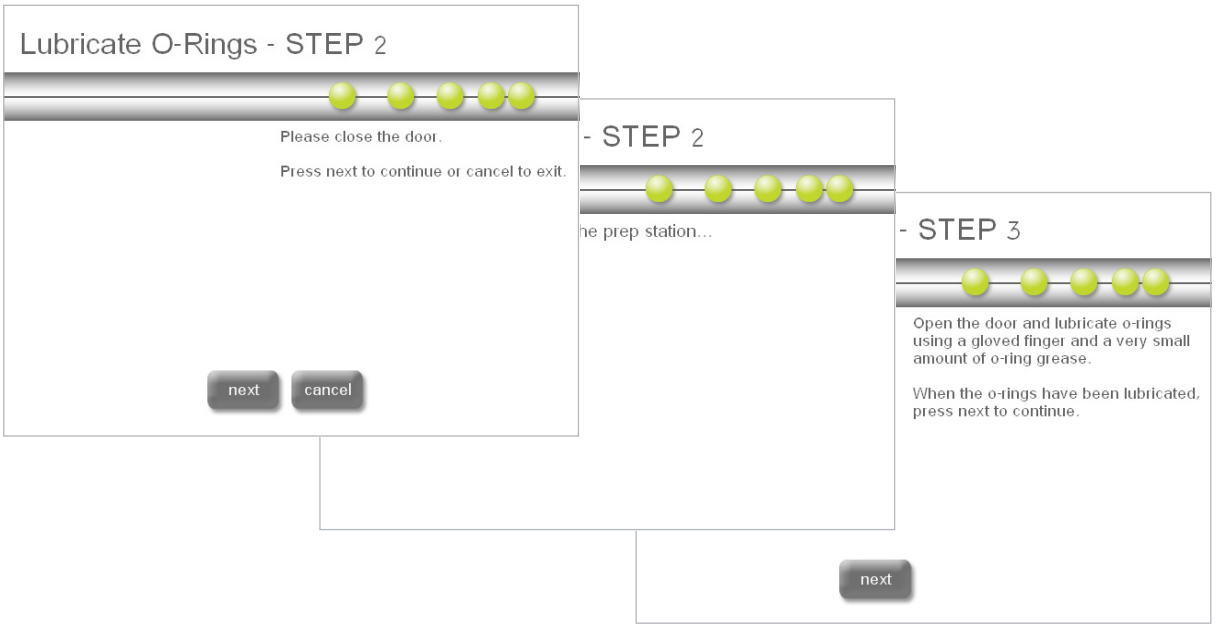
   >>> A series of Lubricate O-rings Maintenance screens appears.

FIGURE 3.17: Lubricate O-rings screen



2. Follow the directions on screen.

FIGURE 3.18: Lubricate O-rings screens



3. To lubricate the O-rings take a small amount of silicone grease and rub the tip around the O-ring using a gloved hand as shown in **FIGURE 3.20** below.

4. Using lint-free paper, thoroughly wipe off any excess silicone grease from the nozzles and Tip Ejectors.
   - Be careful not to get grease into the nozzle itself. If grease does happen to get in the nozzle, un-bend a paperclip and very carefully use the wire to scrape the grease back out.

5. Turn the tip over so that it fits over the O-ring, and gently twirl the tip around each ring to distribute the grease evenly.

IMPORTANT: Excessive grease may cause equipment malfunction. To test before running samples, perform the Calibration Check (described below).

FIGURE 3.20: Lubricating the O-rings

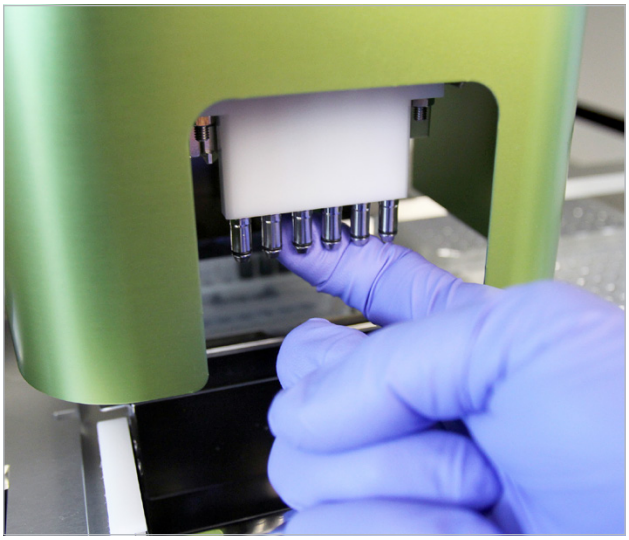


FIGURE 3.21: Lubricate O-rings screens

Completing the Lubricate O-rings process will reset the internal timer, and remove the yellow warning icon if it is present.

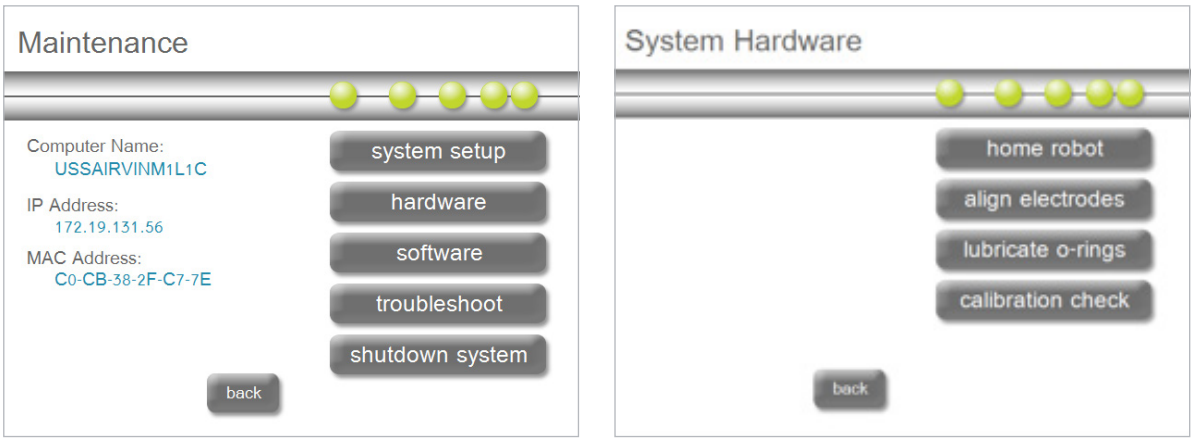
Calibration Check

If the system has been left dormant for a month or more, the calibration state of the system may change. When the check is completed, they can be returned to the rack and used in the next purification procedure.

1. Select **hardware** on the Maintenance menu.

   >>> The System Hardware menu appears (**FIGURE 3.22**).

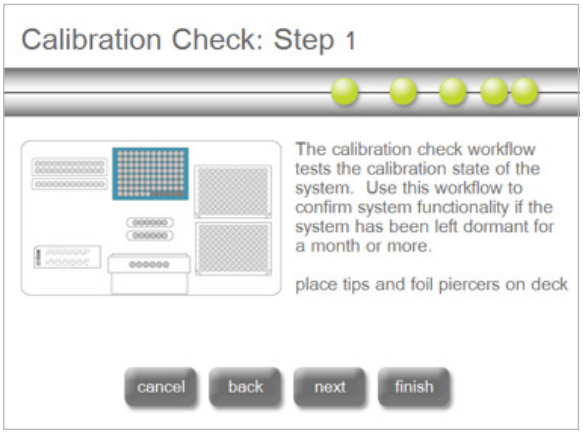
FIGURE 3.22: Maintenance Menu and System Hardware Menu



2. Select **calibration check**.

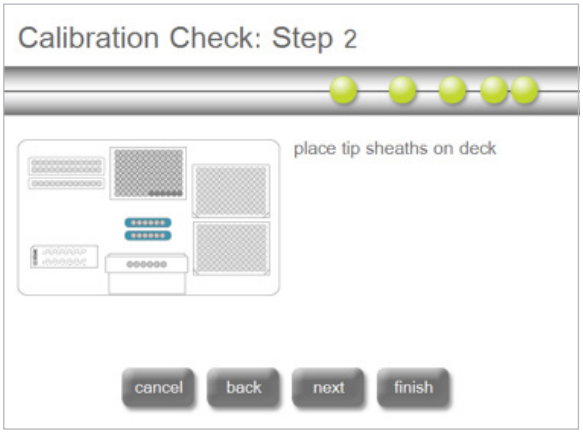
   >>> The Calibration Check screen appears.

FIGURE 3.23: Calibration Check screen



3. Place tips and foil piercers on the deck as instructed. Press **next**.

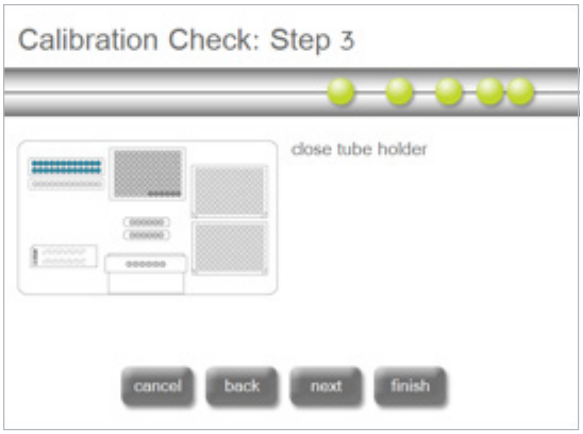
FIGURE 3.24: Calibration Check screen

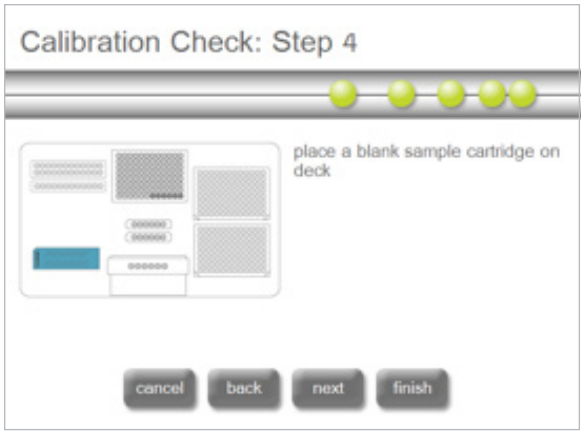


4. Place tip sheaths on the deck. Press **next**.

>>> Two screens follow in succession. Follow as instructed on the screens and press **next**.

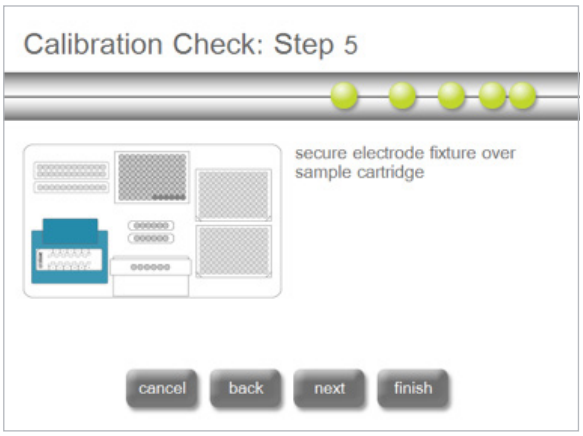
FIGURE 3.25: Calibration Check screens





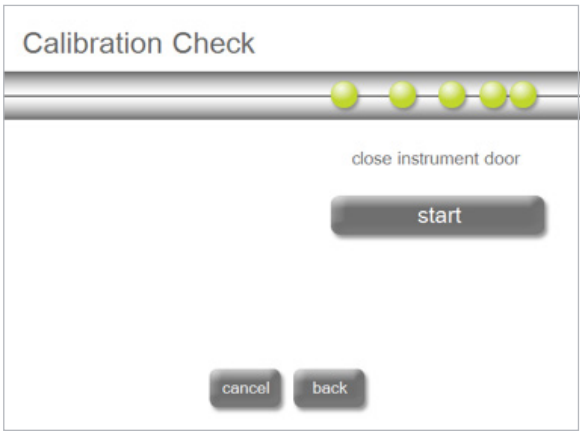
5. Secure the electrode fixture over the sample cartridge. Press **next**.

FIGURE 3.26: Calibration Check screen



6. Close the door and the electrode fixture will tilt back to its home position. Press **start.**

FIGURE 3.27: Calibration Check screen

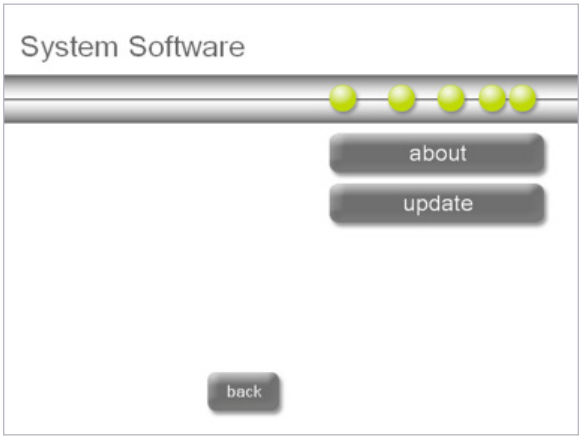


7. **At the completion of the process, the system will instruct you on how to proceed. In the case of failures, follow the on-screen instructions carefully.** Press **finish** to return to the Maintenance menu when the calibration check is complete.

Software

Use the **software** section under the Maintenance menu to update the Prep Station's system software when a new release occurs and also to view the current software version information.

FIGURE 3.28: System Software screen



About

Should you need assistance with your Prep Station, please identify system software version prior to calling NanoString. This information can be found in the **about** section on the System Software menu.

FIGURE 3.29: About screen

Update

Periodically, NanoString will release new version of software for the instruments. You can download the latest version of the software from the NanoString Technologies website. You will be required to transfer the update files to a memory stick prior to updating the software. To update the software, insert your memory stick into the USB port on the side of the touchscreen and select the update button. The system will automatically perform the software update.

Troubleshoot

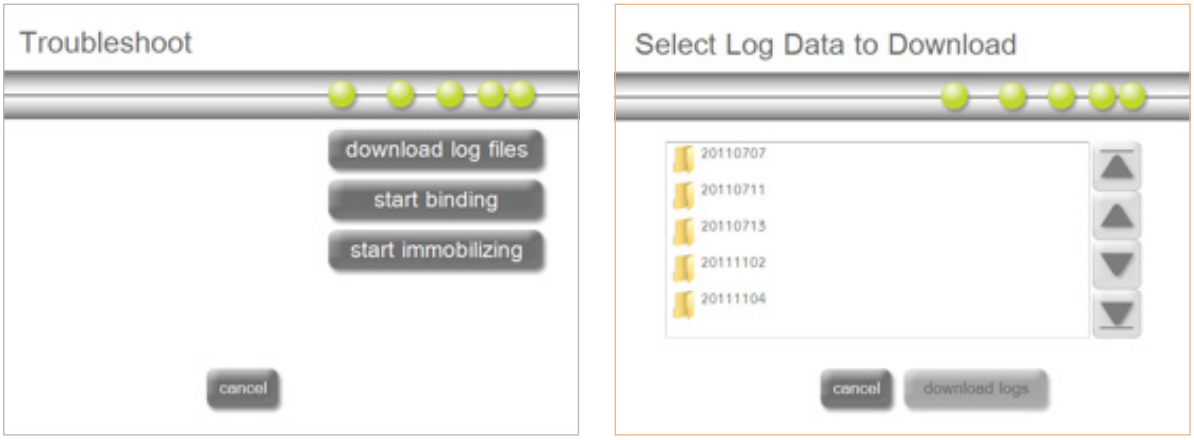
If you experience technical difficulties, NanoString support staff will ask you to download and email log files to them for diagnosis of the issue. The troubleshoot screen is used to gain access to the screen where you can select files to download, start binding and start immobilizing.

Downloading Log Files

Log files are stored by date of a run. Multiple dated folders may be selected for download by touching them. Selected folders will show up highlighted in blue.

1. Insert a memory stick into the Prep Station's front USB port.
2. When the correct folders have been selected, press **download data** and they will be transferred to the memory stick.

FIGURE 3.30: Troubleshoot screens

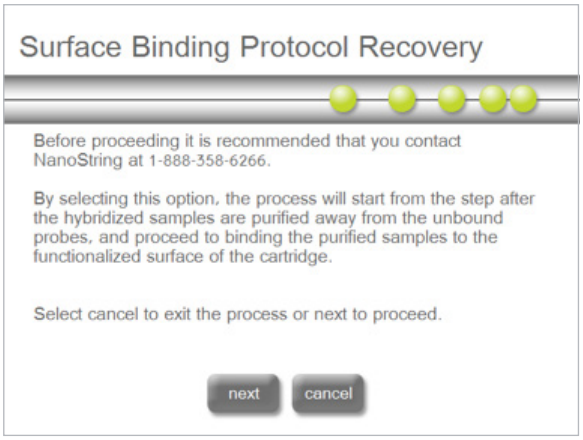


Start Binding

The Start Binding protocol can assist in running specialized procedures or in recovering from failed runs. To Start Binding Protocol Recovery press the **start binding** from the **troubleshoot** screen. Always contact **support@nanostring.com** prior to starting binding recovery.

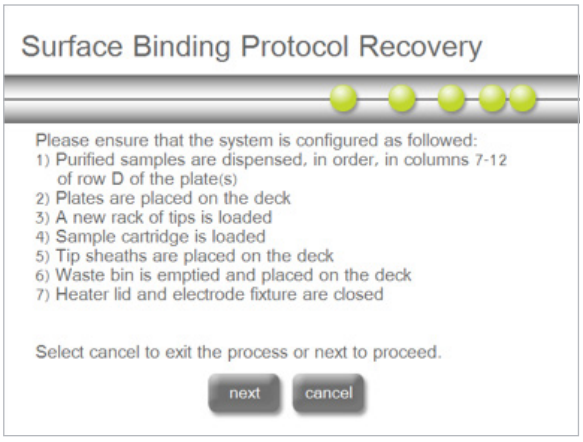
>>> The Surface Binding Protocol Recovery screen appears.

FIGURE 3.31: Start Binding Protocol Recovery screen



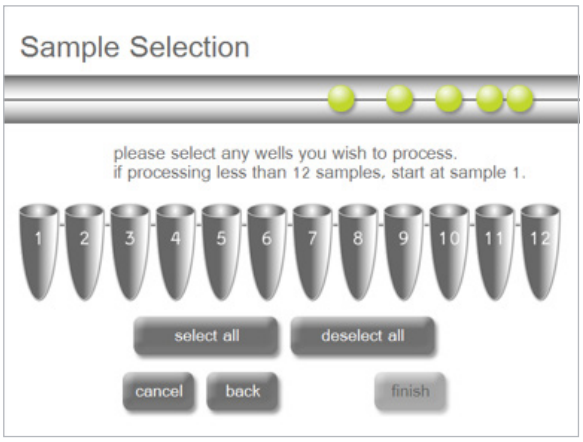
Follow the instructions on the touchscreen and press **next**.

FIGURE 3.32: Surface Binding Protocol Recovery screen



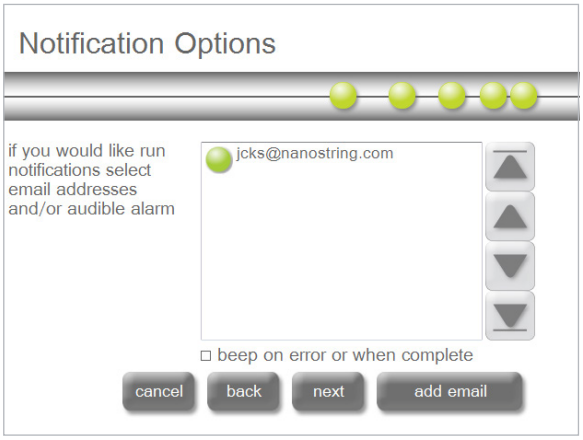
The **Sample Selection** screen appears.

FIGURE 3.33: Sample Selection screen



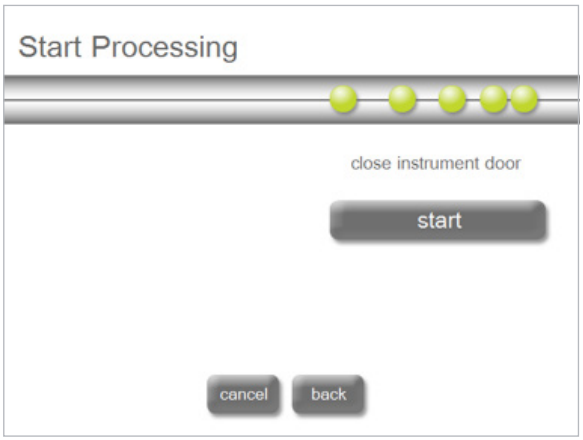
After selecting the samples, press **finish**. The notifications screen appears.

FIGURE 3.34: Notification Options screen



The **Start Processing** screen appears. Press **start** to complete the process.

FIGURE 3.35: Sample Selection screen

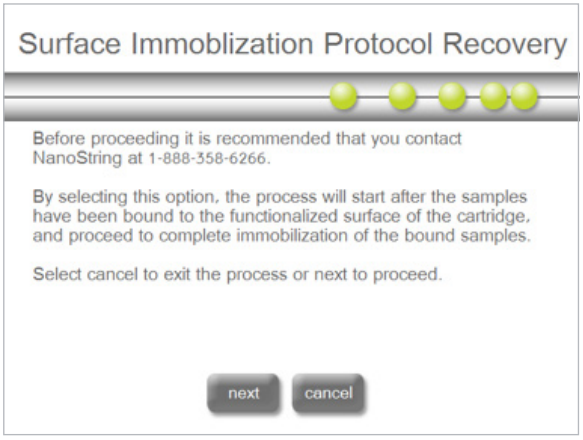


Start Immobilizing

The Start Immobilizing protocol can assist in running specialized procedures or in recovering from failed runs. To Start Immobilizing Protocol Recovery press the **start immobilizing** button from the **troubleshoot** screen. Always contact **support@nanostring.com** prior to starting immobilizing recovery.

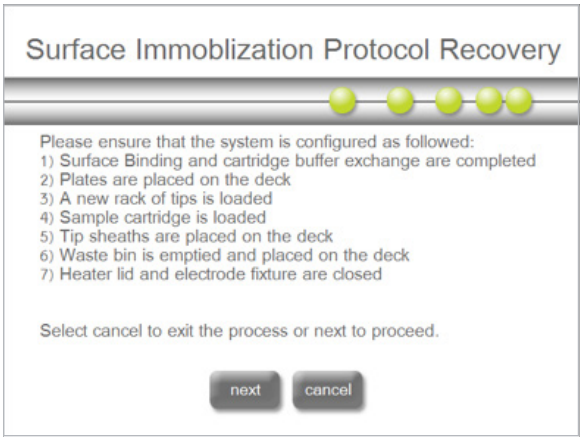
The Surface Immobilization Protocol Recovery screen appears.

FIGURE 3.36: Surface Immobilizing Protocol Recovery screen



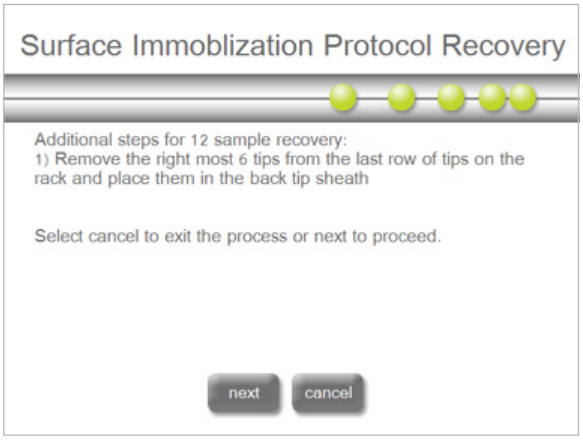
Follow the instructions on the touchscreen and press **next**.

FIGURE 3.37: Surface Immobilization Protocol Recovery screen



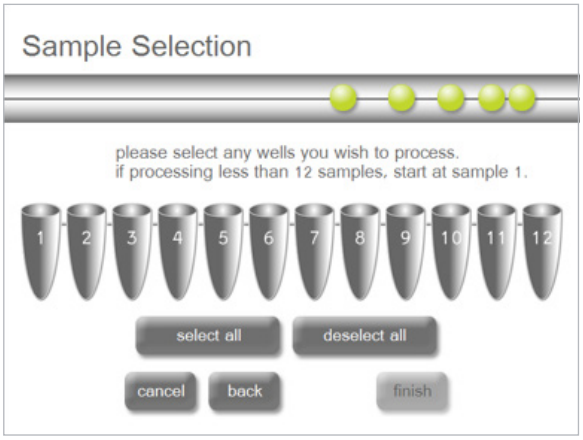
Follow the instructions on the touchscreen and press **next**.

FIGURE 3.38: Surface Immobilization Protocol Recovery screen



The **Sample Selection** screen appears.

FIGURE 3.39: Sample Selection screen

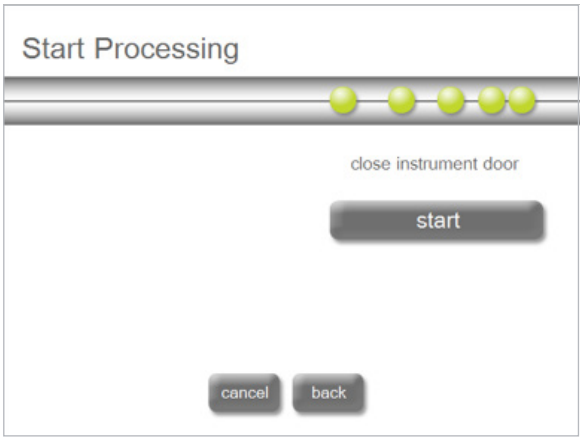


After selecting the samples, press **finish**. The notifications screen appears.

FIGURE 3.40: Notification Options screen

The **Start Processing** screen appears. Press **start** to complete the process.

FIGURE 3.41: Sample Selection screen



System Shutdown

If the system needs to be shut down it is preferable to use the system shutdown button to power off the system before using the hard power switch in the back of the Prep Station.

NanoString Technologies, Inc.
530 Fairview Ave N
Suite 2000
Seattle, Washington 98109

CONTACT US
info@nanostring.com
Tel: (888) 358-6266
Fax: (206) 378-6288
www.nanostring.com

SALES CONTACTS
United States: us.sales@nanostring.com
Europe: europe.sales@nanostring.com
Japan: japan.sales@nanostring.com
Other Regions: info@nanostring.com