SINGULAR GENOMICS

G4X™ Spatial Sequencer Guide

for Multiomic Analysis

Singular Genomics Proprietary

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INTRODUCTION

The Singular Genomics® G4X™ Spatial Sequencer revolutionizes spatial sequencing by integrating distinct modes of detection within a single Formalin-Fixed, Paraffin-Embedded (FFPE) tissue section. These modes provide comprehensive multiomic data, enabling a deeper understanding of tissue architecture and function.

The modes are:

- fH&E™ Staining: Allows for the visualization of tissue morphology using a fluorescent nuclear stain and a cytoplasmic stain. This enables the overlay of molecular findings onto histological features for spatial interpretation.
- Protein Detection (proteomics): Allows for high-resolution imaging of 15+ proteins within the tissue, with an option to add on up to 2 additional custom proteins. This mode enables precise localization of protein expression, providing crucial insights into cellular functions and interactions.
- Transcript Detection (transcriptomics): Performs barcode-free profiling of RNA transcripts with high sensitivity and specificity. This mode offers detailed transcriptomic data at subcellular resolution, essential for understanding gene expression patterns within the tissue context.

This G4X Spatial Sequencer Guide for Multiomic Analysis provides instructions on how to run the G4X Spatial Sequencer to perform spatial sequencing for transcriptomic and proteomic analysis, as well as fH&E™ staining. For instructions on how to prepare flow cells loaded with tissue sections for spatial sequencing, see the *G4X Sample Preparation Guide*. For instructions on how to view and analyze the data, see the *G4X Analysis Viewer Guide*. For Next-Generation Sequencing (NGS), see the *G4 Sequencing Platform User Guide*.

Figure 1 G4X Spatial Sequencer

Spatial Sequencing Process Overview

The process for spatial sequencing on the G4X Spatial Sequencer consists of preparing and setting up the run, imaging, data analysis, and data output. These steps are described below.

NOTE
The G4X spatial sequencing workflow described in this guide starts with spatial flow cells loaded with tissue sections prepared for spatial sequencing. For more information on how to prepare these flow cells, see the *G4X Sample Preparation Guide*.

Step	Sub-step	Performed by
Prepare the run	Set up the sample sheet. See the G4X Sample Sheet Generator.	Operator
	Frame spatial flow cells with tissue sections prepared for spatial sequencing.	Operator
	Prepare the reagents.	Operator
Set up run	Define the run. This may include uploading Custom Target File if needed.	Operator
	Assign a sample sheet to each flow cell.	Operator
	Load the reagents.	Operator
	Pre-run system check	G4X Spatial Sequencer
	Confirm or adjust the tissue alignment.	Operator
Imaging	Sequence transcript padlock probes. This happens in multiple rounds with different sequencing primers, and each round has multiple cycles.	G4X Spatial Sequencer
	P Sequence protein padlock probes. This happens in multiple rounds with different sequencing primers, and each round has one cycle.	G4X Spatial Sequencer
	Perform fH&E™ staining.	G4X Spatial Sequencer
Data analysis	Basecalling. From the images, the instrument software assigns a nucleotide base to a cluster. Basecalling occurs in concurrence with steps in image processing such as registration, stitching, and background subtraction.	G4X Spatial Sequencer
	Cell segmentation based on nuclear staining from the fH&E™ image.	G4X Spatial Sequencer
	Transcript mapping. The sequences are assigned to transcripts.	G4X Spatial Sequencer
	Transcript quantification.	G4X Spatial Sequencer
	P Protein mapping. The single-nucleotide reads are assigned to proteins.	G4X Spatial Sequencer
	P Assignment of protein integrated intensities to segmented cells.	G4X Spatial Sequencer
Data Output	Write images, data, metrics, and log files to the run folder.	G4X Spatial Sequencer
	Move run folder to off-instrument location.	G4X Spatial Sequencer

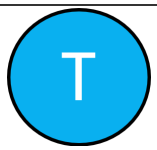
To view and analyze the data, use the G4X Analysis Viewer. For more information, see the *G4X Analysis Viewer Guide*.

NOTE
Unlike the next-generation sequencing workflow, the G4X spatial sequencing workflow does not include a cluster generation step. During G4X sample preparation, the transcript and protein padlock probes are amplified, and there is no need for further cluster generation.

Safety Considerations

Follow operating instructions to curtail potential safety hazards. Ensure that all personnel are trained in the correct operation of the instrument as described in this guide and related safety considerations as described in the *G4X Sequencing Platform Safety and Compliance Guide*.

Icons

ICON	Definition
	**Critical step** that requires extra attention.
	Additional **tips and guidelines**.
	**Hazardous material** involved in the step.
	Indicates if the current section, subsection, or steps are for **transcriptomic only** sequencing. **Skip steps marked with this icon if sequencing a multiomic flow cell.**
	Indicates if the current section, subsection, or steps are for **proteomic** sequencing, used in **multiomic** workflow. **Skip steps marked with this icon if sequencing a transcriptomic only flow cell.**

G4X SPATIAL SEQUENCER OVERVIEW

When sequencing on the G4X Spatial Sequencer you need the following items:

- The G4X Spatial Sequencer
- The case containing the G4X computers
- Singular Control System (SCS)
- Two Uninterruptible Power Supplies (UPS)
- Sequencing Operating System (SeqOS)
- Reagent kits
- Laboratory equipment and consumables

These items are described below.

NOTE
Only personnel authorized by Singular Genomics can uncrate, install, service, or move the instrument. If you must relocate the instrument, contact your Singular Genomics representative.

Instrument Components

The G4X Spatial Sequencer enclosure includes a touchscreen monitor, a status light bar, and three accessible compartments. The user-accessible compartments are intended for loading required reagents and consumables.

A Touchscreen monitor
B Reagent compartment
C Buffer compartment
D Flow cell compartment
E Status lights

Figure 2 G4X Spatial Sequencer components

Reagent Compartment

The reagent compartment contains four reagent cartridge positions and four sample cartridge positions. Each position in the reagent compartment corresponds to a flow cell position on the flow cell stage, numbered 1 through 4 from left to right.

A Sample cartridge positions
B Reagent cartridge positions
C Reagent cartridge door

Figure 3 G4X Spatial Sequencer Reagent compartment

NOTE
Sample cartridges are not used in G4X spatial sequencing and remain empty during a spatial sequencing run. Sample cartridges are used for Next Generation Sequencing.

Buffer Compartment

The buffer compartment holds the waste reservoir provided with the instrument and up to four wash cartridges provided in G4X sequencing kits, for positions 1 through 4, left to right. The waste reservoir contains a removable waste cap that opens the waste port for disposal.

Component	Description
Waste reservoir	During a sequencing run, used reagents and buffers are deposited into the waste reservoir. Always empty the waste reservoir between runs and dispose of the contents according to local laws and regulations.
Wash cartridges	One wash cartridge is required for each flow cell used in a sequencing run. Each position corresponds to a flow cell position on the flow cell stage.

A Waste cap
B Waste port

Figure 4 G4X Spatial Sequencer waste reservoir

NOTE
You can also order an external waste reservoir, which holds more waste. Contact Singular Genomics (see *Customer Care* on page 66) for ordering information and installation of the external waste reservoir.

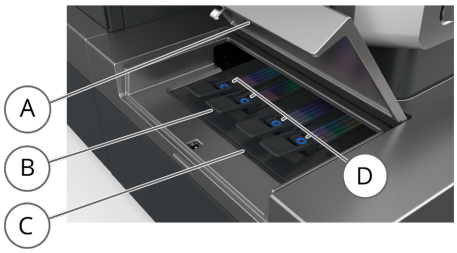
Flow Cell Compartment

The flow cell compartment houses the flow cell stage and contains four magnetic flow cell positions numbered 1 through 4, left to right. Before the run begins, the instrument software performs a check to

ensure that each flow cell is securely positioned on the stage.

Each flow cell position has a light that indicates the status of the position:

Color	Action	Description
White	Solid	Awaiting flow cell. Note that, for a maintenance wash, the light is also white if the flow cell is detected, but no seal check performed.
Green	Solid	Flow cell detected, no seal check performed yet. Note that for a maintenance wash, there is no green light if a flow cell is detected.
Blue	Flashing	Performing seal check
Blue	Solid	Seal check passed
Red	Solid	Seal check failed
	No Light	Flow cell position not used, or all seal check completed successfully for all assigned flow cells.



A Flow cell compartment door
B Flow cells
C Flow cell stage
D Flow cell indicator lights

Figure 5 G4X Spatial Sequencer flow cell compartment

Instrument Status Lights

The instrument enclosure includes a status light bar to indicate instrument status at a glance (see Figure 2).

Color	Action	Description
Green	Solid	Instrument idle
Green	Flashing	Instrument undergoing checks
Blue	Flashing	Instrument sequencing
White	Flashing	Instrument shutting down or SeqOS initializing
Red	Flashing	Instrument sequencing, but an error occurred in one or more flow cells
Red	Solid	Instrument idle, but an error occurred in the previous run
	No Light	Instrument off

G4X Primary and Secondary Computers

The G4X Spatial Sequencer comes with two computers in a single case:

Component	Description
G4X Primary Computer	Hosts the Sequencing Operating System. When you set up a run, you interact with the instrument user interface powered by this computer.
G4X Secondary Computer	A high-performance computer that performs image analysis and base calling.

Front Back

Figure 6 Computer case containing the G4X Primary Computer and G4X Secondary Computer, front and back view.

The G4X computers and their connection ports are for G4X sequencing only and should not be used for other purposes. The G4X Primary and Secondary Computers are a closed system and network. Their use is limited to operation of the platform. These systems never directly connect to the customer network or the internet.

NOTE
Never rearrange connections between the G4X Spatial Sequencer, the G4X Primary Computer, the G4X Secondary Computer, and the SCS.

Singular Control System (SCS)

The Singular Control System (SCS) serves as the bridge linking the G4X Spatial Sequencer with external systems and functions. The SCS is co-located with the G4X and isolates the G4X and its computers from the intranet and the internet, while managing secure data transfer on and off the computers.

Figure 7 Singular Control System (SCS)

SCS Key Functions

Transfer of Sequencing Data	The SCS is directly connected to the secondary computer which processes the sequencing data connected to the instrument. The finalized data is securely transferred to the SCS when processing is completed. The SCS securely transfers the finalized data to customer-owned or managed storage at the local site or in the cloud.
Transitional Storage of Sequencing Data	The SCS provides transitional storage for sequencing results to enable users to start another run without having to wait until data transfer from previous runs is complete.
Secure Remote Connectivity	The SCS establishes a secure remote tunnel to the Singular Secure Customer Network (SCN), using SSL/VPN with AES256/SHA256 encryption.
Security & Protection	Singular Genomics utilizes FortiClient with EMS to secure the SCS and connected systems. FortiClient with EMS provides up-to-date Antivirus, Antimalware, Zero-day, and other features to protect the SCS and customer data. Singular Genomics does not permit other security applications to be installed on the SCS. If needed, Singular Genomics can provide you a feed and/or logs from the SCS.
Software Updates	The SCS enables the G4X Spatial Sequencer to receive and install software and security updates and patches.
Troubleshooting	The SCS enables health monitoring to detect problems early and remote support to fix problems promptly.

Off-Instrument Storage

The G4X Spatial Sequencer has a limited amount of storage available due to the large data sets produced. All run data is automatically transferred to the SCS (for more information, see *Singular Control System (SCS)* on page 7). The SCS provides transitional storage and frees up the G4X Spatial Sequencer to start the next sequencing run. The SCS then automatically transfers the run results to your off-instrument storage.

The G4X Spatial Sequencer supports the following storage solutions:

- On-premises storage (NAS/SAN): CIFS/SMB, NFS storage.
- Cloud-based storage: Amazon S3, Google Cloud Platform (GCP), Azure.
- Other cloud-based providers may be supported. Contact Singular Genomics Customer Care for more information.

NOTE
Singular Genomics manages the configuration of the G4X off-instrument storage.

Uninterruptible Power Supplies

The G4X Spatial Sequencer includes two Uninterruptible Power Supplies (UPS): one for the instrument, the primary computer, and SCS, and one for the secondary computer. The UPSs provide backup battery power in the event of a power outage and safeguard your equipment from power surges.

NOTE
The UPSs are not meant to provide backup power for an entire run. They are meant to provide temporary power during shorter power outages for about 4 minutes, so building backup power can take over after that to complete the run.

Figure 8 Uninterruptible Power Supplies (UPS) for instrument, the primary computer, and SCS, and one for the secondary computer, respectively.

Software User Interface

Operations on the G4X Spatial Sequencer are controlled by the Sequencing Operating System (SeqOS). When operating the G4X Spatial Sequencer or setting up a run, you interact with the software through the SeqOS. You can also upload sample information using the sample sheet. These features are described in this section.

Sequencing Operating System

The G4X Spatial Sequencer operating system SeqOS controls operations on the G4X Spatial Sequencer and provides an intuitive interface to interact with the instrument. Various screens guide the user through loading consumables, specifying run parameters, and monitoring run statistics. This section describes the main SeqOS screens, and the operations that can be performed on these screens.

SeqOS Main Screens

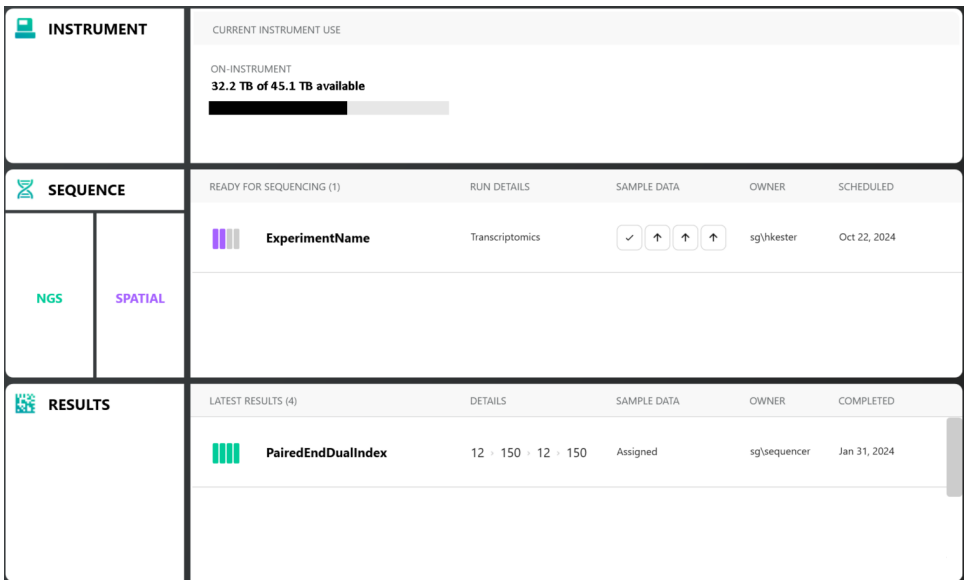


Figure 9 SeqOS Dashboard

The SeqOS main menu options provide access to the following screens:

Screen	Description	Sub Screen
Dashboard	Displays data storage space, available runs, and completed runs. Each section opens a detailed screen.	• **Instrument**–Shows a snapshot of available hard drive space and what portion contains sequencing results. • **Sequence**–Shows a snapshot of runs that are ready for sequencing and details about that run, such as run type, number of cycles, if a sample sheet is assigned, and the run owner. • **Results**–Shows a snapshot of the runs that have completed, the number of cycles for each, if a sample sheet was assigned, and the run owner.
Sequence	Provides the option to set up a new NGS or spatial run or select a run from the queue.	• **NGS**–For Next-Generation Sequencing (NGS), see the G4 User Guide. • **Spatial**–To set up a spatial sequencing run, see *Set Up a Run* on page 27.
Results	Shows details of completed runs. For more information, see *View Results* on page 44.	
Instrument	Provides access to maintenance protocols and instrument settings, each in its own section.	• **Maintenance**–Shows protocols and timing for instrument maintenance. For more information, see *Maintenance Wash* on page 48 • **Storage**–Allows you to set up off-instrument storage. For more information, see *Off-Instrument Storage* on page 8. • **Settings**–Provides a menu to view and change instrument settings. For more information, see *Changing Instrument Settings* on page 13.
Help	Shows contact information for technical support.	Telephone: 442.SG.CARES (442.742.2737)

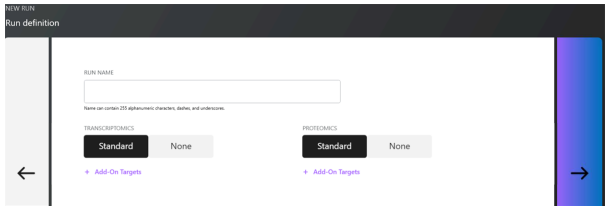
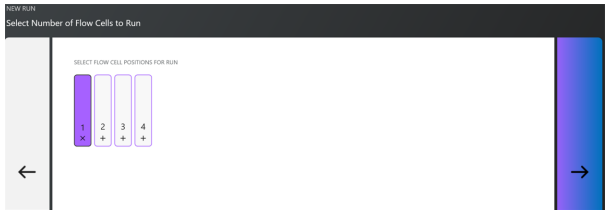
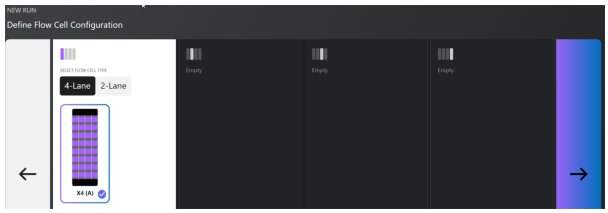
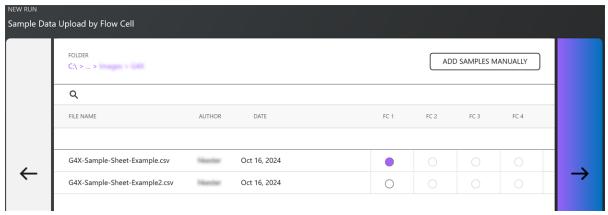
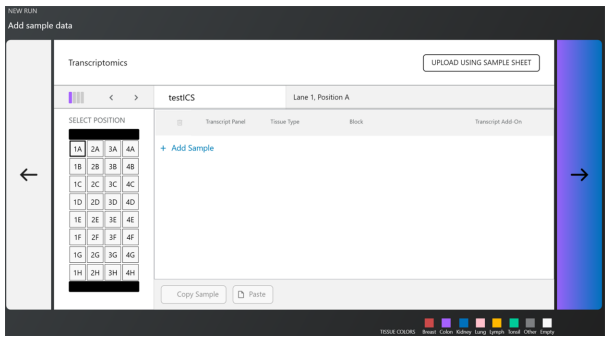
At any time, tap the Singular Genomics icon in the top right corner to access the main menu.

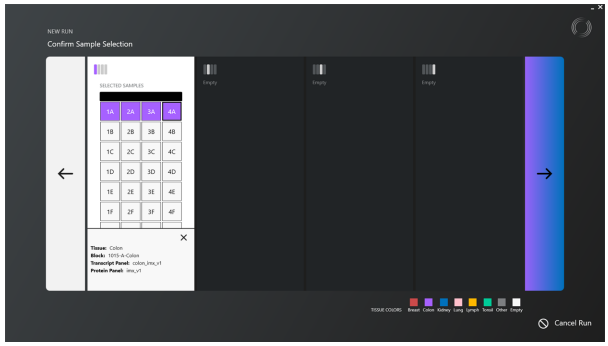
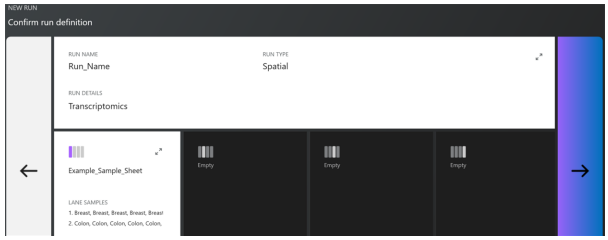
Spatial Run Setup on SeqOS

There are two ways to start a spatial sequencing run:

Run Type	Description
Existing Run	You can select an existing run from the runs listed on the dashboard.
New Run	To create a new run, go to the Dashboard tab, select Spatial, and select the **+**. The New Run screen is divided into four sections.

The New Run option provides access to seven screens:

Screen Name	Description	
Run Definition	Provides field to name the run, which can use up to 255 alphanumeric characters, dashes, or underscores. This screen also allows you to specify the run type (transcriptomics, proteomics, or both) and whether add-on targets or custom panel were used. If add-on targets or a custom panel were used, this screen allows you to upload the Custom Target File.	
Select Number of Flow Cells to Run	Provides the feature to assign the flow cell positions you want to run.	
Define Flow Cell Configuration	Allows you to select the flow cell type for each position you selected.	
Sample Data Upload by Flow Cell	Allows you to upload a sample sheet for the flow cell. Each sample sheet can only be used on one flow cell. You can also indicate that you want to add samples manually, in which case you go to the Add Samples Manually screen.	
Add Samples Manually	Allows you to manually define the samples for the flow cell. You can also indicate that you want to upload using a sample sheet, in which case you go back to the Sample Data Upload by Flow Cell screen.	

Screen Name	Description	
Confirm Sample Selection	Allows you to review the sample selection. If the sample selection is not correct, you can go back to the previous page to edit the information.	
Confirm Run Definition	Allows you to review the run parameters and edit before starting the sequencing run.	

Run Monitoring

After a sequencing run has started, a graphical card appears for each flow cell that lists which step in the run is being performed. Tap the card to view run progress by lane. The elapsed run time is shown in the corner of the screen.

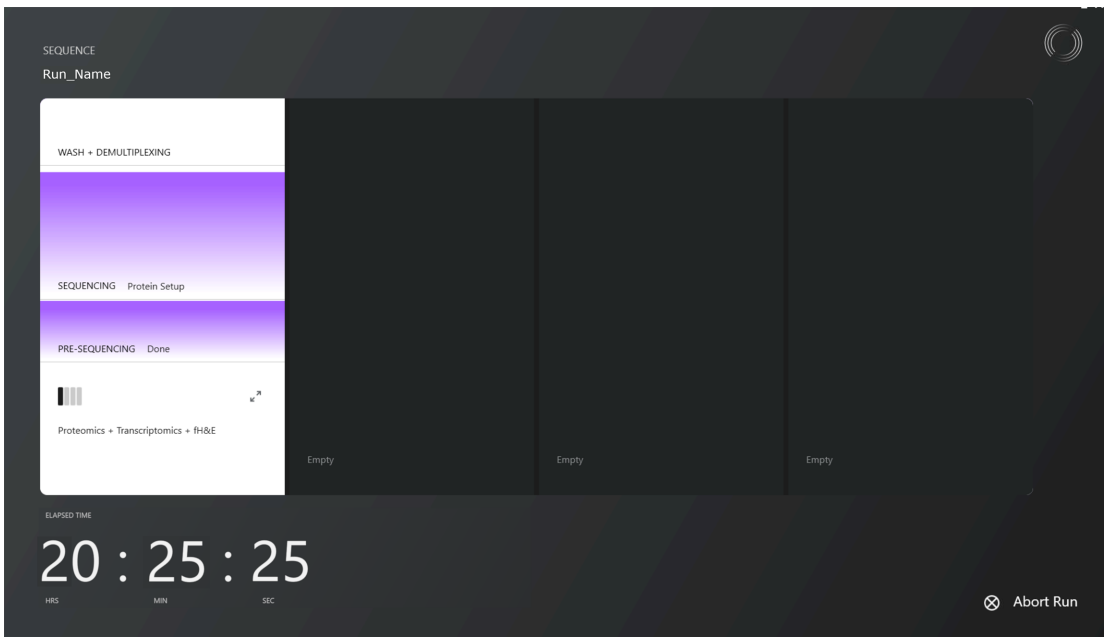


Figure 10 SeqOS Run Monitoring Screen

For a description of run metrics, see *Monitoring Analysis Progress* on page 40.

Changing Instrument Settings

Adjust the following settings via the General tab on the Instrument Settings screen:

Setting	Description
Instrument Name	Change the instrument name.
Run Setup Expert Mode	Expert Mode skips most animations that illustrate the different steps during run setup. This allows for a faster run setup, but only use this mode if you are very familiar with operating a G4X Spatial Sequencer.
Run Start Before/After System Check	Default setting is **Run Start After System Check**. You will have to wait to start the run until the system check passed.

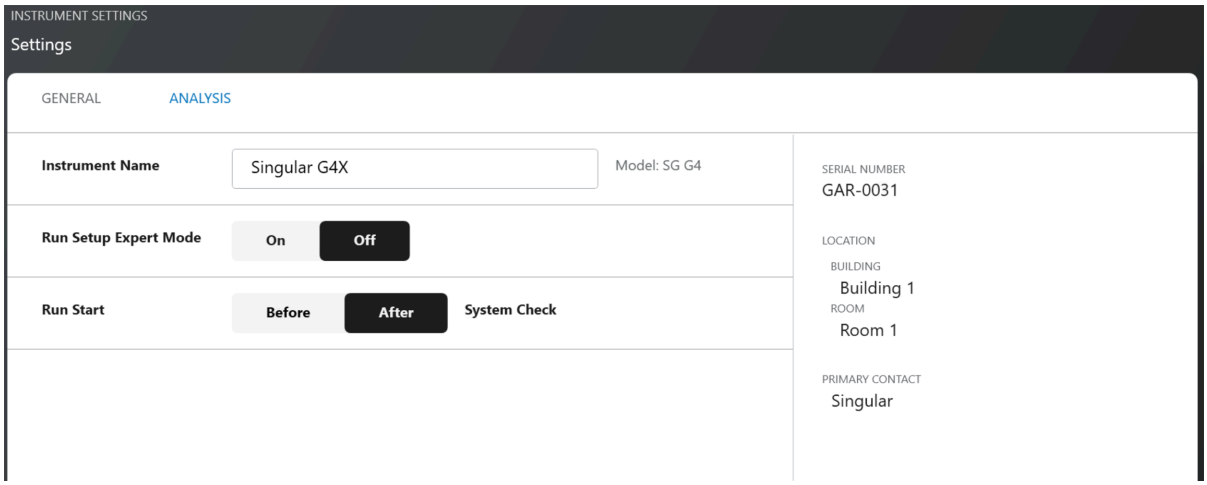
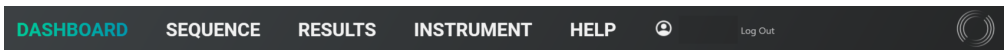


Figure 11 SeqOS Instrument Settings Screen

Switch User

Users are defined by your network settings. The SeqOS allows you to switch users in the following way:

1. Open the main menu by tapping the Singular Genomics icon in the top right corner.
2. Select **Log Out**.
3. Log in with the network credentials for the new user.



G4X Sample Sheet

A sample sheet is a Comma Separated Value (CSV, *.csv) file that stores information about the sample sections and layout of the flow cell to be sequenced . The SeqOS can then uses the information in the sample sheet to set up the run, in addition to set up parameters entered during run setup. The sample sheet is also used in post-run analysis.

Singular Genomics has generated an online G4X Sample Sheet Generator. This tool helps you fill out all

data which minimizes errors in the sample sheet. The G4X Sample Sheet Generator also includes a built in Sample Sheet Checker, which allows you to upload your sample sheet and check for mistakes.

NOTE
You can manually edit the sample sheet in Excel, save as a CSV file, and then upload to the *sample-sheets* folder on the off-instrument storage. Be aware that manually editing the sample sheet increases the risk of errors or incompatibilities. Singular Genomics recommends using the G4X Sample Sheet Generator. See *Create a G4X Sample Sheet* on page 18.

The sample sheet has the following characteristics:

Feature	Description
File Name	Sample sheet file names can use alphanumeric characters, spaces, dashes, or underscores, ending with a .csv extension.
Header Section	Section to enter general information about the run.
Settings Section	Section to enter information about the run settings that apply to all lanes and samples in this run: read types (i.e. Transcript, protein) and flow cell layout. This section is written by the SeqOS during run setup.
Data Section	Section to enter information about the different tissue sections in the lanes.

	A	B	C	D	E	F	G	H
1	[Header]							
2	Date	12/12/2025						
3	Run Name	G4X-100						
4	User Name	G4X User						
5	User Email	g4x@singulargenomics.com						
6	Workflow	Transcript						
7	Assay	Lung Biopsy						
8	Run Notes	Lung Samples Set A						
9	[Settings]							
10	Stage1	Transcript						
11	Stage2							
12	FC Layout	4x8						
13	[Data]							
14	Lane	Sample Position	Tissue Type	Block	Transcript Panel	Transcript Addon	Protein Panel	Protein Addon
15	1	A						
16	1	B						
17	1	C	lung	HuN-29-0020B	lung_imx_v1			
18	1	D						
19	1	E	lung	HuN-29-0020B	lung_imx_v1			
20	1	F						
21	1	G						
22	1	H						
23	2	A						
24	2	B	lung	HuN-29-0020B	lung_imx_v1	lung_supplement1		
25	2	C						

Figure 12 G4X Sample sheet

For a description of what to enter in each field of the sample sheet , see *Create a G4X Sample Sheet* on page 18.

Prior to sequencing on instrument, it is recommended to check the sample sheet using the Sample Sheet Checker, as it will flag any issues with your sample sheet prior to the run on instrument.

When you assign a data sample sheet to a flow cell, the software checks whether the sample sheet is valid.

If an error is detected, you will have to correct the sample sheet before continuing.

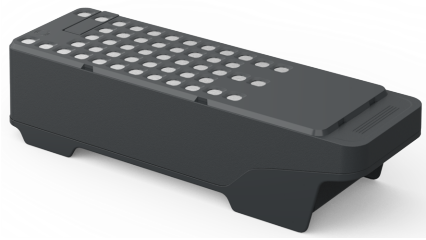
G4X Sequencing Kits

The G4X Sequencing Kit provides consumables designed to support a broad range of applications and integrate seamlessly with the instrument. For more information, see the G4X Sequencing Kit Overview document.

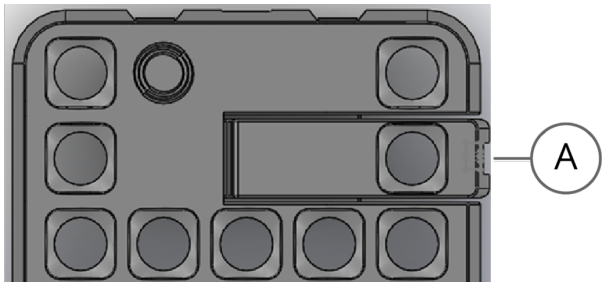
Each sequencing kit includes a reagent cartridge box and wash cartridge box.

Spatial Reagent Cartridge

The Spatial Reagent Cartridge contains reagents required for spatial sequencing on one flow cell. The cartridge is designed with a molded handle for easy loading and foil seals covering each of the reagent wells. During a sequencing run, instrument sippers automatically pierce each foil-sealed well.



On the right side near the rear of the reagent cartridge (opposite end of the handle) is a removable well for the safe disposal of the reagent formamide (see Figure 13). The cartridge enclosure includes a hinged cover that opens to expose the well for easy removal after a sequencing run. For handling instructions, see *Disposal of Used Reagent Cartridges* on page 37.



A Removable formamide well

Figure 13 Reagent cartridge with removable formamide well indicated.

Cleave Buffer Additive

The reagent cartridge comes with a tube of Cleave Buffer Additive. Add this reagent to the well marked with A on the figure below immediately before starting the run (see *Prepare Reagent Cartridge* on page 37).

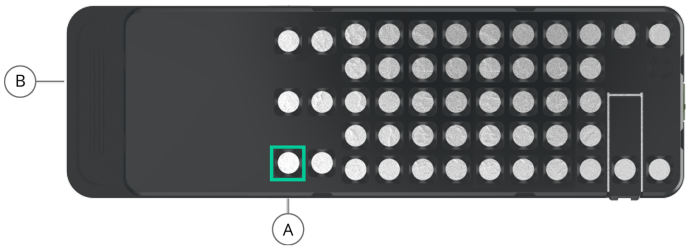


Figure 14 Location of well for Cleave Buffer Additive.

A Well for Cleave Buffer Additive
B Handle

Wash Cartridge

One wash cartridge is required for each flow cell used in the run. Wash buffers are used for priming and washing fluidics lines between steps in the sequencing run.

Product Identifier

The cartridges include an integrated identification chip, the Product Identifier. Before starting a run, the instrument software reads the information from each Product Identifier to confirm the presence and expiration date of the consumable, lot number, and compatibility with specified run parameters.

NOTE
Make sure the Product Identifier remains dry. If the Product Identifier happens to get wet, wipe gently to remove any visible moisture.

Consumables and Equipment

You need to make sure you have the following consumables and equipment in your laboratory. See the G4X Sequencing Platform Site Preparation Guide for more information.

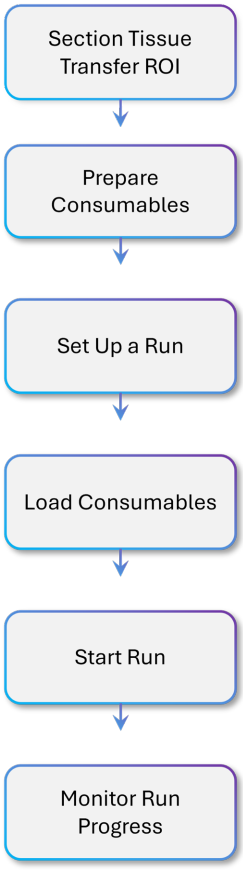
Laboratory Consumables

Consumable	Supplier	Purpose
Lint free optical wipes	General lab supplier	Flow cell cleaning
Alcohol wipes or 80-100% Isopropyl alcohol	General lab supplier	Flow cell cleaning
Disposable gloves, powder-free	General lab supplier	General purpose
10 mL serological pipette	General lab supplier	Mixing well after adding Cleave Buffer Additive
Pipette tips (at least P2, P10, P20, P200, and P1000)	General lab supplier	Sample library preparation
Flow cell frame (1 per flow cell)	Singular Genomics	Framing the flow cell
Flow cell spacer (1 per flow cell)	Singular Genomics	Framing the flow cell
Flow cell tabs (2 per flow cell)	Singular Genomics	Framing the flow cell

Laboratory Equipment

Equipment	Supplier	Purpose
Calibrated P1000 pipette	General lab supplier	Adding Cleave Buffer Additive
Serological pipette controller	General lab supplier	Mixing well after adding Cleave Buffer Additive
Freezer, frost-free -15°C to -25°C	General lab supplier	Storing reagents
Refrigerator 2°C to 8°C	General lab supplier	Storing flow cells and buffers
Hazardous waste container	General lab supplier	Discarding hazardous waste
Flow Cell Framing Tool Suite	Singular Genomics	Framing the flow cell
Optional: Forceps	Singular Genomics	Framing the flow cell

SPATIAL SEQUENCING WORKFLOW



Create a sample sheet and save as a CSV file, or use the Singular Genomics online G4X Sample Sheet Generator (recommended).

Frame flow cells with tissue sections prepared for spatial sequencing.

Thaw the reagent cartridge at 2°C to 8°C for 2 days, or for 2.5 hours in a room temperature water bath.

Set up a new run using the SeqOS on the instrument.

Empty the waste reservoir before every run.

Invert reagent cartridge 5–10 times while holding the lid on and add Cleave Buffer Additive.

When prompted, load the wash cartridges, flow cells, and reagent cartridges.

After the pre-run check is complete, which takes about 30 minutes, confirm or adjust the tissue alignment.

Select Start Run.

While the G4X is sequencing, monitor the run occasionally using the Run Monitoring screen.

Create a G4X Sample Sheet

Create one sample sheet for each flow cell. Use the Singular Genomics G4X Sample Sheet Generator. For more information about the sample sheet, see *G4X Sample Sheet* on page 13.

NOTE

You can also edit the sample sheet manually in Excel and save as CSV file (see *Create a G4X Sample Sheet Manually* on page 20). Be aware that manually editing the sample sheet increases the risk of errors or incompatibilities. Singular Genomics recommends using the G4X Sample Sheet Generator as described in this topic.

1. Fill out the following information for the `Header` section. All fields in this section can be left empty. Do not add rows to the existing template.

Field	Description
Date	Date of sample sheet edit.

Field	Description
Run Name	Name that identifies the run.
User Name	Name of operator.
User Email	Email of operator.
Workflow	Information about specific workflow used.
Assay	Information about specific assay used.
Run Notes	Any other notes for the run.

2. Select the **Run Type** and **Layout** of the flow cell in the `Setting` section.
3. In the `Data` section, enter the following information for each lane:

Field	Description
Transcript Panel	Select the transcript panel that was used to prepare the samples in this lane. Required for transcriptomic and multiomic runs.
Transcript Add-on	For G4X runs with add-on targets or custom panel: Name the Custom Target File for the transcript add-on or custom targets that were used to prepare the samples in this lane. The name specified here must match the .csv filename of the Custom Target File. Filename must be in all lowercase.
P Protein Panel	Select the protein panel that was used to prepare the samples in this lane. Required for multiomic runs.
P Protein Add-on	For G4X multiomic runs with Custom Antibody Add-on: Name the Custom Target File for the protein add-on targets that were used to prepare the samples in this lane. The name specified here has to match the .csv filename of the Custom Target File. Filename must be in all lowercase.

4. For each used tissue position, fill out the following information.

Field	Description
Tissue Type	The type of tissue for this section.
Block	The block that this tissue section was prepared from.

5. Download the sample sheet.
6. Open the G4X Sample Sheet Generator. Navigate to the `Check SampleSheet` tab
7. Click *Upload csv file* to upload your sample sheet.
8. If there are any errors, correct the error and repeat step 7
9. Copy the CSV file (*.csv) in the *sample-sheets* folder on the off-instrument storage accessible to the instrument.

   The SCS monitors the *sample-sheets* folder on the off-instrument storage and automatically moves files from that folder to the SeqOS. The sample sheets are available at *C:\sample-sheets* on the instrument.

Create a G4X Sample Sheet Manually

Singular Genomics recommends using the G4X Sample Sheet Generator, as described in *Create a G4X Sample Sheet* on page 18. However, you can also create a sample sheet manually, as described here. For more information about the sample sheet, see *G4X Sample Sheet* on page 13.

When manually creating a sample sheet, be aware of the following:

- Editing the sample sheet increases the risk of errors or incompatibilities.
- Do not remove any of the fields or rows.
- Allowed characters are alphanumeric and underscore (_) or hyphen (-).

Create one sample sheet for each flow cell the following way:

1. Download a sample sheet template:
   - For a 4-lane flow cell: G4X-sample-sheet-template-4lane.csv.
   - For a 2-lane flow cell: G4X-sample-sheet-template-2lane.csv.
   - For a 2-lane low throughput flow cell: G4X-sample-sheet-template-2lane-lowthroughput.csv.
2. Open the sample sheet template in Microsoft Excel or a text editor software.
3. Fill out the following information for the `Header` section. All fields in this section can be left empty. Do not add rows to the existing template.

Field	Description
Date	Date of sample sheet edit.
Run Name	Name that identifies the run.
User Name	Name of operator.
User Email	Email of operator.
Workflow	Information about specific workflow used.
Assay	Information about specific assay used.
Run Notes	Any other notes for the run.

4. Fill out the following information for the `Setting` section:

Field	Description
Stage1	Enter **Transcript**. This field is required.
P Stage2	Enter **Protein** if running a multiomics run. Leave empty if running a transcriptomic only run.

5. For each used lane, enter the following information in the `Data` section:

Field	Description
Transcript Panel	Enter the transcript panel that was used to prepare the samples in this lane. Required for transcriptomic and multiomic runs. Panels entered should follow the format {panel}_v{n} where panel is the name of panel (kidney, lung, etc) and n is the version number of the panel. Example: kidney_imx_v1. Refer to the panel tube for panel version information. If using a De Novo Panel, select 'custom'.
Transcript Add-on	For G4X runs with add-on targets or custom panel: Name the Custom Target File for the transcript add-on or custom targets that were used to prepare the samples in this lane. The name specified here must match the .csv filename of the Custom Target File. Filename must be in all lowercase.
P Protein Panel	Enter the protein panel that was used to prepare the samples in this lane. Required for multiomic runs. Panels entered should follow the format {panel}_v{n} where panel is the name of panel and n is the version number of the panel. Example: imx_v1. Refer to the panel tube for panel version information.
P Protein Add-on	For G4X multiomic runs with protein add-on panel: Name the Custom Target File for the protein add-on targets that were used to prepare the samples in this lane. The name specified here must match the .csv filename of the Custom Target File. Filename must be in all lowercase.

6. For each used tissue position, fill out the following information in the `Data` section. The tissue layout should align with the template chosen.

Field	Description
Tissue Type	The type of tissue for this section. Required for all sample locations with tissue. If this field is empty, all other fields for this sample location should be empty.
Block	The block that this tissue section was prepared from.

7. Name the sample sheet and save it.

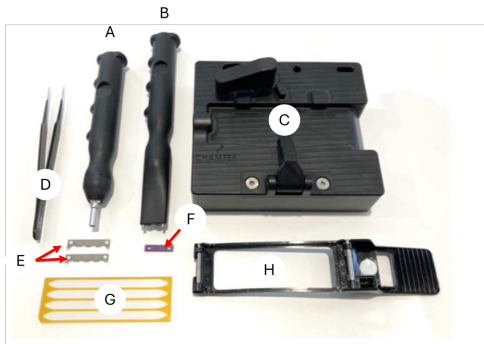
Prepare Consumables

Frame Flow Cell

This topic describes how to frame a spatial flow cell with tissue sections prepared for spatial sequencing as described in the *G4X Sample Preparation Guide*.

NOTE
Frame the flow cell right before the run start.

1. Obtain all parts of the G4X Flow Cell Framing Tool Suite and G4X Tooling Consumables Kit:

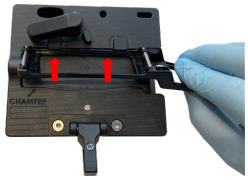


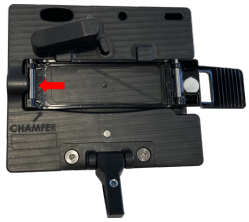
   A Tab press tool
   B Spacer installation tool
   C Flow cell framing base
   D Forceps (optional)
   E Flow cell tabs (2)
   F Flow cell spacer
   G Flow cell with tissue sections prepared for spatial sequencing
   H Flow cell frame

2. Open the latch on the flow cell framing base.

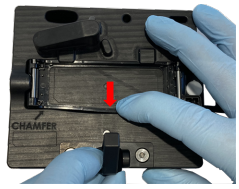
   NOTE
   Left-handed operators may flip the base 180° for easier handling.

3. Place the frame in the base with the bottom side facing up.
4. Ensure the frame slides under the framer ledge and then push in until the dowel pin is flush against the magnet.



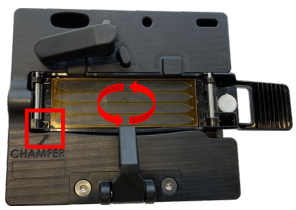


5. Flex the bowed edge of the frame and secure with the latch.



6. Remove any tape or stickers on the flow cell.
7. Write the Flow Cell ID onto tape or a sticker and attach it to the flow cell handle to easily identify the flow cell.

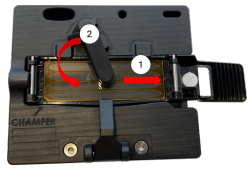
   NOTE
   The Flow Cell ID will not be visible once flow cell is framed.
8. Wipe the flow cell thoroughly with lint-free wipe and isopropyl alcohol.
9. Insert the flow cell into the frame, matching the chamfered corner of the flow cell to the text identifier. Place a finger on the center of the flow cell and push around in a circular pattern to ensure flow cell is able to shift freely in the open frame.



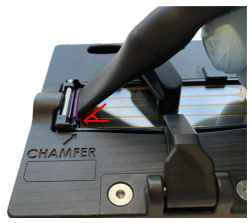
CRITICAL STEP
Proper flow cell placement in frame is vital before proceeding with spacer and tab installation.
If flow cell is not shifting freely in the frame, check if the latch is properly secure on the edge of the frame or if the flow cell is sitting on top of the notches within the frame.

NOTE
The chamfer is the 45-degree angled edge cut along the corner of the slide.

10. Bias flow cell towards frame handle and rotate retaining arm towards the flow cell.

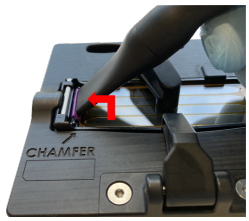


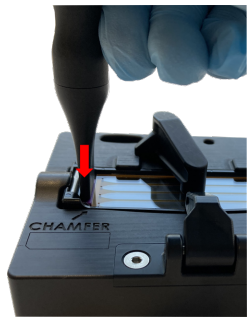
11. Pick up the spacer with the spacer installation tool.
12. Line up the spacer to the spacer pins at an angle of approximately 45°, ensuring the side of the spacer contacts the flow cell edge.



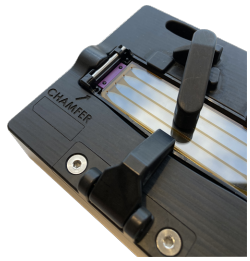
13. Rotate the installation tool and press down until the spacer pins capture the spacer.

CRITICAL STEP
Very little force is required to transfer the spacer. Excess force could result in chipping or cracking of the flow cell.



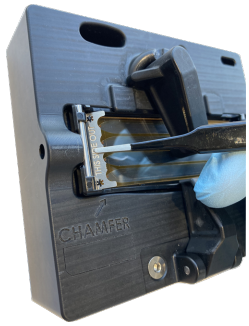


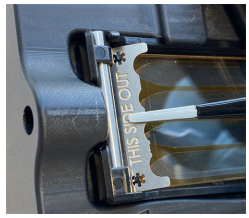
14. Remove the spacer installation tool, ensure that the spacer sits flat inside the frame.



15. Place the flow cell tabs over the frame retention pins. The pins should be visible through the star-pattern of the tab, and the side engraved with 'THIS SIDE OUT' should be facing up and visible.

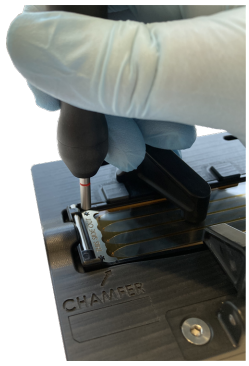
    Optional: If preferred, use forceps for handling the tabs.





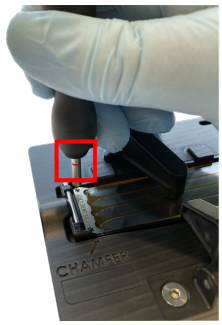
16. Align the tip of the press tool over a retention pin.

    NOTE
    Ensure the flow cell tab does not shift when aligning the press tool over the pin. Both pins should be visible through the star-pattern of the tab.



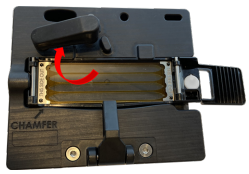
17. Apply slow and steady downward force until:
    - The red line of the press tool is covered, **OR**
    - The red band at the top of the press tool appears

    CRITICAL STEP
    Very little force is required to secure the tab. Ensure that the red line is covered to apply consistent force across all four pins.

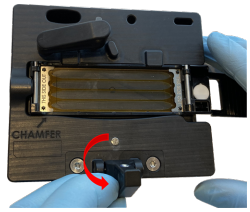


18. Inspect the tab and press again if the tab is not seated flush against flow cell.
19. Repeat 17-18 for all remaining retention pins.

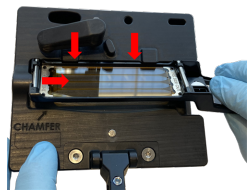
20. Rotate retaining arm off of the flow cell.



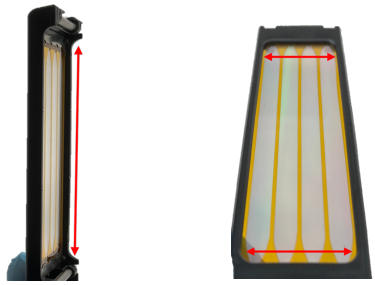
21. Remove the latch from the base.



22. Pull the frame away from the magnet then angle the frame away from the ledge to remove from the base.



23. Inspect that the flow cell is sitting flat in the frame and that the flow cell tabs are flush against the flow cell.



Prepare Reagents

Prepare one Spatial Reagent Cartridge for each flow cell you plan to use for the run. You can either thaw a cartridge for 2 days in the refrigerator, or in a water bath at 20°C-22°C for 2.5 hours.

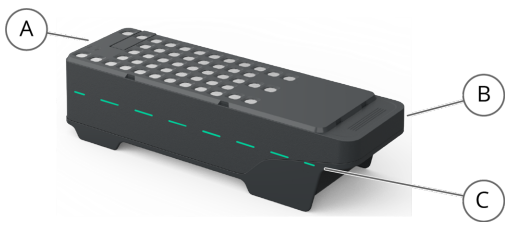
Option 1: Thaw in a Refrigerator

1. Remove reagent cartridges from -15°C to -25°C storage and thaw at 2°C to 8°C for 2 days.
2. After thawing is complete, remove the reagent cartridges from 2°C to 8°C storage and equilibrate to room temperature for 30 minutes.

Option 2: Thaw in a Water Bath

3. Remove reagent cartridges from -15°C to -25°C storage. Place the reagent cartridge in a temperature-controlled water bath (20°C-22°C) containing enough deionized water to submerge the base of the reagent cartridge. If the water bath is not temperature-controlled, thawing time may vary.

   NOTE
   Do not allow the water to exceed the lip of the handle or come into contact with the Product Identifier integrated identification chip as shown below:



A Product Identifier location
B Handle
C Maximum water level

4. Let reagents thaw for 2.5 hours.
5. Remove the cartridge from the water bath by lifting from the handle at an angle to minimize retention of water in the underside of the cartridge. Wipe water off the cartridge, and ensure that there is no moisture on the Product Identifier.
6. For best results, proceed directly to loading the sample and setting up the run. Alternatively, store the reagent cartridge at 2°C to 8°C until ready to set up the run.

The reagent cartridge can be kept at 2°C to 8°C for up to 1 week; make sure to equilibrate to room temperature for 30 minutes before use, and mix well.

NOTE
Just before loading the reagent cartridge onto the instrument, you need to mix the reagents as described in *Load Reagent Cartridges* on page 37.

Set Up a Run

You can set up a run in different ways. The major differences in run setup are listed below:

- You can load an existing run, see *Load an Existing Run* on page 33.
- You can set up a new run, see *Set Up a New Run* on page 28. When setting up a new run, you can define sample data in different ways:
  - Upload a sample sheet before the run
  - Define the samples manually while setting up the run

The main run setup options for the G4X Spatial Sequencer are illustrated in Figure 15.

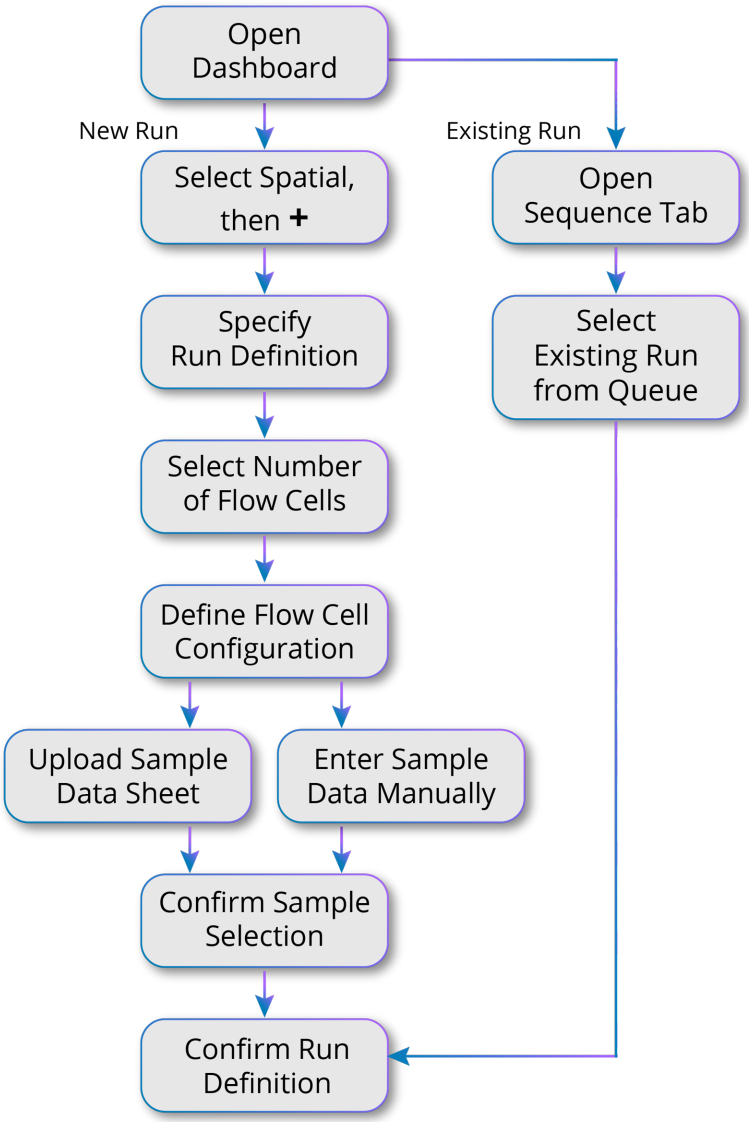


Figure 15 Main run setup options for the G4X Spatial Sequencer.

Set Up a New Run

To specify parameters for a new run, use the Sequencing Operating System. Tap the Singular Genomics icon on the instrument touchscreen to show the main menu.

1. Select **Dashboard** from the main menu.
2. On the Sequence window, select **Spatial**.
3. Select **+** to open the New Run screen.

Specify Run Definition

1. Enter the run name.
2. Set up run type:
   - T a. For a transcriptomic only run, select **Standard** under Transcriptomics and **None** under Proteomics.
   - P b. For a multiomic run, select **Standard** under Transcriptomics and **Standard** under Proteomics.
3. If custom panels or add-on panels are used:
   c. Select the **+Add-on Targets** button under Transcriptomics and/or Proteomics.
   d. Select the Custom Target File to be uploaded. This should be provided along with the custom or add-on panel.
4. Select the **forward arrow** → to proceed.

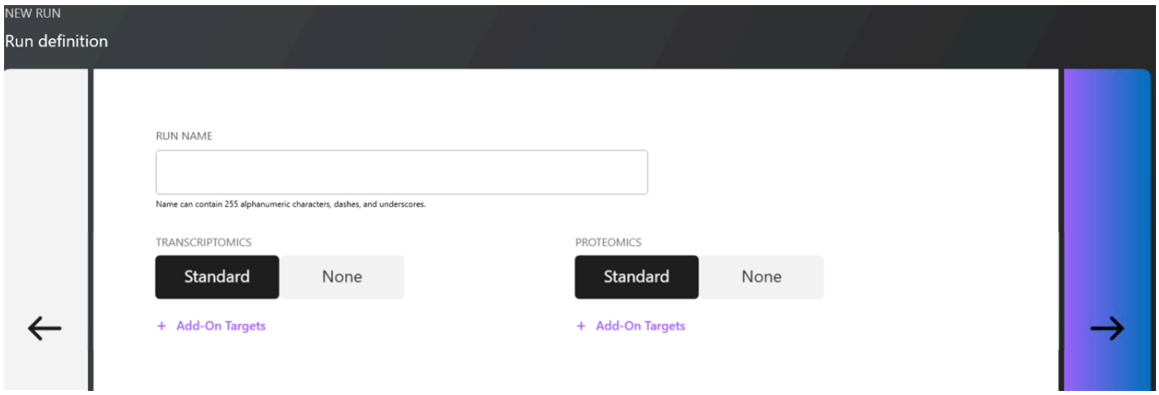


Figure 16 Run Definition Screen

Select Number of Flow Cells to Run

1. Select the flow cell positions that will be used for the run.

   NOTE
   If you frequently perform runs with fewer than 4 flow cells, alternate between the flow cell positions you use. Do not always use the same flow cell positions while leaving other positions always unused.

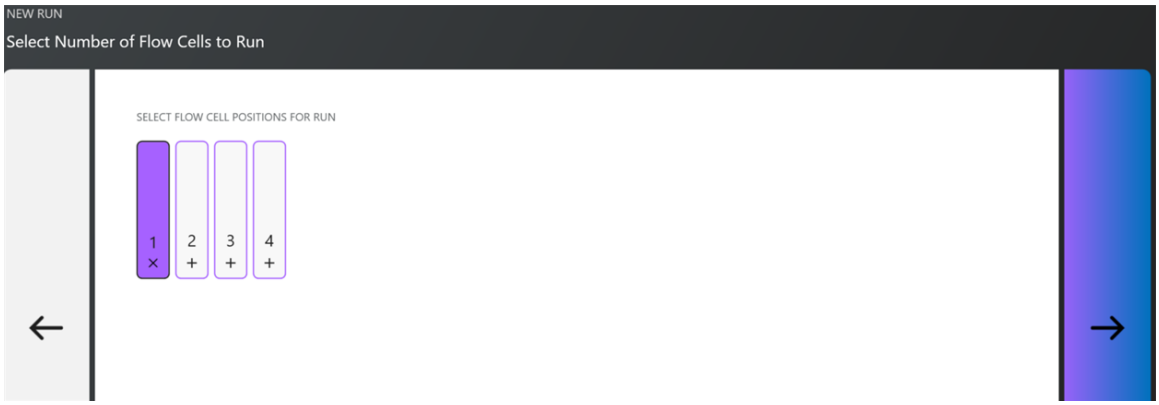


Figure 17 Select Number of Flow Cells to Run Screen

Define Flow Cell Configuration

1. Select the flow cell type for each position used.
2. Select the **forward arrow** → to proceed.

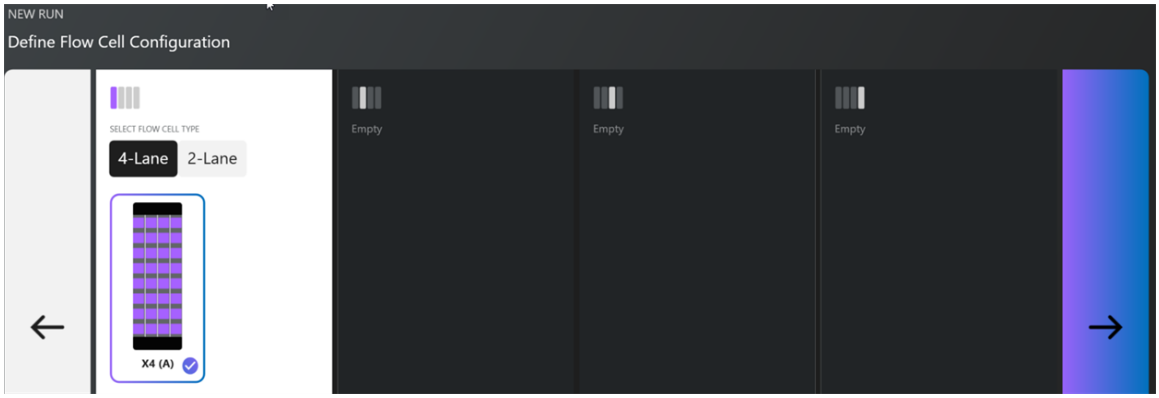


Figure 18 Define Flow Cell Configuration Screen

Upload Sample Data by Flow Cell

The sample sheet stores information about the sample sections and layout of the flow cell to be sequenced (for more information, see *G4X Sample Sheet* on page 13). Assign a sample sheet to a flow cell to provide data for the samples on each lane.

NOTE
If you want to manually define samples without using a sample sheet, select **Add Samples Manually** and refer to *Add Sample Data Manually* on page 30 instead.

1. Check if the sample sheet you want to upload is auto-populated on the screen. If the sample sheet is not listed, do the following:
   - Check if the path under **Folder** on top of the screen is pointing to the folder with the sample sheet. If not, select the path and browse to the folder where the sample sheet is saved. Sample sheets copied from the network storage will be available at *C:\sample-sheets* on the instrument.
   - If you cannot find the sample sheet containing folder, make sure the instrument has access to the folder. If not, either provide permission for the instrument, or move the sample sheet to a folder the instrument has access to.
   - If you have not created a sample sheet for your flow cell yet, see *Create a G4X Sample Sheet* on page 18.
   - To search for the sample sheet in the folder, click on the magnifying glass icon above the table headers to search for the specific sample sheet.
2. For the sample sheet you want to upload, select the flow cell that corresponds to the sample data provided in the sample sheet. Each flow cell requires its own sample sheet.
3. Repeat steps 1-2 for all sample sheets you want to upload.
4. Select the **forward arrow** → to proceed.

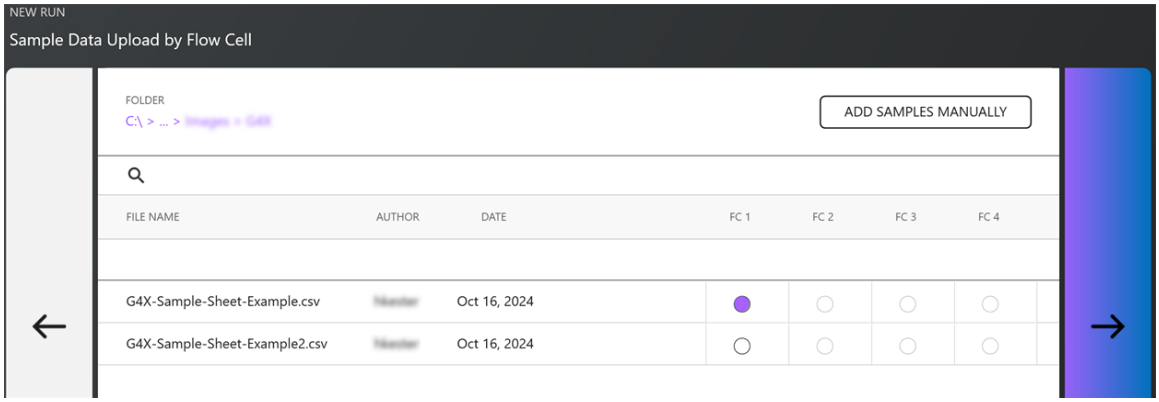


Figure 19 Sample Data Upload by Flow Cell Screen

Add Sample Data Manually

You can also manually enter information about the sample sections and layout of the flow cell to be sequenced.

NOTE
If you want to use a sample sheet to define samples, select **Upload Using Sample Sheet** and refer to *Upload Sample Data by Flow Cell* on page 29 instead.

1. Select the flow cell you want enter sample data for using the chevrons (‹ ›) on top of the screen.
2. Select the sample position you want to assign sample data to (see Figure 20 for an explanation of the sample position numbering).

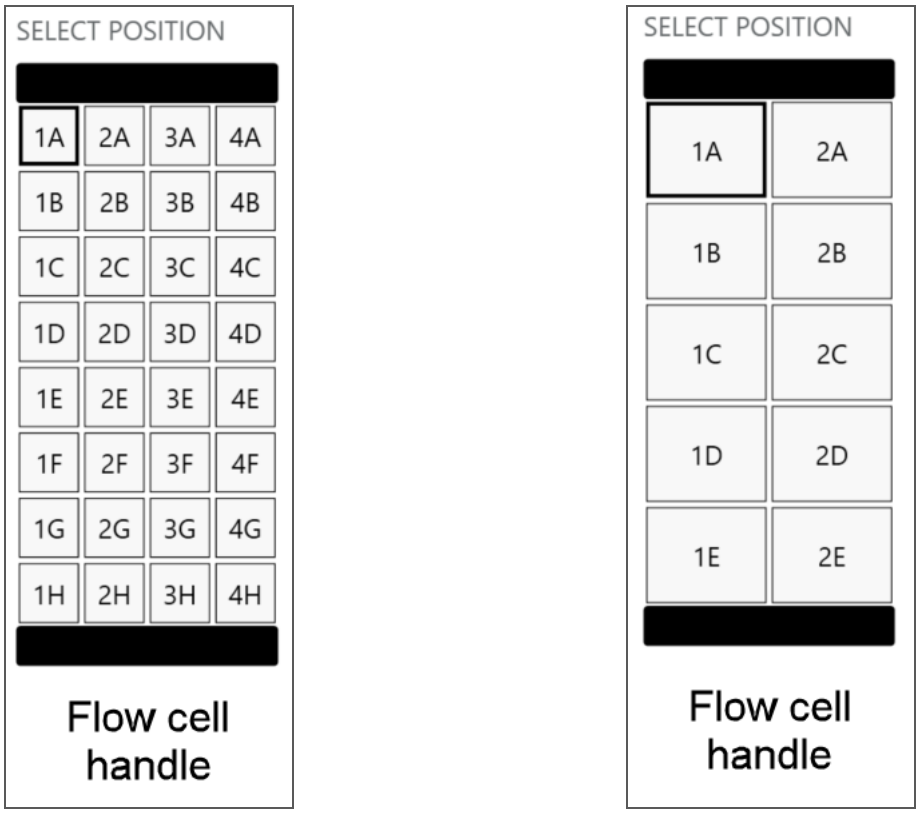


Figure 20 Sample position numbering for 4-lane flow cell (left) and 2-lane flow cell (right). The flow cell is oriented top-down, with the handle positioned at the bottom.

3. Select **+ Add Sample**.
4. Define the sample:
   - Panel (required). All samples in a lane need to have the same panel assigned. If you select a different panel, that panel selection is copied to all other samples in that lane.
   - Tissue type (optional).
   - Block (optional).
   - Add-ons (only needed if using add-on targets). Only allows selection of add-on targets of Custom Target File that was already uploaded in *Specify Run Definition* on page 28.
5. If there are more samples with the same sample data, do the following:
   a. Select **Copy Sample**.
   b. Select the sample position you want to copy the sample data to.
   c. Select **Paste**.
   d. Repeat steps b-c until you have copied the content to all sample positions that need the same sample data.
6. Repeat steps 1-5 until all samples that need sample data manually assigned have been set up.
7. Select the **forward arrow** → to proceed.

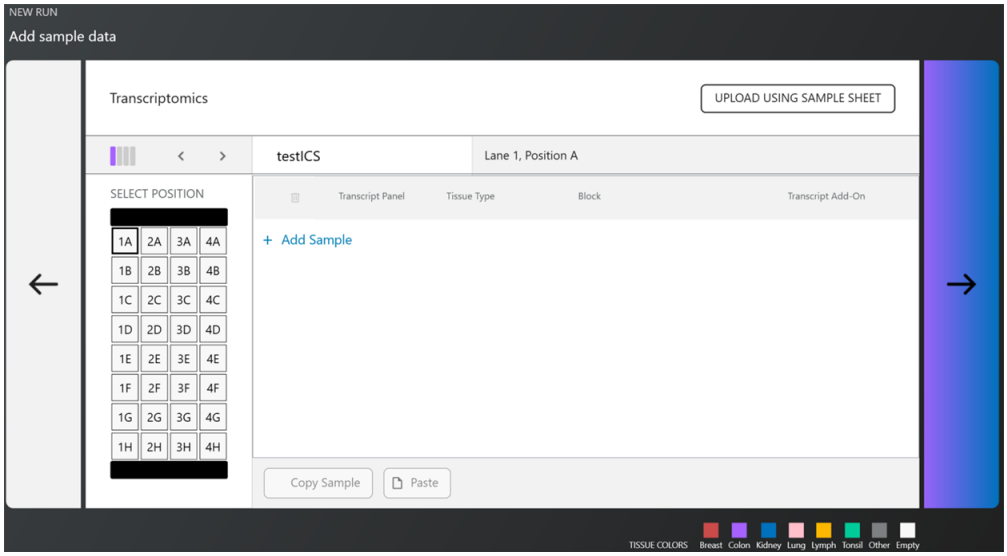


Figure 21 Add Sample Data Manually Screen

Confirm Sample Selection

The Confirm Sample Selection screen allows you to check whether the sample data is correct.

1. Select a sample. The tissue, block, and panel for that sample will appear below the flow cell.
2. Check the sample data. If something is wrong, select the back arrow to return to correct the error.
3. Repeat steps 1-2 until all samples have been checked.
4. Select the **forward arrow** → to proceed.

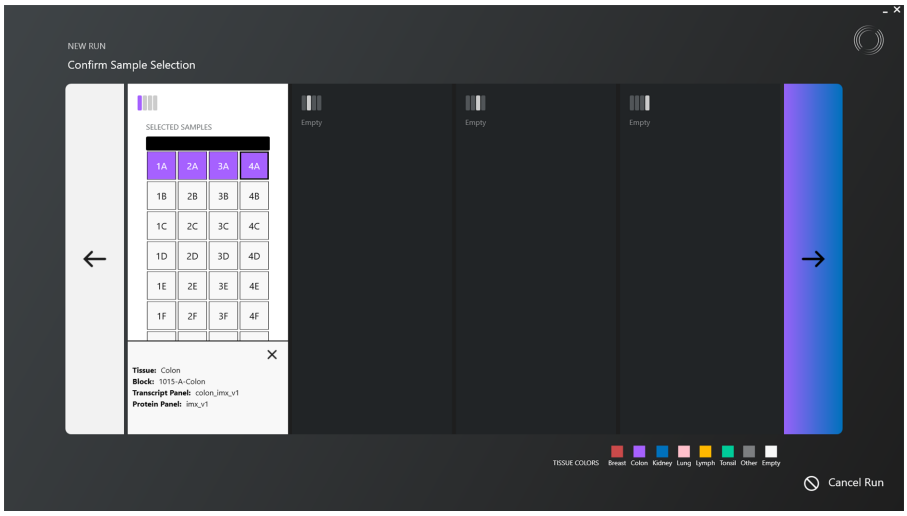


Figure 22 Confirm Sample Selection Screen

Confirm Run Definition

The Confirm Run Definition screen allows you to check whether the run definition is correct.

1. Check the run definition and flow cell settings. If something is wrong, select the back arrow to return to correct the error.

2. Select the **forward arrow** → to proceed.

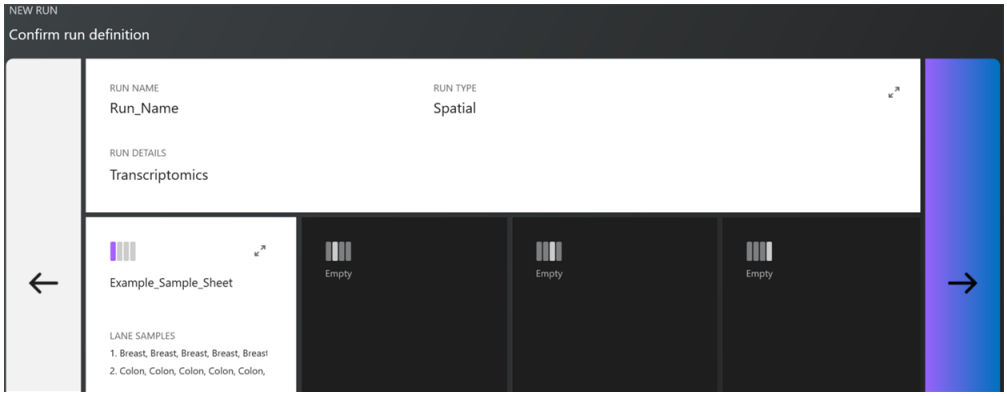


Figure 23 Confirm Run Definition Screen

Load an Existing Run

To start a run, you can load an existing run that has been created before on the instrument.

1. Open the Sequencing Operating System from the instrument touchscreen monitor.
2. Tap the Singular Genomics icon to show the main menu and select **Dashboard**.
3. Scroll through the available sequencing runs and select the run you are ready to start.
4. Review parameters specified for the run.
5. If you need to make changes to the sample data information or upload a new sample sheet, select the **expand** ↙↗ icon, then select the sample sheet you want to change. Select **Save** when finished.
6. Select the **forward arrow** → to proceed. The instrument automatically prepares for loading.

> Do not reload a run that has started system checks. In that case, generate a new run.

Load Consumables

Follow the on-screen prompts to load consumables. Always empty the internal waste reservoir before each run.

> Avoid pinching your fingers when closing the doors or drawers of the consumable compartments.

Empty Waste Reservoir

The G4X Spatial Sequencer comes with an internal waste reservoir, but you can also order an external waste reservoir (Contact Singular Genomics, see *Customer Care* on page 66). The external waste reservoir has a larger capacity. For instructions on how to empty your waste reservoir, see one of the following topics:

- *Empty Internal Waste Reservoir* on page 34.
- *Empty External Waste Reservoir* on page 34.

Empty Internal Waste Reservoir

If the internal waste reservoir is not empty, SeqOS displays a warning during run set up and you need to empty it.

1. Open the buffer compartment drawer. Select the **forward arrow** → to proceed.
2. Carefully remove the waste reservoir and open the waste port without spilling.

- Waste reservoir may be full.
- Always wear gloves when handling waste.

3. Empty the contents into the appropriate hazardous waste container.
4. Reinstall the waste cap.
5. Using a dry low-particulate wipe, clean any spills on the waste reservoir and any liquid in the drawer.
6. Replace the empty waste reservoir in the buffer compartment.
7. Change gloves.
8. Select the **forward arrow** → to proceed.

FORMAMIDE WARNING

Be aware that this set of reagents contains formamide, a suspected carcinogen and reproductive toxin, and other potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations through a licensed hazardous waste contractor. Refer to SDS for more information.

Empty External Waste Reservoir

1. If the waste in the external waste container reaches the waste limit (

, around the 15 Liter mark), empty the waste container the following way.

   NOTE
   The waste is at least 15 L. Consider having a trained EH&S technician experienced in dealing with heavy waste containers perform the emptying procedure.

   a. Wear appropriate personal protective equipment for the hazard you are handling.

FORMAMIDE WARNING
Be aware that this set of reagents contains formamide, a suspected carcinogen and reproductive toxin, and other potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations through a licensed hazardous waste contractor. Refer to SDS for more information.

b. Push down on the metal ring at the waste tube port and pull out the waste tube connector.
c. Empty the waste container in the appropriate waste disposal according to local regulations and institute's guidelines.
d. Push down on the metal ring at the waste tube port and insert the waste tube connector.
e. Release the metal ring.
f. Push down on the waste tube connector to make sure it is properly inserted in the waste container.

Load Wash Cartridges

1. Remove used wash cartridges from the previous run. Dispose of used cartridges according to local laws and regulations.
2. Place the new wash cartridges in the corresponding positions the following way:
   a. Hold the wash cartridge by the handle at the midpoint.
   b. Place the wash cartridge into the slot with the far end (where the ports are) first.
   c. Pivot the side nearest to you down so the wash cartridge is fully seated.
   d. Select the **forward arrow** → to proceed.
3. Close the buffer compartment drawer. Select the **forward arrow** → to proceed.

NOTE
If the G4X Spatial Sequencer doesn't recognize a Product Identifier, select the **Manual Entry** button in the bottom left of the screen (▦). Fill out the requested product properties for the position, then select **Save**. See also *Product Identifier Not Recognized* on page 59 for more information.

Load Flow Cells

1. Open the flow cell compartment door. Select the **forward arrow** → to proceed.
2. Remove used flow cells from the previous run.

The flow cell stage may still be hot from the previous run. Be careful not to burn your fingers.

3. Carefully remove any seal tape covering the inlet and outlet ports.

4. Hold the spatial flow cell by the handle and remove any seal tape adhesive using a lint free optical wipe with ethanol or isopropanol, and clean the top glass surface gently. After cleaning, avoid touching the glass surface.
5. Ensure that the gaskets of each flow cell position are completely level and clean by running a gloved finger all the way around the blue perimeter gasket and over the two port gaskets at the front and rear. If a gasket is raised in an area, carefully press the gasket down in that area to make sure the gasket is entirely level.

   NOTE
   Raised gaskets at the far end of the flow cell position can be difficult to see. Running a gloved finger carefully at the rear makes it easier to detect. Do not use IPA to wipe the gasket area.
6. The flow cell positions are numbered 1 through 4, left to right, and the expected positions are illuminated in white (see Figure 5). In the positions selected in run parameters, place flow cells the following way:
   a. Carefully place the toe end of the flow cell (the side furthest from the handle) in first. The flow cell should click into place.
   b. Gently lower the heel end (handle end) of the flow cell into place. Do not push the flow cell down but allow the magnets to catch.
7. Enter flow cell ID:
   a. Select the **Manual Entry** button in the bottom left of the screen ( ) and navigate to the Flow Cell cartridge type.
   b. Flow cell type should be automatically read from the product identifier.
   c. Type in the Flow Cell ID.
8. Select the **forward arrow** → to proceed. The instrument software confirms that each flow cell is fully engaged. A prompt appears if reseating is necessary.

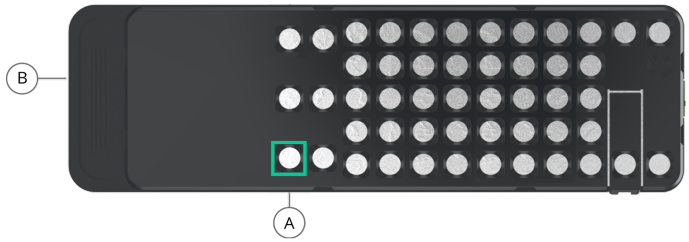
   CRITICAL STEP
   After loading the flow cell in the instrument, use gloved fingers to gently press down on the top corners of the flow cell. This helps the flow cell to come into full contact with the nest

9. If reseating is necessary: flow cells that are not engaged properly are illuminated in red. Take out the flow cell and reseat. If that doesn't work, see *Flow Cell Not Engaged* on page 60. Select the **forward arrow** → to proceed.
10. Close the flow cell compartment door. Select the **forward arrow** → to proceed.
11. Instrument will confirm that each flow cell is seated properly. A prompt appears if reseating is necessary.
12. If reseating is necessary: flow cells that are not seated properly are illuminated in red. Take out the flow cell and reseat.
13. Close the flow cell compartment door. Select the **forward arrow** → to proceed.

    NOTE
    For an explanation of the flow cell position light colors, see *Flow Cell Compartment* on page 5

Prepare Reagent Cartridge

1. Invert Spatial Reagent Cartridge 5-10 times while holding the lid on, then gently tap the cartridge on the bench surface. Wipe down any condensation that may have formed on the Spatial Reagent Cartridge. Gently pat the Product Identifier to dry it.
2. Place one tube of Cleave Buffer Additive per reagent cartridge in a room temperature water bath for 5 minutes.
3. Dry the tube and ensure the Cleave Buffer Additive has thawed completely. If necessary, briefly warm the tube with your hands. Invert the tube 10 times to mix.
4. Puncture the foil of the bottom right well.



A Well for Cleave Buffer Additive
B Handle

5. Add 500 µL Cleave Buffer Additive into the well. If needed, briefly spin down the content.
6. Mix the solution in the well 10 times with a 10 mL serological pipette, taking care to not introduce bubbles.
7. Dry up liquid on the exterior of the well and gently tap the cartridge on the table to release any bubbles.
8. Set aside until prompted to load reagent cartridges onto the G4X Spatial Sequencer.

Load Reagent Cartridges

1. Open the reagent compartment door. Select the **forward arrow** → to proceed.
2. Remove used reagent cartridges from the previous run.

> The reagent sippers are sharp. Be careful not to touch them, because you can injure your hands.

3. Holding the cartridge by the handle, guide the Spatial Reagent Cartridge into position. Ensure that each cartridge is fully positioned toward the rear of the compartment. Select the **forward arrow** → to proceed.

   NOTE
   If the G4X Spatial Sequencer doesn't recognize a Product Identifier, select the **Manual Entry** button in the bottom left of the screen (▦). Fill out the requested product properties for the position, then select **Save**. See also *Product Identifier Not Recognized* on page 59 for more information.

Disposal of Used Reagent Cartridges

1. Wearing protective gloves, lift the tab to open the hinged cover toward the rear of the reagent cartridge. Bend the cover back to expose the removable well.

2. Carefully lift the well from the reagent cartridge, holding it upright to avoid spilling through the pierced foil seal.
3. Dispose of the removable well as hazardous waste (see Figure 13 on page 15).
4. Empty the remaining contents of the reagent cartridge into the appropriate hazardous waste container.

FORMAMIDE WARNING
Be aware that this set of reagents contains formamide, a suspected carcinogen and reproductive toxin, and other potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations through a licensed hazardous waste contractor. Refer to SDS for more information.

Start the Run

1. Confirm the run parameters. The software checks the run parameters. If there are incompatible or missing settings, the software returns to the Run Parameters screen. Add or correct the run data.
2. Select the **forward arrow** → to proceed. The instrument initiates a pre-run system check, which takes approximately 5–10 minutes.
3. If any error messages appear, resolve the issue using the on-screen instructions before continuing. If you get a flow rate error, see *Flow Rate Check Failed* on page 61.
4. Hit **Start** to proceed after the system check completes and all errors are resolved.

   NOTE
   If you get warned for insufficient onboard disk space, you may need to remove older data to create enough free onboard disk space for the current run. See *Storage Maintenance* on page 54 for more information.
5. The screen will display an estimated time until the Confirm Tissue Alignment screen appears.
6. Once the Confirm Tissue Alignment screen appears, check whether all samples are properly aligned in the image, and whether the sample information on the screen is correct.

   Clicking on a sample will show the tissue, block, and panel assigned for that sample.

   If an image is not properly aligned, select the flow cell and align the image manually with the **Up** and **Down** buttons. Misalignment may result in loss of data.

Figure 24 Confirm Tissue Alignment screen

7. If there are any incorrect sample information:
   a. Wrong tissue type selected: On the Tissue Type drop-down, choose the correct tissue type.
   b. Wrong panel selected: On the panel drop down, choose the correct panel. If add-on or custom panel is used, you can only select the Custom Target File that was already uploaded through *Specify Run Definition* on page 28. Only one panel is allowed per lane.
   c. Empty section selected as sample: Select Remove Sample to remove sample information.
   d. Sample not selected: Select sample location and select Add New Sample and include all sample information.

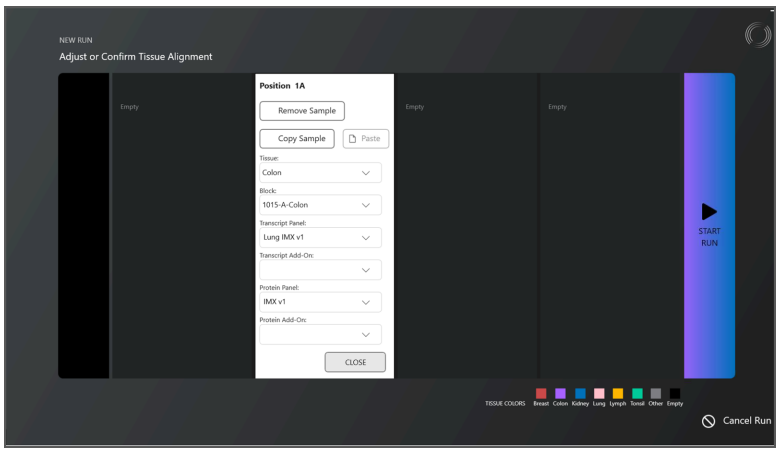


Figure 25 Example of information shown on screen when clicking on a sample

8. When the system check is successfully completed and the tissues are aligned, the run is ready to start. Select **Start Run** to start sequencing.

Avoid bumping the instrument or causing vibrations on the lab bench after starting the sequencing run.

Monitoring Analysis Progress

Analysis starts after all images are obtained. The following metrics are available for each sample on each lane of the flow cell:

Metric	Description
Total Cells Detected	Number of cells detected.
Median Decoded Transcripts/Cell	Median of transcripts decoded in each cell.
Median Unique Genes/Cell	Median of unique genes identified in each cell.
%High Quality Transcripts	Percentage of transcripts with quality scores greater than or equal to Q20.
Median Cell Size	Median cell size in μm^2.
%False Discovery Rate	Percentage of false discovery rates.

Metrics continue to change as analysis progresses. Final full run metrics are calculated when the analysis is complete (see *View Results* on page 44).

Post-Run Wash

An automatic post-run wash begins after the last sequencing cycle completes. The post-run wash cleans the fluidics lines and removes any contaminants from the previous run using wash reagents. The post-run wash takes approximately 35 minutes.

NOTE
Empty the waste reservoir if the instrument will be idle for longer periods, or if the waste starts developing unpleasant odors. See *Empty Waste Reservoir* on page 53.

Run Transfer Completed

Run data is automatically transferred from the G4X Spatial Sequencer to the SCS, and from there to your off-instrument storage, as described in *Off-Instrument Storage* on page 8. To see if the data transfer to your off-instrument storage is complete, look for the transfer_complete file in the run folder in your off-instrument storage.

DATA ANALYSIS AND OUTPUT FILES

Data analysis begins during the sequencing run and continues beyond completion of the last sequencing cycle. The Results screen indicates when analysis is complete. For more information about the outputs, refer to documentation of the G4X output at https://docs.singulargenomics.com/g4x_data/g4x_output/.

G4X Output Folder

The G4X folder has a structure as shown in Figure 26 below. An aggregated report is generated at each level.

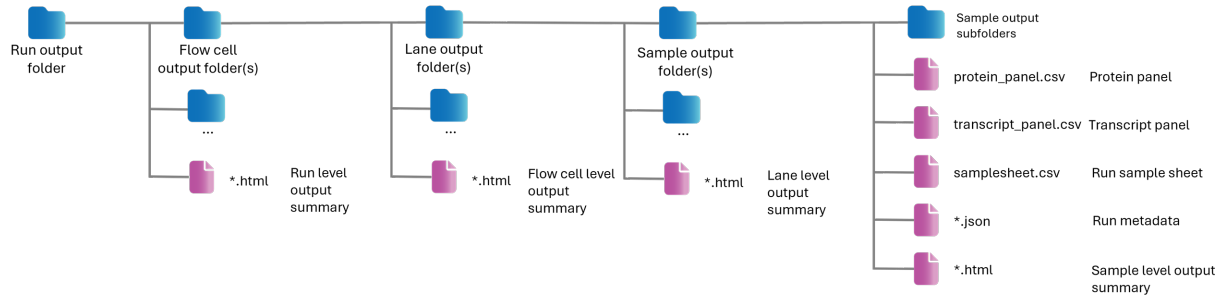


Figure 26 G4X Spatial Sequencer Output Folder Structure

Table 1 Description of the G4X Spatial Sequencer output files

Data File	Type	Folder	Description
Run level output summary	.html	Run output folder	Summarizes metrics output of samples in the run.
Flow cell level output summary	.html	Flow cell output folder (s)	Summarizes metrics output of samples in the flow cell.
Lane level output summary	.html	Lane output folder	Summarizes metrics output of samples in the lane.
Run metadata	.json	Sample output folder	Json file containing information about the run (instrument, run number, panel, time, version).
Run sample sheet	.csv	Sample output folder	Sample sheet of the run.
Protein panel	.csv	Sample output folder	Lists all targeted protein.
Transcript panel	.csv	Sample output folder	Lists all targeted transcripts.
Output summary	.html	Sample output folder	Summarizes metrics output of the sample.

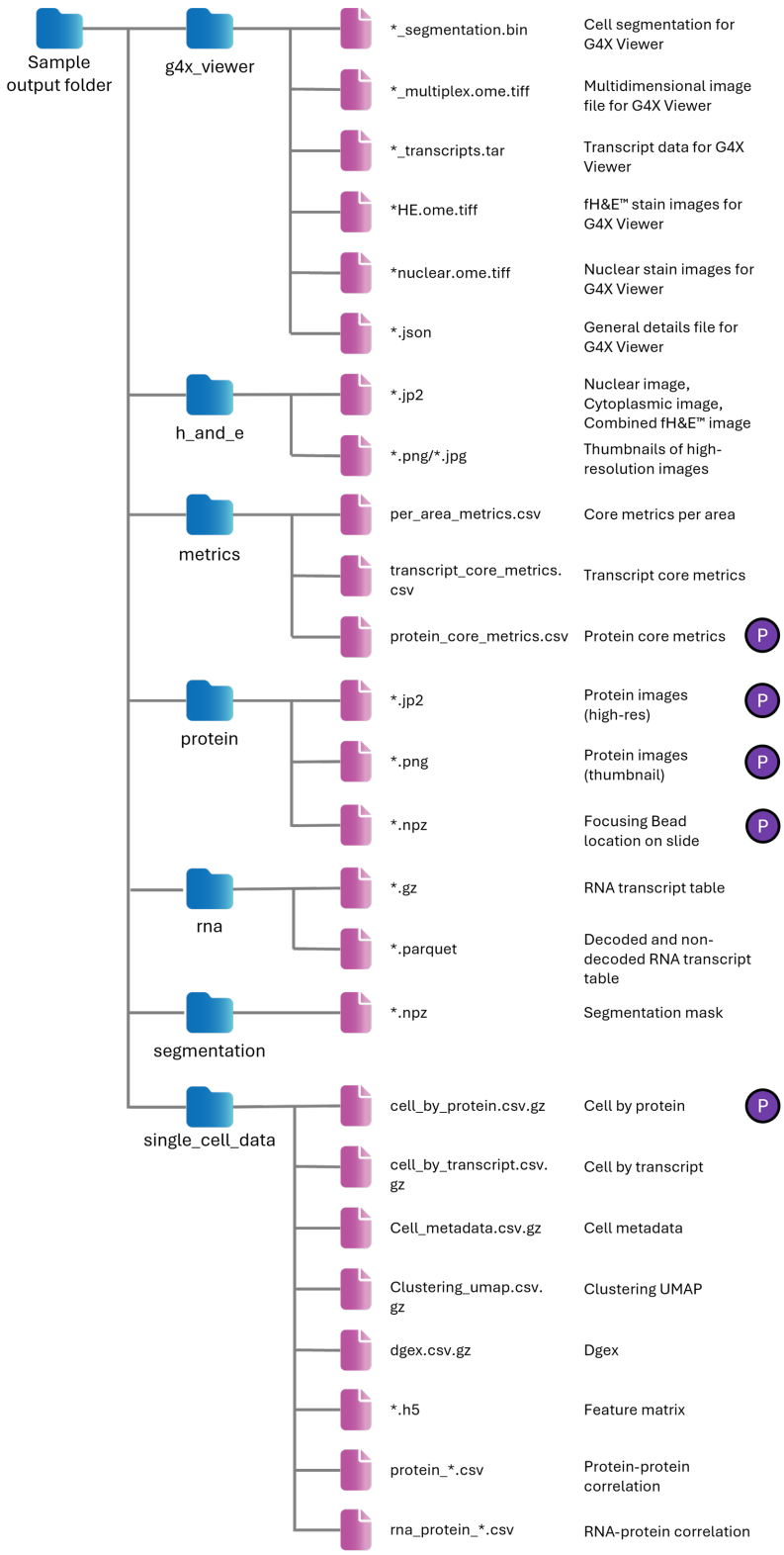


Figure 27 G4X Spatial Sequencer Sample Output Subfolder structure

Table 2 Description of the G4X Spatial Sequencer Sample output files

Data File	Type	Folder	Description
Cell segmentation for G4X Viewer	.bin	g4x_viewer	Contains cell segmentation information. Input to G4X Viewer.
Multidimensional image file for G4X Viewer	<sample>.ome.tiff	g4x_viewer	Contains multidimensional OME-TIFF image file. Input to G4X Viewer.
Transcript data for G4X Viewer	.tar	g4x_viewer	Contains transcript and metadata. Input to G4X Viewer.
fH&E™ stain images for G4X Viewer	<sample>_ HE.ome.tiff	g4x_viewer	Contains transformed fH&E™ image from run. Input to G4X Viewer.
Nuclear stain images for G4X Viewer	<sample>_ nuclear.ome.tiff	g4x_viewer	Contains combined cytoplasmic and nuclear images from run. Input to G4X Viewer.
General details file for G4X Viewer	<sample>_run_ metadata.json	g4x_viewer	Contains run information and extra core metrics information.
Nuclear image	.jp2	h_and_e	High resolution image for nuclear stain in JP2 encoding format.
Cytoplasmic image	.jp2	h_and_e	High resolution image for cytoplasmic stain in JP2 encoding format.
Combined fH&E™ image	.jp2	h_and_e	High resolution combined image for nuclear and cytoplasmic stain in JP2 encoding format.
Thumbnails of high-resolution images	.png/.jpg	h_and_e	Smaller thumbnail for each of the nuclear, cytoplasmic, and combined nuclear and cytoplasmic images.
Core metrics per area	.csv	metrics	Contains per-area metrics for the sample, separated into images before images are stitched to a full sample image
Transcript core metrics	.csv	metrics	Contains core transcript metrics for the sample
Protein core metrics	.csv	metrics	Contains core protein metrics for the sample
Protein images (high-res)	.jp2	protein	Single high resolution image per protein.
Protein images (thumbnail)	.png/.jpg	protein	Single thumbnail per high resolution image.
RNA transcript table	.csv.gz	rna	X/Y/Z coordinates, assigned gene name, confidence score, and assigned cell id for each transcript.
Decoded and non-decoded RNA transcript table	.parquet	rna	Contains all decoded and non-decoded transcripts and associated metadata.
Segmentation mask	.npz	segmentation	The pixel segmentation mask in compressed numpy array format.

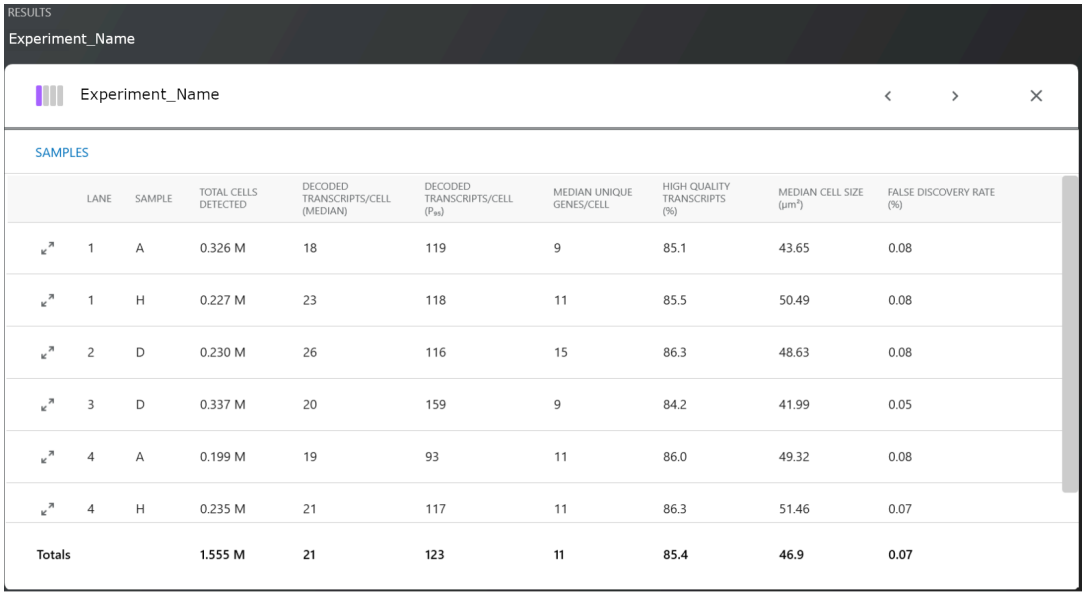
Data File	Type	Folder	Description
Cell by protein	.csv.gz	single_cell_data	Matrix of median protein intensity per cell.
Cell by transcript	.csv.gz	single_cell_data	Matrix of genes per cell.
Cell metadata	.csv.gz	single_cell_data	Spatial coordinates, cell area, and other metrics per cell.
ClusteringUMAP	csv.gz	single_cell_data	Clustering and UMAP coordinates per cell. Several resolutions.
Dgex	csv.gz	single_cell_data	Differential gene expression for clusters described above.
Feature matrix	.h5	single_cell_data	All of the single-cell information in HDF5 format.
Protein-protein correlation	.csv	single_cell_data	Data on protein-protein correlation.
RNA-protein correlation	.csv	single_cell_data	Data on RNA-protein correlation.

View Results

The Results screen provides access to metrics. A summary of metrics per flow cell are displayed on the flow cell tabs of the result screen.

Figure 28 Result page

Selecting a flow cell tab allows access to Results tabs with more detailed information. A summary for each sample on each lane will be shown.



	LANE	SAMPLE	TOTAL CELLS DETECTED	DECODED TRANSCRIPTS/CELL (MEDIAN)	DECODED TRANSCRIPTS/CELL (P_{95})	MEDIAN UNIQUE GENES/CELL	HIGH QUALITY TRANSCRIPTS (%)	MEDIAN CELL SIZE (μm^2)	FALSE DISCOVERY RATE (%)
	1	A	0.326 M	18	119	9	85.1	43.65	0.08
	1	H	0.227 M	23	118	11	85.5	50.49	0.08
	2	D	0.230 M	26	116	15	86.3	48.63	0.08
	3	D	0.337 M	20	159	9	84.2	41.99	0.05
	4	A	0.199 M	19	93	11	86.0	49.32	0.08
	4	H	0.235 M	21	117	11	86.3	51.46	0.07
Totals			**1.555 M**	**21**	**123**	**11**	**85.4**	**46.9**	**0.07**

Figure 29 Result summary of each sample in each lane of the flow cell

Selecting a sample shows the metrics for the selected sample, along with the fH&E™ image of the sample. If running a multiomic run, protein images are also available to view. To select a specific protein marker, pick the protein from the drop down menu.

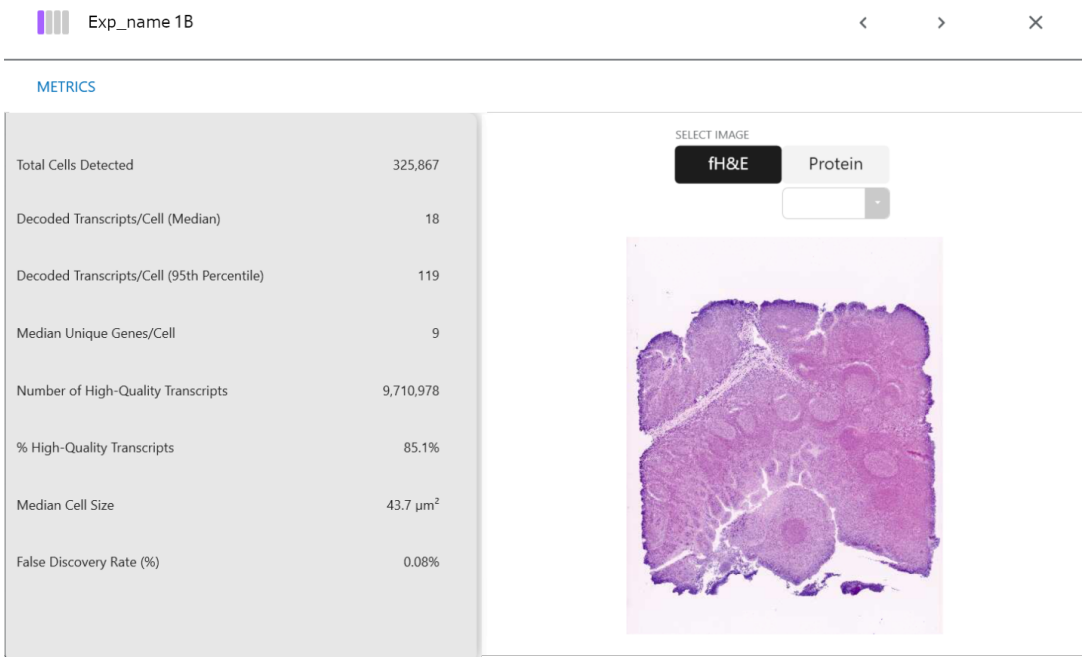


Figure 30 Detailed summary of sample metric

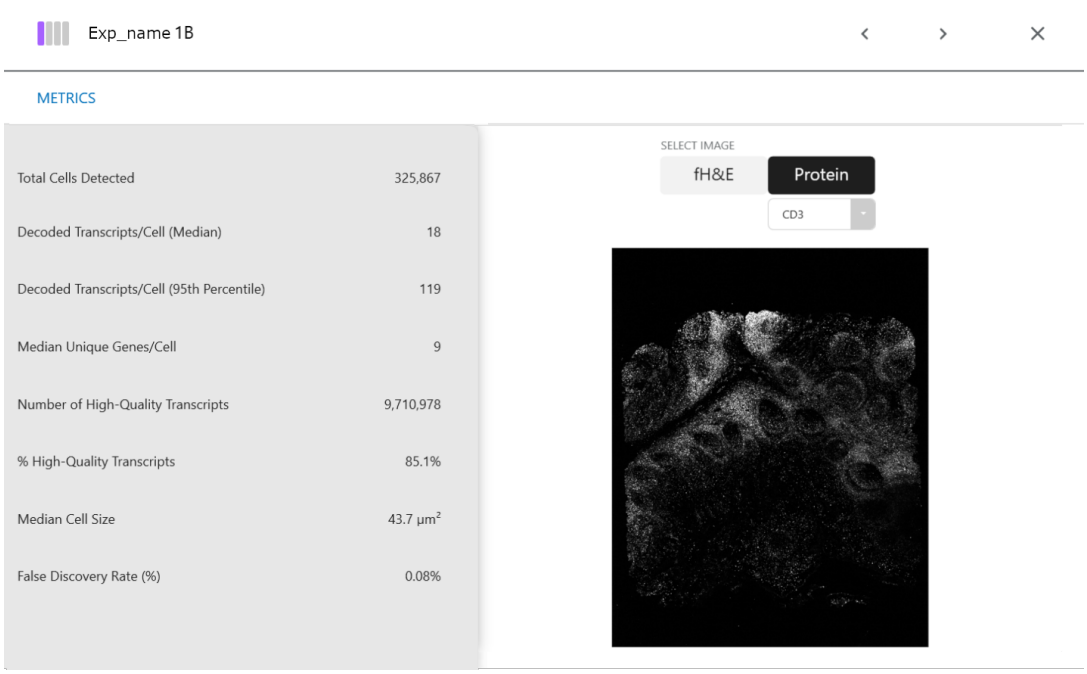


Figure 31 Protein image in sample metric summary

MAINTENANCE

In order to keep the G4X Spatial Sequencer in good working order, you need to perform the maintenance tasks listed below regularly.

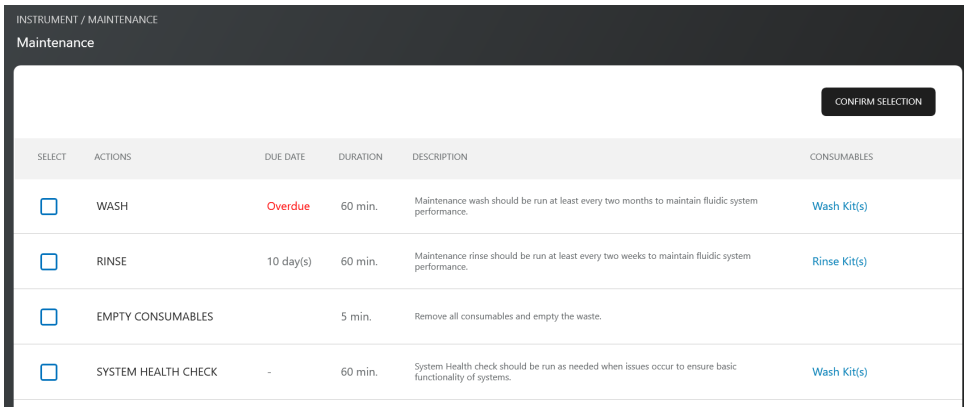


Figure 32 SeqOS Maintenance Screen. Selecting the Wash Kit(s) or Rinse Kit(s) links provides access to guides for loading the reagents.

Fluidics Maintenance

The SeqOS keeps track of the maintenance washes and rinses, and provides due dates and reminders for the next wash or rinse. There are other occasions you should perform fluidics maintenance. These occasions are listed below.

Occasion	Action
Whenever recommended by the SeqOS	Maintenance wash or rinse
2 weeks after last maintenance wash or rinse for each position	Maintenance wash or rinse
2 months after last maintenance wash for each position	Maintenance wash
If a run fails on a position	Maintenance wash or rinse for that position
Instrument or position will be idle for 1 week to 1 month	After the last run before the idle period: • Maintenance rinse • Empty waste reservoir • Remove empty consumables
Position has been idle for 1 week or more	Before the first run after the idle period: • Maintenance wash
Instrument or position will be idle for more than 1 month	• Empty waste reservoir • Remove empty consumables • Contact *Customer Care* to schedule a service appointment for draining your system

Maintenance Wash

Maintenance washes and rinses are very similar, but a maintenance wash uses an NaOH solution in certain cartridge well rows (see below), while a maintenance rinse uses water in those well rows. The timing of maintenance washes is described in *Maintenance* on page 47

Consumables & Equipment

- Milli-Q water (or better).
- Reagent-grade (or better) 5% sodium hypochlorite (NaOCl), for example: https://www.sigmaaldrich.com/US/en/product/sigald/239305.
- 1 M NaOH stock solution.
- G4 Maintenance Kit (part number 700002), which has the following components:

Item	Description	Quantity
Maintenance Sample Cartridge	Empty sample cartridge (reusable)	4
Maintenance Reagent Cartridge	Empty maintenance reagent cartridge (reusable)	4
Maintenance Wash Cartridge	Empty wash cartridge (reusable)	4
Maintenance Flow Cell	Maintenance flow cell (reusable)	4

- Instead of the Maintenance Flow Cell, you can also use a regular used NGS flow cell for each position that needs a maintenance wash or rinse.

Perform a Maintenance Wash

To initiate a maintenance wash from the SeqOS prompt, do the following :

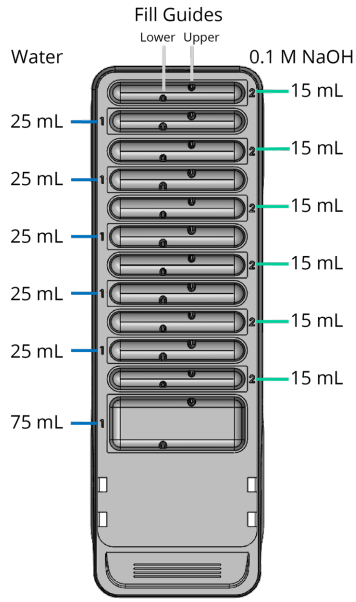
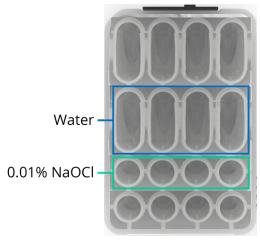
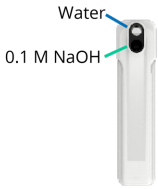
1. For each position you want to wash, make fresh:
   a. 2 mL 0.01% sodium hypochlorite by diluting sodium hypochlorite stock solution in water.
   b. 200 mL 0.1 M NaOH by diluting 20 mL of 1 M NaOH stock solution in 180 mL water.

   If you are washing multiple positions, multiply volumes by the number of positions.
2. Rinse all wells of the maintenance cartridges with Milli-Q water to remove any dust or particulates that may have accumulated in the cartridges.

CRITICAL STEP
Use of dirty maintenance cartridges may result in clogs in the system

3. Fill the maintenance cartridges with the following reagents:

   NOTE
   The Maintenance Reagent Cartridge has lower and upper fill guides in every well. As long as you fill the well above the lower fill guide and below the upper fill guide, there is enough volume for the maintenance wash.

Cartridge	Location (counted from top of picture)	Wash Reagents	Volume	Image
Maintenance Reagent Cartridge	Rows 2, 4, 6, 8, 10 Rows marked 1	Water	In between fill guides (25 mL)	
	Rows 1, 3, 5, 7, 9, 11 Rows marked 2	0.1 M NaOH	In between fill guides (15 mL)	
	Row 12 Row marked 1	Water	In between fill guides (75 mL)	
Maintenance Sample Cartridge	All wells in row 2	Water	1.2 mL per well	
	All wells in row 3	0.01% sodium hypochlorite	0.4 mL per well	
Maintenance Wash Cartridge	Top (bulk) compartment	Water	200 mL	
	Small rinse well	0.1 M NaOH	9 mL	
Maintenance Flow Cell*				

* A regular used NGS Flow Cell is also acceptable

4. Start the wash protocol using one of the following methods:
   - Select the **Start Wash** in the SeqOS prompt.
   - Go to the **Instrument | Maintenance** section in the Sequencing Operating System and select **Wash**.
5. Unselect the positions you do not want to wash (see Figure 33). By default all positions are selected.

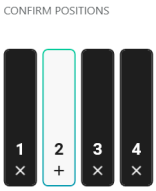


Figure 33 Confirming maintenance wash positions with positions 1, 3, and 4 selected, and position 2 unselected.

6. Load the consumables as prompted.

The flow cell stage may still be hot from the previous run. Be careful not to injure your hands.

The reagent sippers are sharp. Be careful not to touch them, because you can injure your hands.

Be careful when loading consumables to avoid spilling, because the cartridges are not sealed. If you do spill buffer, promptly clean up with a dry low-particulate wipe before proceeding the system health check.
Make sure to always wear gloves when handling reagents, waste, or spills.

FORMAMIDE WARNING
Be aware that waste contains formamide, a suspected carcinogen and reproductive toxin, and other potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations through a licensed hazardous waste contractor.

7. Follow the instructions in the Sequencing Operating System. For an explanation of the flow cell position light colors, see *Flow Cell Compartment* on page 5
8. If you performed a maintenance wash and the instrument or position will not be used for 1 week or more, perform a maintenance rinse.
9. After the maintenance wash has finished, rinse all maintenance cartridges the following way:
    a. Rinse all wells in the maintenance cartridges with Milli-Q water. Make sure no NaOH or sodium hypochlorite remains in the wells.
    b. Put the maintenance cartridges upside down and let dry in the air.
    c. Store the maintenance cartridges in a closed dust free box when dry and not in use.

CRITICAL STEP
Failure to clean and correctly store maintenance cartridges can result in fluidic system damage to the G4X Spatial Sequencer.

Maintenance Rinse

Maintenance washes and rinses are very similar, but a maintenance rinse uses water in certain cartridge well rows (see below), while a maintenance wash uses an NaOH solution in those well rows. The timing of maintenance rinses is described in *Maintenance* on page 47

Consumables & Equipment

- Milli-Q water (or better).
- G4 Maintenance Kit (part number 700002), which has the following components:

Item	Description	Quantity
Maintenance Sample Cartridge	Empty sample cartridge (reusable)	4
Maintenance Reagent Cartridge	Empty maintenance reagent cartridge (reusable)	4
Maintenance Wash Cartridge	Empty wash cartridge (reusable)	4
Maintenance Flow Cell	Maintenance flow cell (reusable)	4

- Instead of the Maintenance Flow Cell, you can also use a regular used NGS flow cell for each position that needs a maintenance wash or rinse.

Perform a Maintenance Rinse

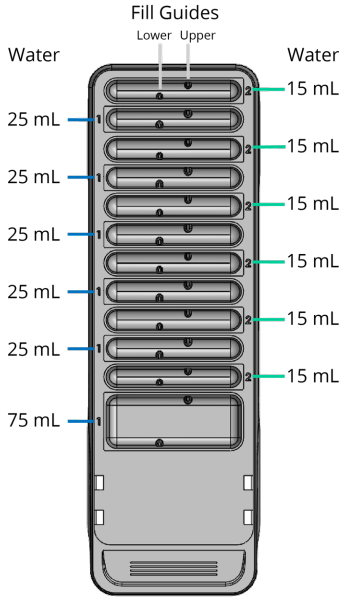
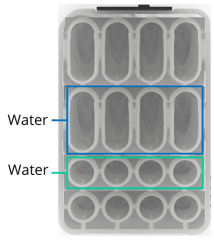
To initiate a maintenance rinse from the SeqOS prompt, do the following :

1. Rinse all wells of the maintenance cartridges with water to remove any dust or particulates that may have accumulated in the cartridges.

CRITICAL STEP
Use of dirty maintenance cartridges may result in clogs in the system

2. Fill the maintenance cartridges with the following reagents:

NOTE
The Maintenance Reagent Cartridge has lower and upper fill guides in every well. As long as you fill the well above the lower fill guide and below the upper fill guide, there is enough volume for the maintenance rinse.

Cartridge	Location (counted from top of picture)	Rinse Reagents	Volume	Image
Maintenance Reagent Cartridge	Rows 2, 4, 6, 8, 10 Rows marked 1	Water	In between fill guides (25 mL)	
	Rows 1, 3, 5, 7, 9, 11 Rows marked 2	Water	In between fill guides (15 mL)	
	Row 12 Row marked 1	Water	In between fill guides (75 mL)	
Maintenance Sample Cartridge	All wells in row 2	Water	1.2 mL per well	
	All wells in row 3	Water	0.4 mL per well	
Maintenance Wash Cartridge	Top (bulk) compartment	Water	200 mL	
	Small rinse well	Water	9 mL	
Maintenance Flow Cell*				

* A regular used NGS Flow Cell is also acceptable

3. Start the rinse protocol one of these two ways:
   - Select the **Start Rinse** in the SeqOS prompt.
   - Go to the **Instrument | Maintenance** section in the Sequencing Operating System and select **Rinse**.
4. Unselect the positions you do not want to rinse (see Figure 34). By default all positions are selected.

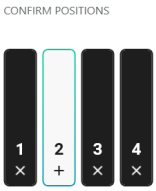


Figure 34 Confirming maintenance rinse positions with positions 1, 3, and 4 selected, and position 2 unselected.

5. Load the consumables as prompted.

The flow cell stage may still be hot from the previous run. Be careful not to injure your hands.

The reagent sippers are sharp. Be careful not to touch them, because you can injure your hands.

Be careful when loading consumables to avoid spilling, because the cartridges are not sealed. If you do spill buffer, promptly clean up with a dry low-particulate wipe before proceeding the system health check.
Make sure to always wear gloves when handling reagents, waste, or spills.

FORMAMIDE WARNING
Be aware that waste contains formamide, a suspected carcinogen and reproductive toxin, and other potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations through a licensed hazardous waste contractor.

6. Follow the instructions in the Sequencing Operating System. For an explanation of the flow cell position light colors, see *Flow Cell Compartment* on page 5
7. After the maintenance rinse has finished, rinse all maintenance cartridges the following way:
   a. Rinse all wells in the maintenance cartridges with Milli-Q water.
   b. Put the maintenance cartridges upside down and let dry in the air.
   c. Store the maintenance cartridges in a closed dust free box when dry and not in use.

CRITICAL STEP
Failure to clean and correctly store maintenance cartridges can result in fluidic system damage to the G4X Spatial Sequencer.

Empty Waste Reservoir

Empty the waste reservoir and consumables if the instrument will be idle for longer than a week, or if the waste starts developing unpleasant odors.

NOTE
This procedure is only applicable if you have an internal waste reservoir. If you have an external waste reservoir and the instrument will be idle for longer than a week or the waste develops

unpleasant odors, empty the waste as described in *Empty External Waste Reservoir* on page 34.

1. Go to the **Instrument | Maintenance** section in the Sequencing Operating System and select **Empty Consumables**.
2. Select **Confirm Selection**.
3. Remove consumables.
4. Open the buffer compartment drawer. Select the **forward arrow** → to proceed.
5. Carefully remove the waste reservoir and open the waste port without spilling.

- Waste reservoir may be full.
- Always wear gloves when handling waste.

6. Empty the contents into the appropriate hazardous waste container.
7. Reinstall the waste cap.
8. Using a dry low-particulate wipe, clean any spills on the waste reservoir and any liquid in the drawer.
9. Replace the empty waste reservoir in the buffer compartment.
10. Change gloves.
11. Select the **forward arrow** → to proceed.

FORMAMIDE WARNING

Be aware that this set of reagents contains formamide, a suspected carcinogen and reproductive toxin, and other potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations through a licensed hazardous waste contractor. Refer to SDS for more information.

Remove Empty Consumables

If the instrument will be idle for longer than a week, we recommend removing any remaining empty consumable cartridges the following way:

1. Go to the **Instrument | Maintenance** section in the Sequencing Operating System and select **Empty Consumables**.
2. Follow the instructions in the Sequencing Operating System.

Computer Maintenance

In order to keep the G4X Spatial Sequencer in good working order, you need to keep the instrument computer local storage clean and install instrument software updates soon after you are prompted.

Storage Maintenance

The G4X Spatial Sequencer can only contain a limited number of runs in local storage, depending on the size of the runs. It is also good practice to clean up the G4X local storage once the data has been moved to your off-instrument storage.

NOTE

The G4X Spatial Sequencer performs a final check for disk space at the pre-run checks before you start after consumables have been loaded.

If the instrument indicates that storage is getting full, you should also clear up space. You can get two types of warnings:

- If you get a yellow warning, the local storage has enough space for smaller runs.
- If you get a red warning, you should delete older data to make space for the current run before proceeding.
- Refer to this table to see how much space is required for each run

Estimated space needed per fully loaded flow cell for each run type

Flow cell type	Transcriptomic only run	Multiomic run
X2s	6.98 TB	7.72 TB
X2	17.44 TB	20.00 TB
X4	14.01TB	15.92 TB

Clean up the G4X local storage the following way:

1. Check if the run has been fully moved to your off-instrument storage location.
2. If your transcript panel was incorrectly specified, it is possible to repeat the demultiplexing with an updated panel.
3. Navigate to the Results Tab in SeqOS.
4. Select the More Options icon at the end of the line (•••) for the run that will be deleted.
5. Clean up storage using one of the following options
   - **Delete Images Only** to delete only images, but not the run outputs. When images are deleted, it is no longer possible to repeat analysis or demultiplexing.
   - **Delete** to delete run images along with the run outputs.

The Sequencing Operating System (SeqOS) will delete the run data for that run. Log files for the run will be retained for 30 days after the run finished.

Software Updates

In order to keep the G4X Spatial Sequencer in good working order, you need to periodically update software in the following time frames:

- SeqOS updates: generally, Singular Genomics manages the SeqOS updates. If you are prompted by the Sequencing Operating System to update, install soon after you get the prompt.
- Operating System and Security Updates: Updates are managed automatically by the SCS and/or through major updates performed by the G4X Spatial Sequencer. These are all managed by Singular Genomics.

Do not install Windows updates yourself, Singular Genomics manages the process. Also do not enable automatic Windows updates; updates during a run result in run failure.

- Driver updates: Alteration or changes to the system configuration, drivers, or other similar changes can directly affect reliability and performance of the G4X Spatial Sequencer. Contact Singular Genomics Customer Care if you have any questions.

Make sure your IT department is aware of and agrees with these guidelines. If there are concerns, check with Singular Genomics Customer Care.

System Health Check

If the G4X Spatial Sequencer is not performing as expected, Singular Genomics Customer Care may ask you to perform a system health check to assess its condition. Perform the system health check as described below, and send the log files to Singular Genomics Customer Care.

Consumables & Equipment

- Milli-Q water (or better).
- G4 Maintenance Kit (part number 700002), which has the following components:

Item	Description	Quantity
Maintenance Sample Cartridge	Empty sample cartridge (reusable)	4
Maintenance Reagent Cartridge	Empty maintenance reagent cartridge (reusable)	4
Maintenance Wash Cartridge	Empty wash cartridge (reusable)	4
Maintenance Flow Cell	Maintenance flow cell (reusable)	4

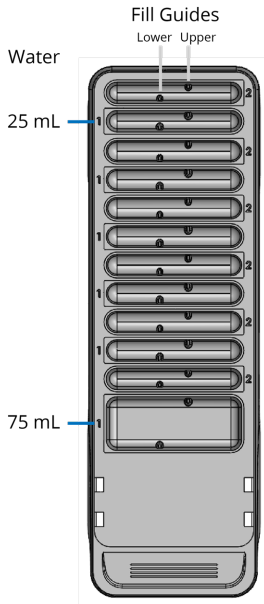
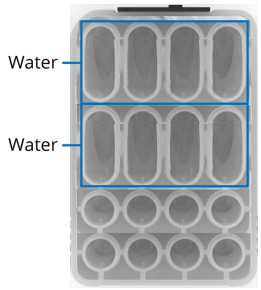
- Instead of the Maintenance Flow Cell, you can also use a regular used NGS flow cell for each position that needs a maintenance wash or rinse.

Performing a System Health Check

To initiate a system health check, do the following :

1. Rinse all wells of the maintenance cartridges with water to remove any dust or particulates that may have accumulated in the cartridges.
2. Fill the following wells of the maintenance cartridges with water:

   NOTE
   The Maintenance Reagent Cartridge has lower and upper fill guides in every well. As long as you fill the well above the lower fill guide and below the upper fill guide, there is enough volume for the system health check.

Cartridge	Location (counted from top of picture)	Reagent	Volume	Image
Maintenance Reagent Cartridge	Row 2 Row marked 1	Water	In between fill guides (25 mL)	
	Row 12 Row marked 1	Water	In between fill guides (75 mL)	
Maintenance Sample Cartridge	All wells in rows 1 and 2	Water	1.2 mL per well	
Maintenance Wash Cartridge	Top (bulk) compartment	Water	200 mL	

3. Go to the **Instrument | Maintenance** section in the Sequencing Operating System and select **System Health Check** to start the protocol.
4. Unselect the positions you do not want to perform a system health check on (see Figure 35). By default all positions are selected.

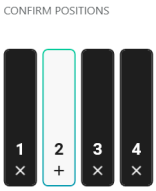


Figure 35 Confirming system health check positions with positions 1, 3, and 4 selected, and position 2 unselected.

5. Load the consumables as prompted.

The flow cell stage may still be hot from the previous run. Be careful not to injure your hands.

The reagent sippers are sharp. Be careful not to touch them, because you can injure your hands.

Be careful when loading consumables to avoid spilling, because the cartridges are not sealed. If you do spill buffer, promptly clean up with a dry low-particulate wipe before proceeding the system health check.
Make sure to always wear gloves when handling reagents, waste, or spills.

FORMAMIDE WARNING
Be aware that waste contains formamide, a suspected carcinogen and reproductive toxin, and other potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations through a licensed hazardous waste contractor.

6. Follow the instructions in the Sequencing Operating System. For an explanation of the flow cell position light colors, see *Flow Cell Compartment* on page 5
7. After the system health check has finished, rinse all maintenance cartridges the following way:
   a. Rinse all wells in the maintenance cartridges with Milli-Q water.
   b. Put the maintenance cartridges upside down and let dry in the air.
   c. Store the maintenance cartridges in a closed dust free box when dry and not in use.

CRITICAL STEP
Failure to clean and correctly store maintenance cartridges can result in fluidic system damage to the G4X Spatial Sequencer.

Sending Log Files

When the system health check is completed, the zipped support log package should be generated automatically and will pop up in a Windows explorer window. Send the support log package to Customer Care for review.

TROUBLESHOOTING

Informational messages, error alerts, and warning alerts are color-coded based on the severity of alert.

- Informational messages appear in gray and provide advanced notice of a pending task, such as upcoming annual maintenance.
- Warning alerts appear in yellow and indicate that attention is recommended or that you need to acknowledge the alert before proceeding.
- Error alerts appear in red and indicate that attention is required.

A list of troubleshooting topics is available at:

https://knowledge.singulargenomics.com/

Cannot Select Sample Sheet

If SeqOS cannot find or select the sample sheet, check the following:

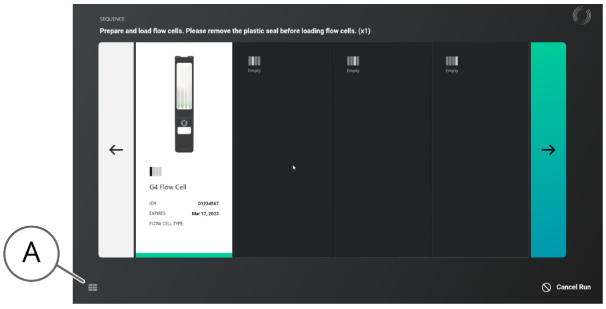
- Make sure the sample sheet is saved in an off-instrument location SeqOS can access, and navigate to that location.
- Make sure the sample sheet is a .csv file and not .xls or other file type.
- Make sure there is no mismatch between the sample sheet parameters and the run parameters. For example, if the run format is different, SeqOS cannot select the sample sheet.
- Make sure that the panels have version numbers on them, for example, lung_imx_v1 as opposed to lung.

Correct the sample sheet using the information in *G4X Sample Sheet* on page 13.

Product Identifier Not Recognized

If the G4X Spatial Sequencer does not recognize a Product Identifier, enter the product properties manually the following way:

1. Select the **Manual Entry** button in the bottom left of the screen.



A Product Properties Manual Entry Button

Figure 36 Position of Product Properties Manual Entry Button.

The *Product Properties Manual Entry* screen opens.

2. Make sure the proper position for the missing product properties is selected.
3. *For Expert Mode only:* Make sure the cartridge type for the missing product properties is selected.
4. Fill out the requested product properties. The product properties can be found here:

- For the flow cell and sample cartridge, there is a sticker on the box with product properties.
- For wash buffer cartridge and the reagent cartridge, there is a sticker on the cartridge with product properties.

NOTE
If you manually enter the flow cell properties, make sure that you select the right flow cell type, and that the flow cell type for all lanes that are used is the same. An erroneous flow cell type entry may result in run failure.

5. Select **Save**.

Once the missing product properties are entered, the SeqOS leads you to the next step.

Flow Cell Not Engaged

If a flow cell is not engaged properly it is illuminated in red. SeqOS displays a red message indicating which flow cell did not seal properly. Perform the following to reseat the flow cell:

1. Take out the flow cell.
2. Ensure the gaskets of the flow cell position is completely level by running a gloved finger all the way around the blue perimeter gasket and over the two port gaskets at the front and rear. If a gasket is raised in an area, carefully press the gasket down in that area to make sure the gasket is entirely level (see Figure 37).

NOTE
Raised gaskets at the far end of the flow cell position can be difficult to see. Running a gloved finger carefully at the rear makes it easier to detect. Do not use IPA to wipe the gasket area.

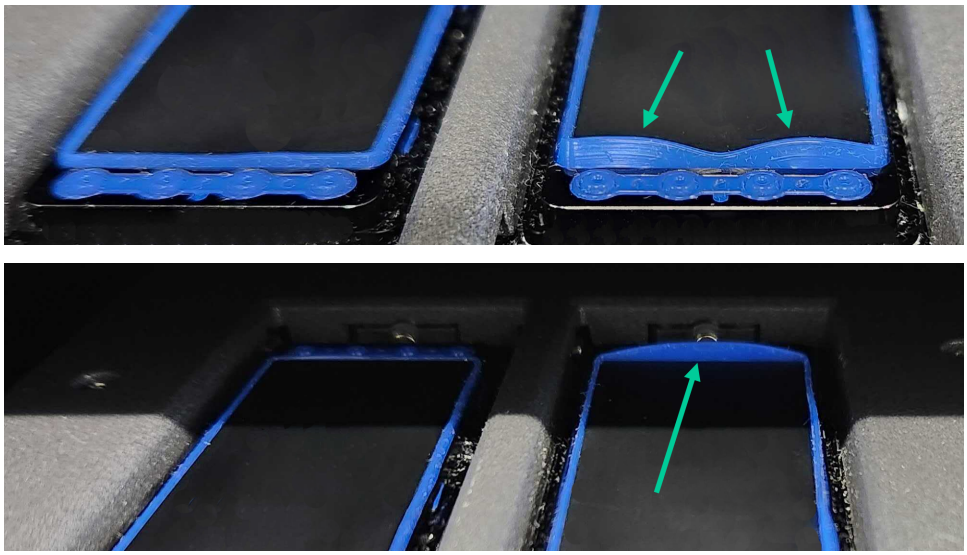


Figure 37 Raised gaskets at the front end and far end of a flow cell position, indicated by arrows.

3. Reseat the flow cell carefully the following way:
   a. Carefully place the toe end of the flow cell (the side furthest from the handle) in first. The flow cell should click into place.
   b. Gently lower the heel end (handle end) of the flow cell into place. Do not push the flow cell down but allow the magnets to catch.
4. Select **Retry**.
5. If that does not work, gently place a finger on either side of the flow cell (not touching the glass) and apply slight pressure temporarily to help the vacuum seal form. Release pressure as soon as you feel the flow cell suck down, which is no more than 8 seconds from the beginning of the flashing light.

It is important to not hold the flow cell after the check has engaged, even if you do not feel the flow cell shift down. Holding down the flow cell too long can cause a false 'pass'.

Use gloves and do not leave fingerprints on the flow cell surface. Be careful not to push too hard on the flow cells.

6. If that still does not work, perform the following:
   a. Take out the flow cell and reclean it, paying particular attention to the area around the ports of the flow cell where the seal tape was removed from. Clean by holding the flow cell by the handle and using a lint free optical wipe with ethanol or isopropanol. After cleaning, avoid touching the glass surface.
   b. Ensure both the blue perimeter and port gaskets are flush with the nest and clean. Gently push the gaskets down with gloved fingertips, running a finger across the whole length of them, to ensure they are clean and flush with the nest.
   c. Reseat the flow cell carefully as described in step 5 and select **Retry**.
7. Select the **forward arrow** → to proceed.

Flow Rate Check Failed

The flow cell that failed the flow rate check is illuminated in red. SeqOS displays a red message indicating which flow cell does not have a proper flow rate. Reseating the flow cell usually restores the flow rate; perform the following to reseat the flow cell:

1. Take out the flow cell.
2. Ensure the gaskets of the flow cell position is completely level by running a gloved finger all the way around the blue perimeter gasket and over the two port gaskets at the front and rear. If a gasket is raised in an area, carefully press the gasket down in that area to make sure the gasket is entirely level (see Figure 38).

   NOTE
   Raised gaskets at the far end of the flow cell position can be difficult to see. Running a gloved finger carefully at the rear makes it easier to detect. Do not use IPA to wipe the gasket area.

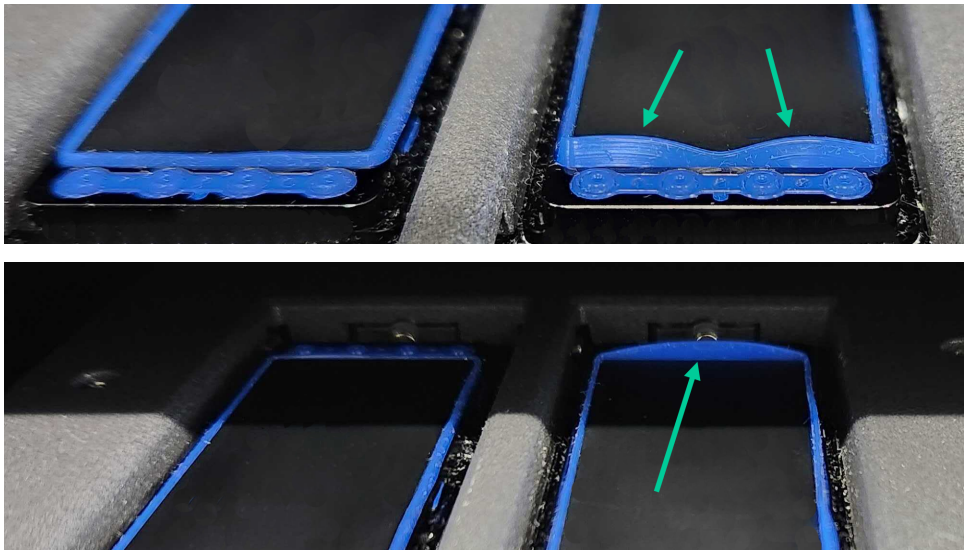


Figure 38 Raised gaskets at the front end and far end of a flow cell position, indicated by arrows.

3. Reseat the flow cell carefully the following way:
   a. Carefully place the toe end of the flow cell (the side furthest from the handle) in first. The flow cell should click into place.
   b. Gently lower the heel end (handle end) of the flow cell into place. Do not push the flow cell down but allow the magnets to catch.
4. Select **Retry**.
5. If that does not work, gently place a finger on either side of the flow cell (not touching the glass) and apply slight pressure temporarily to help the vacuum seal form. Release pressure as soon as you feel the flow cell suck down, which is no more than 8 seconds from the beginning of the flashing light.

> It is important to not hold the flow cell after the check has engaged, even if you do not feel the flow cell shift down. Holding down the flow cell too long can cause a false 'pass'.
>
> Use gloves and do not leave fingerprints on the flow cell surface. Be careful not to push too hard on the flow cells.

6. If that still does not work, perform the following:
   a. Take out the flow cell and reclean it, paying particular attention to the area around the ports of the flow cell where the seal tape was removed from. Clean by holding the flow cell by the handle and using a lint free optical wipe with ethanol or isopropanol. After cleaning, avoid touching the glass surface.
   b. Ensure both the blue perimeter and port gaskets are flush with the nest and clean. Gently push the gaskets down with gloved fingertips, running a finger across the whole length of them, to ensure they are clean and flush with the nest.
   c. Reseat the flow cell carefully as described in step 5. Leave the flow cell door open and select **Retry**.
   d. Observe the flow cell during the Flow Rate Check. Note if any lanes have significant bubbles and if the Flow Rate Check fails again, contact Customer Care.

7. Close the flow cell door if it is open. Select the **forward arrow** → to proceed.

Waste Reservoir Not Empty

The G4X Spatial Sequencer comes with an internal waste reservoir, but you can also order an external waste reservoir (Contact Singular Genomics, see *Customer Care* on page 66). The external waste reservoir holds more waste.

If the internal waste reservoir is not empty, SeqOS displays a warning during run set up. Make sure the waste reservoir is empty by performing the procedure described in *Empty Internal Waste Reservoir* on page 34.

Window Freezes

If the system does not respond after selecting a button, wait 15 seconds. This will usually result in the window becoming responsive again.

Selecting a button too many times before SeqOS can finish its task can result in the window freezing or becoming unresponsive.

Starting Up and Shutting Down

Starting Up the Instrument

If the instrument had to be shut down for any reason, use the following instructions to safely restore power and properly prepare the instrument for operation.

1. Make sure that the instrument power cable and both computer power cables are connected to the respective UPSs and the UPSs are plugged into the wall.
2. Make sure that only devices supplied by Singular Genomics are connected to the instrument, computers, or SCS; all other external, non-standard devices (for example, an external keyboard) should be disconnected.
3. Power on the SCS by pushing the power button on the SCS.
4. Wait 7 minutes.
5. Power on the instrument by toggling the power switch adjacent to the power cable to the **I** position.
6. Power on the G4X Primary Computer the following way:
   a. Toggle the power switch on the back of the computer to the **I** position.
   b. Press the power button on the front of the computer in the section labeled *G4X Primary*.
7. Wait for 2 minutes
8. Power on the G4X Secondary Computer by pressing the power button on the front of the computer in the section labeled *G4X Secondary*.

   The indicator LED adjacent to the power button should turn on.
9. Wait 7 minutes.
10. Start SeqOS.

If the G4X Primary Computer fails to reboot or has a blank screen, see *Blank Screen After Restart* on page 64.

Shutting Down the Instrument

Singular Genomics recommends that you leave the instrument on at all times. However, in the event that you have to shut down the instrument, use the following instructions to safely shut down the instrument and instrument software.

1. Close SeqOS.
2. Power off the SCS by holding the power button down for 5 seconds.
3. Wait 5 seconds.
4. Power off the G4X Primary Computer by using the **Shut down** option in the Windows menu.
5. Power off the G4X Secondary Computer the following way:
    a. Press the power button on the front of the computer in the section labeled *G4X Secondary* once. Lights should turn off in about 10 seconds.
    b. Toggle the power switch on the back of the computer to the **O** position.
6. Power off the instrument by toggling the power switch on the back of the instrument to the **O** position.

Restarting Instrument and Primary Computer

Restarting both instrument and Primary Computer can fix miscommunications between G4X and SeqOS. Follow these steps when advised by Customer Support:

1. Close SeqOS.
2. Power off the G4X Primary Computer by using the **Shut down** option in the Windows menu.
3. Shut down the instrument by toggling the power switch adjacent to the power cable to the **O** position.
4. Wait 5 minutes.
5. Make sure that only devices supplied by Singular Genomics are connected to the instrument , computers, or SCS; all other external, non-standard devices should be disconnected.
6. Power on the instrument by toggling the power switch adjacent to the power cable to the **I** position.
7. Power on the G4X Primary Computer by pressing the power button on the front of the computer in the section labeled *G4X Primary*.
8. Wait 2 minutes.
9. Start SeqOS.

Blank Screen After Restart

If the G4X Primary Computer fails to reboot or has a blank screen, perform the following:

1. Hold the power button for the Primary Computer until the LED turns off.

    The Secondary Computer should remain on.
2. Unplug the Primary Computer A/C power cord from the wall.
3. Wait 5 minutes.
4. Make sure that only devices supplied by Singular Genomics are connected to the instrument , computers, or SCS; all other external, non-standard devices should be disconnected.
5. Plug the Primary Computer A/C power cord back into the wall.
6. Power on the G4X Primary Computer by pressing the power button on the front of the computer in the section labeled *G4X Primary*.
7. Confirm Windows starts and is visible on the monitor within 2-3 minutes.

8. Start SeqOS.

Restarting SCS and Secondary Computer

If there is a network disruption and data transfer is interrupted, you may need to restart both SCS and Secondary Computer. Follow these steps when advised by Singular Genomics Customer Support:

SCS Restart

The SCS is the small black box next to the main computer tower.

1. Make sure that only devices supplied by Singular Genomics are connected to the instrument , computers, or SCS; all other external, non-standard devices should be disconnected.
2. Power off the SCS by holding the power button down for 5 seconds.
3. Wait 5 seconds.
4. Power on the SCS by pushing the power button on the SCS.
5. Wait 7 minutes.

Secondary Computer Restart

6. Power off the G4X Secondary Computer by pushing the power button on the front of the computer in the section labeled **G4X Secondary** for 1 second.

   Lights should turn off in about 1-2 minutes.
7. Wait 10 seconds.
8. Press the power button on the front of the computer in the section labeled **G4X Secondary**.

   The indicator LED adjacent to the power button should turn on and you should hear the fans turn on.

Secondary Computer Restart, Alternative Method

If the regular *Secondary Computer Restart* method described above fails, try the following alternative method for restarting the Secondary Computer:

9. Power off the Secondary Computer by holding down the power button on the front of the computer in the section labeled **G4X Secondary**.

   Lights should turn off in about 10 seconds.
10. Wait 30 seconds.
11. Press the power button on the front of the computer in the section labeled **G4X Secondary**.

    The indicator LED adjacent to the power button should turn on and you should hear the fans turn on.

CUSTOMER CARE

Our team is committed to ensuring your success. Field application scientists, field service engineers, and customer care specialists are dedicated to responding quickly and effectively to your inquiries. From preparing your lab for the arrival of your instrument, to onsite training in system operation and maintenance and continued support, we'll be with you every step of the way.

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Additional Resources

Resource	Description
G4X Sequencing Platform Safety and Compliance Guide	Information about operational safety considerations, compliance statements, and instrument labeling.
G4X Sequencing Platform Site Preparation Guide	Information for preparing your site for delivery, installation, and operation of the G4X Spatial Sequencer.
Networking, Security, and Remote Access for the G4 Sequencing Platform Technical Note	Specifications for connecting the instrument to a network and settings for antivirus software.
Training videos	Visual instructions for run setup and instrument operation.

Revision History

Document #	Revision	Release Date	Description of Change
600041	Rev. 3	January 2026	• Removed Quenching Solution, replace with Cleave Buffer Additive instructions • Updated file output structure • Updated terminology to align across all guides
600041	Rev. 2	November 2025	• Updated Maintenance Rinse protocol • Removed deprecated links • Updated file output structure
600041	Rev. 1	August 2025	• Included multiomics • Added new sample sheet checker • Added customization workflow
600041	Rev. 0	December 2024	Initial Early Access Program release.