SINGULAR GENOMICS

G4® Sequencing Platform

User Guide

Singular Genomics Proprietary

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INTRODUCTION

Using innovative high-throughput technology, the Singular Genomics® G4® Sequencing Platform combines advancements in speed, flexibility, and accuracy in a versatile benchtop sequencer.

- **Speed**—High-resolution optical detection, streamlined fluidics, and dramatically decreased chemistry time per cycle shortens overall sequencing duration and enables an increase in daily data output.
- **Flexibility**—Designed to support a broad range of applications, the G4 Sequencing Platform can sequence up to four independent flow cells in a single run. Additionally, each flow cell has four independent lanes that allow libraries to be sequenced separately while retaining high-throughput capacity.
- **Accuracy**—Optimizations in clustering, imaging, and signal processing is designed to produce 85% or more of bases having a base call accuracy of Q30 (99.9%) or greater.

Figure 1 G4 Sequencing Platform

NOTE
The G4 Sequencing Platform supports Max Read™ sequencing. See the Max Read Kit User Guide for more information, including Max Read reagent information and instructions on how to prepare and sequence Max Read libraries (available from https://singulargenomics.com/g4/support/).

Sequencing Overview

Sequencing comprises cluster generation, imaging, base calling, and data output.

- **Cluster generation**—Samples transferred from the sample cartridge to the flow cell bind to complementary oligos within each nanowell on the flow cell. The clustering process creates clonal copies of a single DNA molecule until each nanowell fills to create a cluster.
- **Imaging**—Directly following cluster generation, clusters are imaged during sequencing using the proprietary 4-color Rapid SBS chemistry to encode and detect each of the four nucleotides. Imaging is performed continuously for each lane. For two index paired reads, the order of sequencing is Index 1–read 1–Index 2–read 2.
- **Base calling**—From the images, the instrument software assigns a nucleotide base to a cluster, measures the quality of that call, and assigns a quality score. For more information, see *Quality Scores* on page 41.

- **Data Output**—The results of a sequencing run are recorded in FASTQ files. Each file contains the sequence for every cluster in a lane that passed the quality filter and an encoded quality score for each base in the sequence. The G4 Sequencing Platform also applies on-instrument demultiplexing if indices are specified. For more information, see *FASTQ Files* on page 41.

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 *G4 Sequencing Platform Safety and Compliance Guide*.

G4 SEQUENCING PLATFORM OVERVIEW

When sequencing on the G4 Sequencing Platform you need the following items:

- The G4 Sequencing Platform
- The case containing the G4 primary and secondary computers
- Singular Control System (SCS)
- Two Uninterruptible Power Supplies (UPS)
- The instrument software
- 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.

See also the video: G4 Overview

Instrument Components

The G4 Sequencing Platform 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 G4 Sequencing Platform 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, and the positions are numbered 1 through 4, left to right.

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

Figure 3 G4 Sequencing Platform Reagent Compartment

Buffer Compartment

The Buffer Compartment holds the waste reservoir provided with the instrument and four wash cartridges provided in G4 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 G4 Sequencing Platform waste reservoir

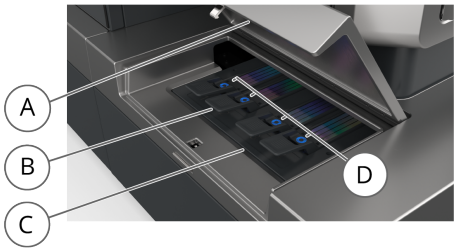
NOTE
You can also order an external waste reservoir, which holds more waste and is needed for Max Read sequencing. Contact Singular Genomics (see *Customer Care* on page 72) for ordering information and installation of the external waste reservoir.

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 G4 Sequencing Platform 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

G4 Primary and Secondary Computers

The G4 Sequencing Platform comes with two computers in a single case:

Component	Description
G4 Primary Computer	Hosts the G4 SeqOS. When you set up a run, you interact with the instrument user interface powered by this computer.
G4 Secondary Computer	A high-performance computer that performs image analysis and base calling.

Front Back

Figure 6 Computer case containing the G4 Primary Computer and G4 Secondary Computer, front and back view.

The G4 computers and their connection ports are for G4 sequencing only and should not be used for other purposes.

NOTE
Never rearrange connections between the G4 Sequencing Platform, the G4 Primary Computer, the G4 Secondary Computer, and the SCS.

Singular Control System (SCS)

The Singular Control System (SCS) serves as the bridge linking the G4 Sequencing Platform with external systems and functions. The SCS isolates the G4 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. For more information, see *Internet Connectivity and Secure Tunnels* on page 1 and *Singular Secure Customer Network (SCN)* on page 12.
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 G4 Sequencing Platform 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 G4 Sequencing Platform 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 10). The SCS provides transitional storage and frees up the G4 Sequencing Platform to start the next sequencing run. The SCS then automatically transfers the run results to your off-instrument storage.

The G4 Sequencing Platform G4 Sequencing Platform 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 G4 off-instrument storage.

Singular Secure Customer Network (SCN)

The Singular Secure Customer Network (SCN) is a Singular Genomics network that controls connections into the SCS. The SCN has the following characteristics:

- The SCN is a highly controlled and closed network hosted in Singular Genomics datacenters.
- No customer sequencer result data is ever transferred to the SCN or other destinations not explicitly defined or authorized by the customer.
- The SCS tunnels terminate in the SCN environment. Special access and separate credentials are required to access this environment from within the Singular Genomics corporate network.
- The Singular Genomics datacenter meets SOC1 and SOC2 standards.
- Only cleared and authorized Singular Genomics personnel are authorized to access the SCN. Users must remotely connect to the Singular Genomics Corporate Network first or be physically located in a Singular Genomics office. From inside the Singular Genomics corporate network authorized users connect to the SCN with their separate credentials.
- All activity by Singular Genomics users accessing the SCN is monitored and logged.
- The remote capabilities of the SCS are optional and can be deactivated upon request by the customer. However, this limits the ability to update and support the G4 Sequencing Platform at customer sites by Singular Genomics. Updates and support then need to be done at the customer sites in-person and delays response time.

Uninterruptible Power Supplies

The G4 Sequencing Platform includes two Uninterruptible Power Supplies (UPS): one for the instrument, and one for the primary and secondary computers. 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 and computers, respectively.

Software User Interface

Operations on the G4 Sequencing Platform are controlled by the G4 SeqOS (SeqOS). When operating the G4 Sequencing Platform or setting up a run, you interact with the software through the SeqOS. You can also upload sample library information using the sample sheet. These features are described in this section.

G4 SeqOS

The G4 Sequencing Platform operating system SeqOS controls operations on the G4 Sequencing Platform 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

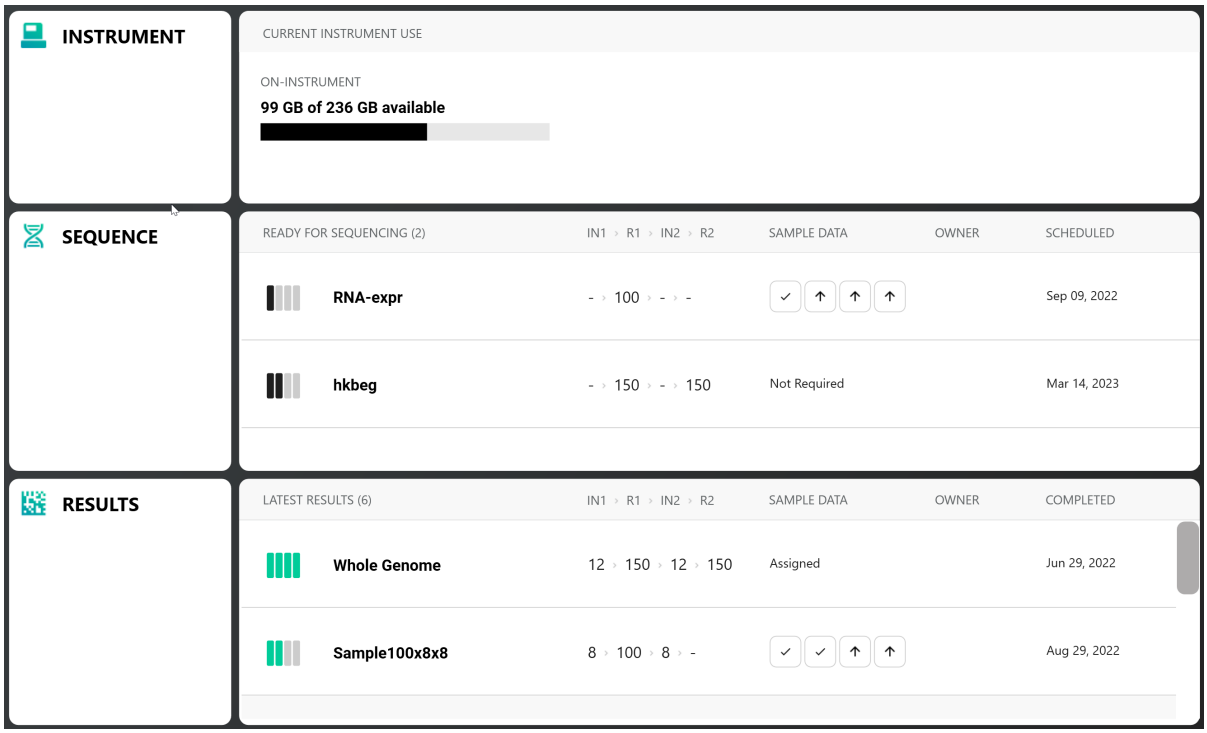


Figure 9 SeqOS Dashboard

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

Screen	Description	Sub Screen
Dashboard	Provides an overview of the data storage space, available runs, and completed runs. Each area of the Dashboard opens a screen with detailed information.	• **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 run or select a run from the queue. For more information, see *Set Up a Run* on page 31.	

Screen	Description	Sub Screen
Results	Shows details of completed runs. For more information, see *View Results* on page 42.	
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 50 • **Storage**—Allows you to set up off-instrument storage. For more information, see *Off-Instrument Storage* on page 11. • **Settings**—Provides a menu to view and change instrument settings. For more information, see *Changing Instrument Settings* on page 16.
Help	Shows contact information for technical support.	Telephone: 442.SG.CARES (442.742.2737)

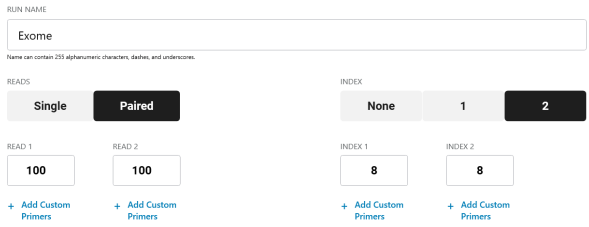
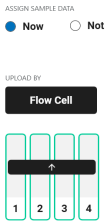
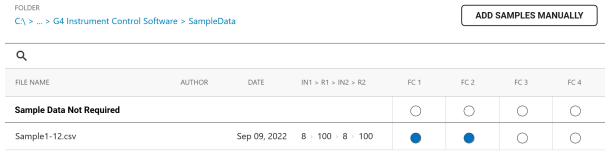
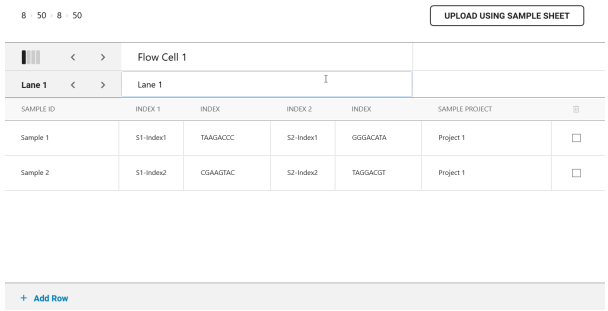
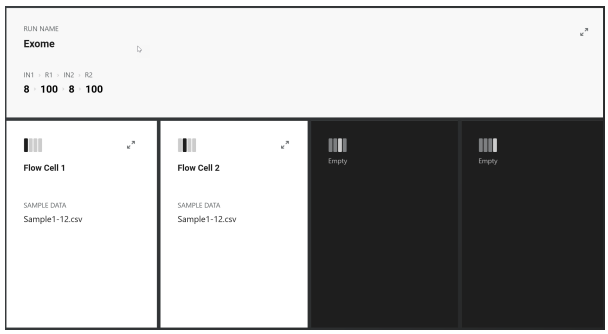
At any time, tap the Singular Genomics icon in the top right corner to access the main menu.

Run Setup on SeqOS

There are two ways to start a 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 Sequence tab and select the **+**. The New Run screen is divided into four sections.

The New Run option provides access to five 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 provides options to specify read type (single or paired), number of cycles for each read, whether the run uses indices, number of cycles for each read and index, and whether custom primers are used.	
Sample Sheet Upload Parameters	Provides the feature to assign a sample sheet for each flow cell in the run. Sample data is needed for demultiplexing a run on-instrument. If lanes only contain single libraries, you can also select that a sample sheet is not required. You will have to assign the flow cell positions you want to run.	
Sample Data Upload	Allows you to upload a sample sheet for the 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 a sample sheet, in which case you go back to the Sample Data Upload screen.	
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.

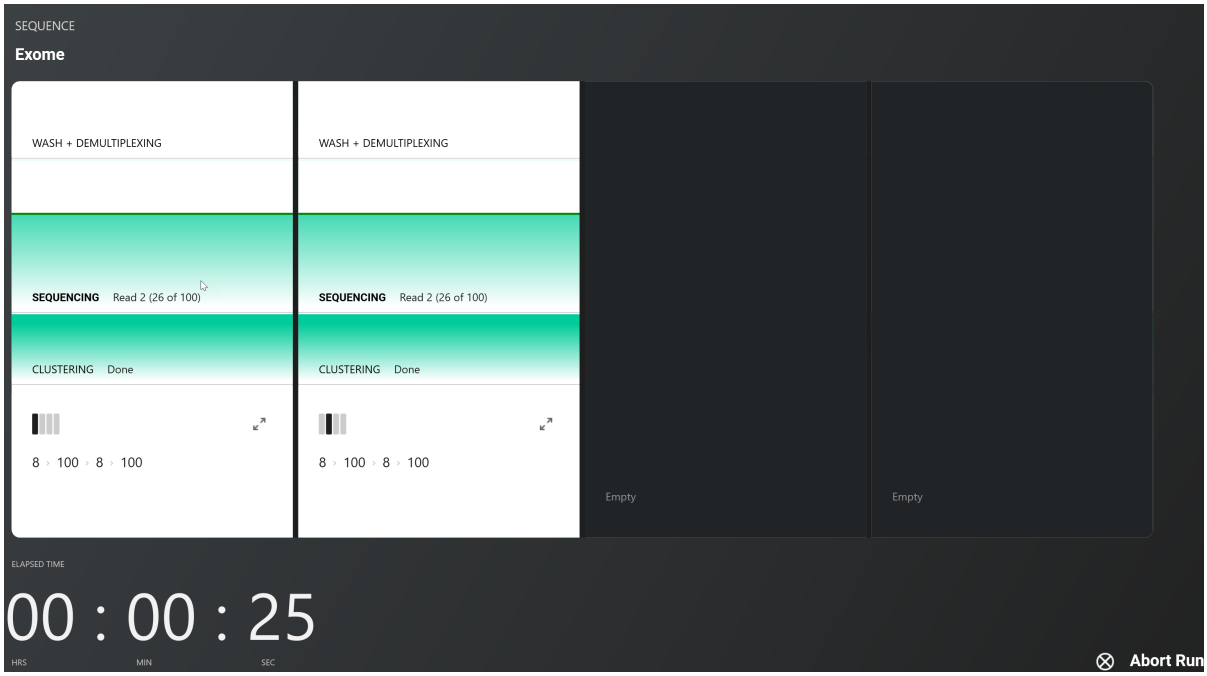


Figure 10 SeqOS Run Monitoring Screen

For a description of run metrics, see *Monitoring Run Progress* on page 39.

Changing Instrument Settings

Adjust the following settings via 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 G4 Sequencing Platform.
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. If you set this to **Run Start Before System Check**, you can click **Start Run** before the system check starts. The run will then start automatically after the system check is complete, so you don't have to stay with the instrument. However, if the system check fails, the run will not start, so always check if your run indeed started.

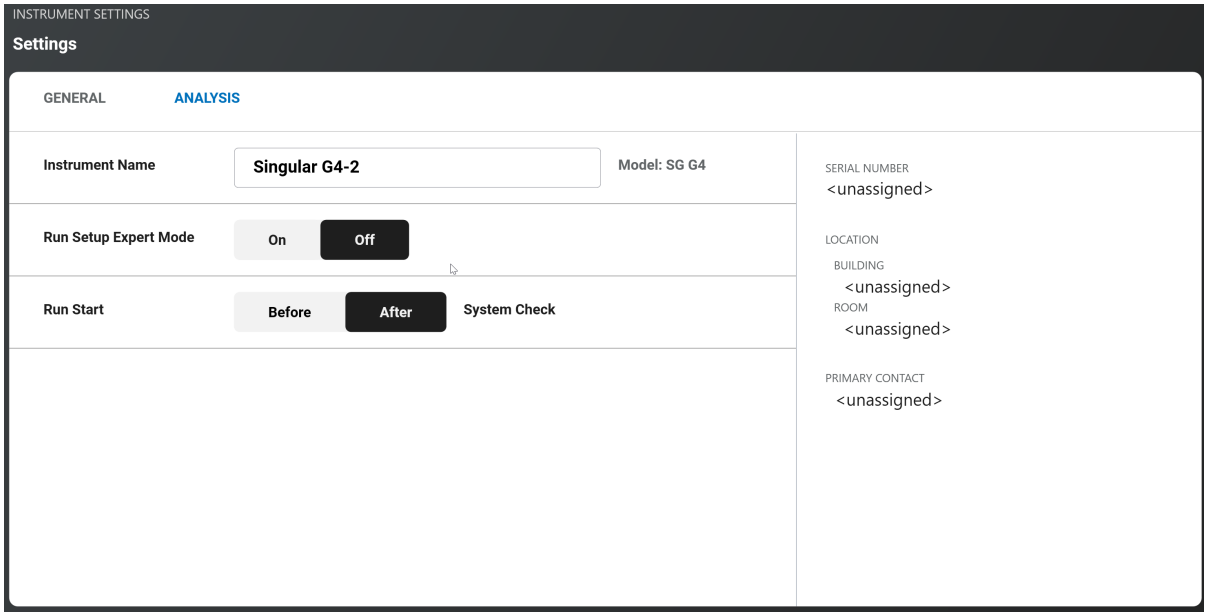


Figure 11 SeqOS Instrument Settings Screen

Switch User

Users are defined by your network settings. The SeqOS allow you to switch users 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.

Sample Sheet

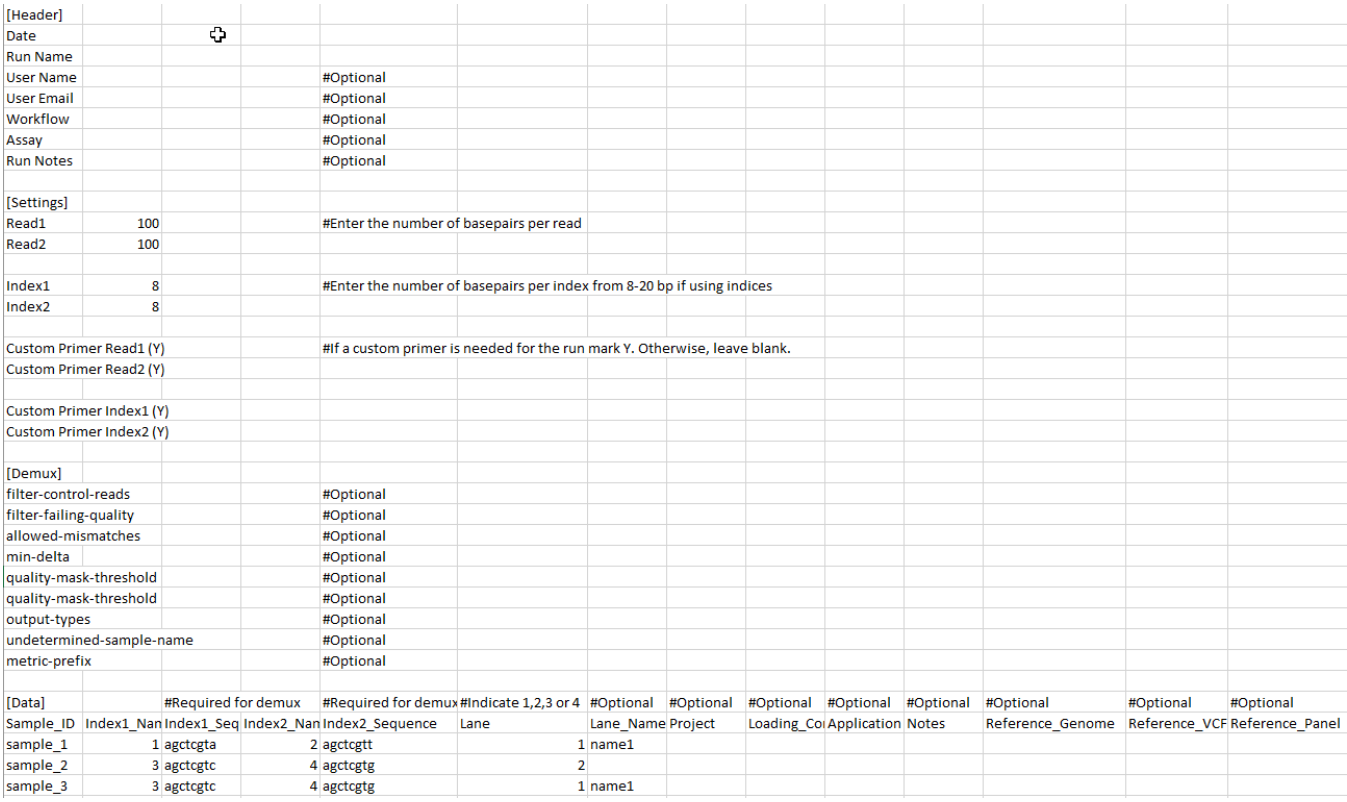
A sample sheet is a Comma Separated Value (CSV, *.csv) file that stores information about the sample libraries to be sequenced on a flow cell. You can edit the sample sheet in Excel, save as CSV file, and then upload to the *sample-sheets* folder on the off-instrument storage. The SeqOS can then use the information in the sample sheet on the off-instrument storage to set up the run.

NOTE
Singular Genomics has generated an online Sample Sheet Generator. This tool helps you fill out all data, which minimizes errors in the sample sheet.

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	General information about the run.

Feature	Description
Settings Section	Information about the run settings that apply to all lanes and samples in this run: reads, indices, custom primers.
Demux Section	*Optional*: section for custom demultiplexing settings. Not needed for default demultiplexing. See *Custom Demultiplexing Settings in the Sample Sheet* on page 27 for more information.
Data Section	Information about the different sample libraries.
Sample Sheet Template	Sample sheet templates are located in your off-instrument storage in a folder called *G4 Run Templates*.



[Header]													
Date													
Run Name													
User Name				#Optional									
User Email				#Optional									
Workflow				#Optional									
Assay				#Optional									
Run Notes				#Optional									
[Settings]													
Read1	100			#Enter the number of basepairs per read									
Read2	100												
Index1	8			#Enter the number of basepairs per index from 8-20 bp if using indices									
Index2	8												
Custom Primer Read1 (Y)				#If a custom primer is needed for the run mark Y. Otherwise, leave blank.									
Custom Primer Read2 (Y)													
Custom Primer Index1 (Y)													
Custom Primer Index2 (Y)													
[Demux]													
filter-control-reads				#Optional									
filter-failing-quality				#Optional									
allowed-mismatches				#Optional									
min-delta				#Optional									
quality-mask-threshold				#Optional									
quality-mask-threshold				#Optional									
output-types				#Optional									
undetermined-sample-name				#Optional									
metric-prefix				#Optional									
[Data]		#Required for demux		#Required for demux	#Indicate 1,2,3 or 4	#Optional	#Optional	#Optional	#Optional	#Optional	#Optional	#Optional	#Optional
Sample_ID	Index1_Nan	Index1_Seq	Index2_Nan	Index2_Sequence	Lane	Lane_Name	Project	Loading_Co	Application	Notes	Reference_Genome	Reference_VCF	Reference_Panel
sample_1	1	agctcgta	2	agctcgtt	1	name1							
sample_2	3	agctcgtc	4	agctcgtg	2								
sample_3	3	agctcgtc	4	agctcgtg	1	name1							

Figure 12 Sample sheet

For a description of what to enter in each field of the sample sheet , see *Create a Sample Sheet* on page 25.

When you assign a data sample sheet to a flow cell using the SeqOS, the software checks whether the sample sheet is valid. If an error is detected, you will have to correct the sample sheet before continuing. If you detect an error in the data sample sheet that was not detected by the software, for example, a library has the wrong index assigned, you can repeat the demultiplexing with a corrected sample sheet (see *Fixing Improper Demultiplexing* on page 63).

NOTE
You do not have to upload a sample sheet. You can also define the samples manually when you set up the run on the SeqOS, or not provide sample data at all. However, without index information the G4 Sequencing Platform does not perform on-instrument demultiplexing.

G4 Sequencing Kits

The G4 Sequencing Kit provides consumables designed to support a broad range of applications and integrate seamlessly with the instrument. Sequencing kits are available in various sizes depending on the number of cycles to be performed. For more information, see the G4 Sequencing Kit Overview document.

Each sequencing kit includes a flow cell box, sample cartridge box, reagent cartridge box, and wash cartridge box.

NOTE
The G4 Sequencing Platform supports Max Read™ sequencing. See the Max Read Kit User Guide for more information, including Max Read reagent information and instructions on how to prepare and sequence Max Read libraries (available from https://singulargenomics.com/g4/support/).

See also the video: Preparing For a Run

Flow Cell

The G4 flow cell is a single-use substrate contained within glass in a plastic housing. Each flow cell contains four fluidically independent lanes, which enable independent sample loading.

Each lane is patterned with millions of nanowells each containing oligos on which sample libraries are attached at the beginning of a run. All four lanes are processed simultaneously during a sequencing run.

Sample Cartridge Box

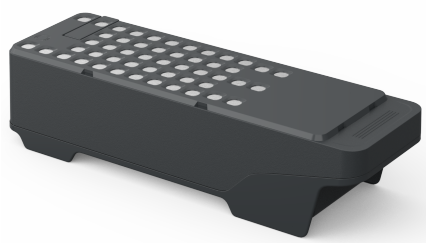
The sample cartridge box contains the sample cartridge and four tubes with reagents.

Item	Description
Sample Cartridge	The sample cartridge consists of 16 wells arranged in four columns that correspond to the four lanes on the flow cell. The first row is reserved for loading samples to be sequenced. Remaining foil-sealed wells contain reagents used for priming lines and performing the post-run instrument wash. Visually inspect the cartridge to make sure that the foil seal covering all other wells remains intact. Keep the sample cartridge in the box until ready for use and avoid extended light exposure. 
1 M NaOH	One tube of 1 M NaOH, used for denaturing libraries. Always use a fresh, unopened tube with each sample cartridge.
200 mM Tris pH 7.0	One tube of 200 mM Tris pH 7.0, used for neutralizing denaturing libraries.
2x Sample Loading Buffer	Two tubes of 2x Sample Loading Buffer, used to dilute libraries in Sample Loading Buffer.

NOTE
When denaturing and diluting libraries, always use the reagents (1 M NaOH, 200 mM Tris pH 7.0, and 2x Sample Loading Buffer) that are provided in the sample cartridge box.

Reagent Cartridge

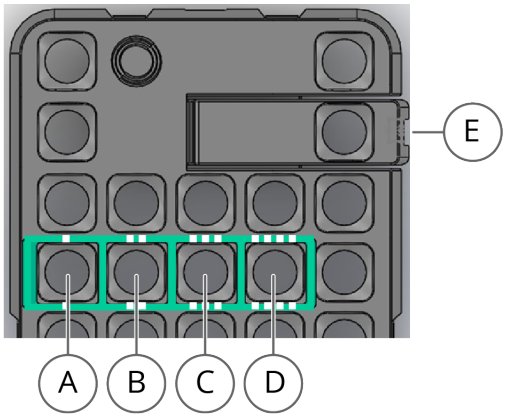
The reagent cartridge contains reagents required for cluster generation and sequencing on one flow cell. The cartridge is designed with a molded handle for easy loading and foil seals covering each of the reagent wells. During a sequencing run, instrument sippers automatically pierce each foil-sealed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

If you are using custom sequencing primers for read 1, read 2, Index 1, or Index 2, load them in positions 1, 2, 3, or 4 respectively within the green outline on the reagent cartridge (see Figure 13). Load custom primers just after inverting and mixing the reagent cartridge and just before loading consumables for the run. See *Load Reagent Cartridges* on page 37 for more information.

NOTE
If you need to dilute the custom sequencing primers, make sure to use the 5X Primer Annealing Buffer (PAB) to arrive at a final buffer concentration of 1xPAB. Stock 5XPAB is available for purchase from Singular Genomics (ordering # 700,140) or is included in selected library preparation kits.



A Custom read 1 primer
B Custom read 2 primer
C Custom Index 1 primer
D Custom Index 2 primer
E Removable formamide well

Figure 13 Reagent cartridge with custom sequencing primer wells and removable formamide well indicated.

NOTE

If you designed your own custom sequencing primers, you must validate them yourself. Singular Genomics cannot guarantee performance or compatibility of custom sequencing primers.

Quenching Solution

The reagent cartridge comes with a tube of Quenching Solution. This reagent needs to be added to the bottom left well of the reagent cartridge just before you start the run (see *Prepare Reagent Cartridge* on page 30).

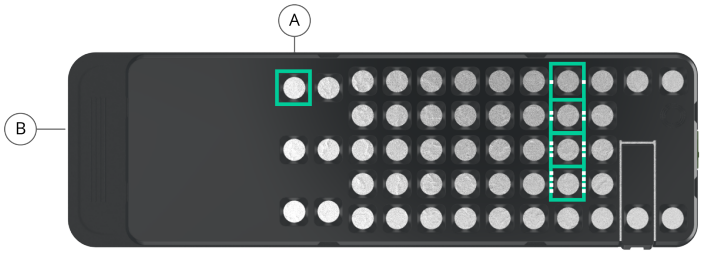


Figure 14 Location of well for Quenching Solution.

A Well you need to add Quenching Solution to
B Handle

NOTE
The Quenching Solution contains DMSO, which has a melting temperature close to room temperature. It may be necessary to briefly warm the tube with your hands.

Wash Cartridge

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

Product Identifier

Each Singular Genomics sequencing consumable includes 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 G4 Sequencing Platform Site Preparation Guide for more information.

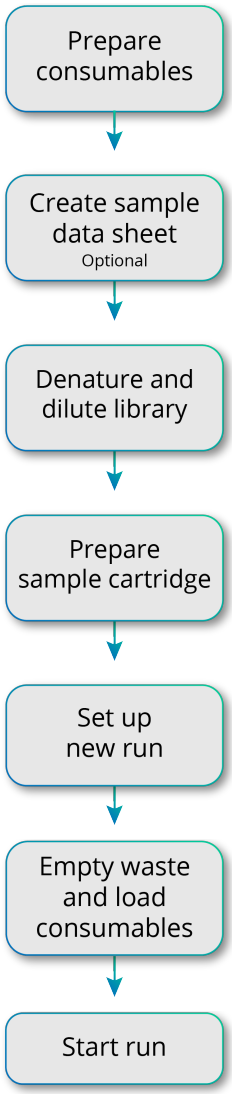
Laboratory Consumables

Consumable	Supplier	Purpose
5X Primer Annealing Buffer (5X PAB)	Singular Genomics, # 700,140	Custom primer dilution
10 mM Tris-HCl (pH 8.0) with 1 mM EDTA (TE)	General lab supplier	Sample library dilution
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
Molecular-grade water	General lab supplier	Various dilutions

Laboratory Equipment

Equipment	Supplier	Purpose
Centrifuge	General lab supplier	Sample library preparation
Calibrated pipettes (at least P2, P10, P20, P200, and P1000)	General lab supplier	Sample library preparation
Microcentrifuge tube racks	General lab supplier	Sample library preparation
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

SEQUENCING WORKFLOW



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

Allow the thawed reagent cartridge and flow cell to equilibrate to room temperature for 30 minutes before loading consumables.

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

Denature libraries and dilute to loading concentration. Optional: use the Loading Concentration Calculator.

Load 300 µL in each well of the sample cartridge.

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 Quenching Solution and custom primers if used.

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

After the pre-run check is complete, select Start Run.

NOTE

The G4 Sequencing Platform supports Max Read™ sequencing. See the Max Read Kit User Guide for more information, including Max Read reagent information and instructions on how to prepare and sequence Max Read libraries (available from https://singulargenomics.com/g4/support/).

Prepare Consumables

Prepare Reagents

Prepare one reagent cartridge for each flow cell you plan to use for the run. Ensure that the reagent cartridge selected is appropriate for the number of cycles that will be sequenced. 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.

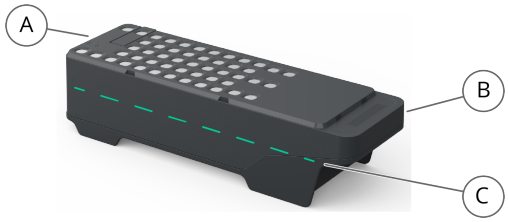
   NOTE
   The 300 cycle reagent cartridges sometimes take a little longer than 2 days to thaw. In that case, place the reagent cartridge in a water bath containing enough room temperature deionized water to submerge the base of the reagent cartridge for 30 to 60 minutes.

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

1. 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

2. Let reagents thaw for 2.5 hours.
3. 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.
4. 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. However, if you plan to use custom sequencing primers for your run, mix the cartridge *before* adding your custom primers. After mixing, add your custom primers to the designated wells in the reagent cartridge (see *Reagent Cartridge* on page 20).

Equilibrate Reagents to Room Temperature

1. Remove wash cartridge from 2°C to 8°C storage and equilibrate to room temperature for 30 minutes before use.

Prepare Flow Cells

1. Remove flow cell boxes from 2°C to 8°C storage for each position you plan to use for the run.
2. Equilibrate flow cell boxes to room temperature for 30 minutes before loading onto the instrument.

Create a Sample Sheet

Create one sample sheet for each flow cell. There are two ways to generate a sample sheet:

- Use the Singular Genomics online Sample Sheet Generator (recommended).
- Generate a sample sheet manually as described below.

NOTE
You do not have to upload a sample sheet. You can also define the samples manually when you set up the run on the SeqOS, or not provide sample data at all. However, without index information the G4 Sequencing Platform does not perform on-instrument demultiplexing.

1. Download a sample sheet template from your off-instrument storage in a folder called *G4 Run Templates*.

NOTE
If you do not plan on specifying demultiplexing options, use the template without Demux section (FlowCellTemplate.csv). Do not use FlowCellTemplateDemuxFields.csv, since you need to edit the demultiplexing options in order for that template to work properly.

2. Fill out the following information for the Header section. Fields marked with an asterisk * are required.

Field	Description
Date	Date of sample sheet edit.*
Run Name	Run name.*
User Name	Name of operator.
User Email	Email of operator.
Workflow	Allows you to track the analysis pipeline.
Assay	Allows you to track the library prep used.
Run Notes	Any other notes for the run.

3. Fill out the following information for the Settings section. Fields marked with an asterisk * are required.

   NOTE
   Do not enter an index length that is longer than the physical length of the indices. This may interfere with the binding of the sequencing primers in subsequent sequencing steps.

	Description
[read 1]	Length of read 1 in bp, maximum 150 bp.*
[read 2]	Length of read 2 in bp, maximum 150 bp.
[Index 1]	Length of Index 1 in bp, minimum 8 bp, maximum 24 bp.
[Index 2]	Length of Index 2 in bp, minimum 8 bp, maximum 24 bp.
Custom Primer Read1 (Y)	If a custom sequencing primer is needed for read 1 enter **Y**. If not, leave blank.
Custom Primer Read2 (Y)	If a custom sequencing primer is needed for read 2 enter **Y**. If not, leave blank.
Custom Primer Index1 (Y)	If a custom sequencing primer is needed for Index 1 enter **Y**. If not, leave blank.
Custom Primer Index2 (Y)	If a custom sequencing primer is needed for Index 2 enter **Y**. If not, leave blank.

   NOTE
   Custom sequencing primers can only be set at the run level, you cannot use custom sequencing primers on one flow cell and Singular Genomics sequencing primers on another flow cell within the same run. Note also that different lanes within a flow cell cannot have different sequencing primers.

4. Fill out the following information for the samples section. Note that within a single lane, no two samples can have the same combination of Sample_ID, Index1_Name, Index2_Name, Index1_Sequence and Index2_Sequence. Fields marked with an asterisk * are required.

	Description
Sample_ID	Sample ID.*
Index1_Name	Name of Index 1. Leave empty if no Index 1 is used.
Index1_Sequence	Sequence of Index 1. Required for demultiplexing. Leave empty if no Index 1 is used.
Index2_Name	Name of Index 2. Leave empty if no Index 2 is used
Index2_Sequence	Sequence of Index 2. Required for demultiplexing. Leave empty if no Index 2 is used
Lane	Lane number, a single number from 1 to 4. Required.
Lane_Name	Lane name.
Project	Allows you to organize libraries in a project.
Loading_Concentration	Loading concentration on lane.
Application	Allows you to track the library prep used.
Notes	Any other notes for the sample library.
Reference	Library reference.

NOTE
If you want to sequence a library in multiple lanes, copy the line for that library in the sample sheet and change the lane number.

5. Name the sample sheet and copy as CSV file (*.csv) in the *sample-sheets* folder on the off-instrument storage.

   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.

NOTE
For custom demultiplexing options, add a Demux section in between the Settings and Data sections and specify options as described here: *Custom Demultiplexing Settings in the Sample Sheet* on page 27.

Custom Demultiplexing Settings in the Sample Sheet

For custom demultiplexing options, add a Demux section in between the Settings and Data sections (see Figure 12 on page 18) and specify options as described here. The default setting is applied if the option is not specified in the Demux section.

NOTE
The Demux section is optional and is intended for advanced users who have a good understanding of demultiplexing and filtering settings. For other users, do not add a Demux section and use the default demultiplexing settings.

Demultiplexing Option	Default	Description
filter-control-reads	Not present (false)	If flag is present, filter out reads marked as control reads in their FASTQ headers.
filter-failing-quality	Not present (false)	If flag is present, filter out reads marked as failing quality control in their FASTQ headers.
allowed-mismatches	3	The number of mismatches allowed, in total, between expected and observed index bases in order to match a read to a sample. Note that the default in the off-instrument demultiplexing software is 1 (see *Demultiplexing Off-instrument* on page 63).
min-delta	1	The minimum number of mismatches by which the best match for a read is better than the next-best match for a read in order to accept the best match. Note that the default in the off-instrument demultiplexing software is 2 (see *Demultiplexing Off-instrument* on page 63).
quality-mask-threshold	Not present	Mask to N template bases in all input reads whose base quality is below the specified value(s). If a single value is specified, it is applied to all input reads/FASTQs. If multiple values are provided in consecutive lines, then the first value applies to index 1, the second to index 2, the third to read 1, and the fourth to read 2. Note that sample barcode/index and UMI bases are never masked, even if a quality-mask-threshold is specified.

Demultiplexing Option	Default	Description
output-types	T	The types of bases/reads for which output files should be generated. A single string containing one or more of T (template), B (sample barcode), M (UMI), and S (skipped).
undetermined-sample-name	Undetermined	The name used as a prefix to generate FASTQ files for reads that did not match to any sample.
metric-prefix	N/A	Prepend this prefix to all output metric file names.

NOTE
If you use the template FlowCellTemplateDemuxFields.csv, make sure to review and edit the demultiplexing options. Some options need values in order for demultiplexing to work properly (`allowed-mismatches, min-delta, quality-mask-threshold, output-types, undetermined-sample-name, and metric-prefix`).

Denature and Dilute Libraries

Consumables

- Molecular-grade water
- TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- [Recommended] 10 nM PhiX DNA Control from Singular Genomics, part number 700,120

Procedure

The following procedure is an example dilution starting with 4 nM libraries or higher and resulting in 15, 20, 25, and 30 pM libraries ready for loading onto the instrument, with 2% PhiX control (optional). Prepare 330 µL diluted library per lane of a flow cell, so you can load a minimum of 300 µL per well of the sample cartridge.

The final dilution of the library may need to be optimized for different samples and applications. For other starting concentrations and variations, see the Loading Concentration Calculator.

Adding a low-percentage PhiX control to your library is optional but recommended. Adding 2%–4% PhiX control to each lane acts as a sequencing control and helps with troubleshooting. Always use the Singular Genomics PhiX DNA Control as PhiX control. Other PhiX libraries may not contain the proper indices and adapters. Be aware that due to relative template seeding density differences between the sample library and PhiX spike-in, you may have to empirically adjust slightly to achieve the desired number of PhiX reads.

1. Make fresh 0.2 M NaOH in a microcentrifuge tube and invert tube several times to mix. Use within 12 hours.

	Volume
Molecular-grade water	800 µL
1 M NaOH	200 µL
TOTAL	**1 mL**

2. If the starting library concentration is higher than 4 nM, dilute the library in a microcentrifuge tube to 4 nM with TE. Vortex and then centrifuge briefly.

3. Dilute the optional 10 nM Singular Genomics PhiX control in a microcentrifuge tube to 150 pM with TE. Vortex and then centrifuge briefly. Store unused diluted PhiX control in 10 µL aliquots in 0.2 mL tubes at -25°C to -15°C to minimize freeze-thaw cycles.
4. Denature the library and PhiX control in separate new microcentrifuge tubes. Vortex and then centrifuge briefly.

	Sample Library (4 nM)	PhiX Control (150 pM)
Library	8 µL	5 µL
0.2 M NaOH	8 µL	5 µL
Total volume, final concentration	**16 µL, 2 nM**	**10 µL, 75 pM**

5. Incubate for 5 minutes at room temperature.
6. To the same microcentrifuge tubes, add 200 mM Tris-HCl pH 7.0 to neutralize, then vortex to mix and centrifuge briefly.

	Sample Library (2 nM)	PhiX Control (75 pM)
200 mM Tris-HCl pH 7.0	8 µL	5 µL
Total volume, final concentration	**24 µL, 1.3 nM**	**15 µL, 50 pM**

7. To the same microcentrifuge tubes, add water to dilute the sample library to 200 pM, then vortex to mix and centrifuge briefly.

	Sample Library (1.3 nM)	PhiX Control (50 pM)
Molecular-grade water	136 µL	-
Total volume, final concentration	**160 µL, 200 pM**	**15 µL, 50 pM**

8. Place the 200 pM library and optional PhiX control on ice until ready to proceed.
9. Dilute the 200 pM library and optional PhiX control in Sample Loading Buffer to the desired loading concentration in 330 µL per lane in a new microcentrifuge tube. Vortex and then centrifuge briefly. Loading concentrations of 15 pM, 20 pM, 25 pM, and 30 pM are shown but final concentration may differ for your application. We recommend a titration run for determining the optimal loading condition when you do a new application.

	Library				Library + 2% PhiX			
	15 pM	20 pM	25 pM	30 pM	15 pM	20 pM	25 pM	30 pM
Water	140.2 µL	132 µL	123.7 µL	115.5 µL	138.7 µL	130.1 µL	121.3 µL	112.5 µL
Library, 200 pM	24.8 µL	33 µL	41.3 µL	49.5 µL	24.3 µL	32.3 µL	40.4 µL	48.5 µL
2x Sample Loading Buffer	165 µL	165 µL	165 µL	165 µL	165 µL	165 µL	165 µL	165 µL
Optional PhiX control, 50 pM	-	-	-	-	2.0 µL	2.6 µL	3.3 µL	4.0 µL
TOTAL	**330 µL**	**330 µL**	**330 µL**	**330 µL**	**330 µL**	**330 µL**	**330 µL**	**330 µL**

If you plan to load the diluted library in multiple lanes, multiply the volumes by the number of lanes.

10. Keep on ice until ready to use, no longer than 2 hours.

See also the video: How to Prepare Your Library For Loading

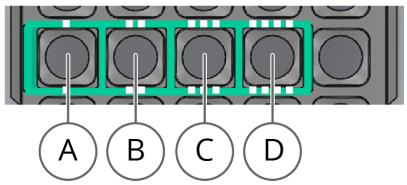
Prepare Sample Cartridge

1. Obtain a sample cartridge for each flow cell.
2. Equilibrate the sample cartridge to room temperature for 30 minutes.
3. Remove the sample cartridge from the sealed bag. Avoid extended light exposure.
4. Pipette 300 µL diluted library into each well in row 1. Make sure that you load libraries into the appropriate well associated with each lane as listed in the sample sheet. Make sure to pipette to the bottom of the well and avoid introducing bubbles. The sticker on the front of the sample cartridge has space for writing down the sample name for each well.
5. Set aside until prompted to load sample cartridges onto the G4 Sequencing Platform.

See also the video: How to Load Samples in The Sample Cartridge

Prepare Reagent Cartridge

1. Invert reagent cartridges 5–10 times while holding the lid on, then gently tap the cartridge on the bench surface.
2. If using custom sequencing primers, load them in the correct wells on the reagent cartridge the following way:
   a. Find the appropriate wells for your custom sequencing primers, which are marked by the green sticker, and are used in the following order:



A Custom read 1 primer
B Custom read 2 primer
C Custom Index 1 primer
D Custom Index 2 primer

   b. Pierce the foil of the wells to be used with a DNAse-free 1 mL pipette tip.
   c. Load 2 mL of each 1 µM custom sequencing primer in the appropriate wells.

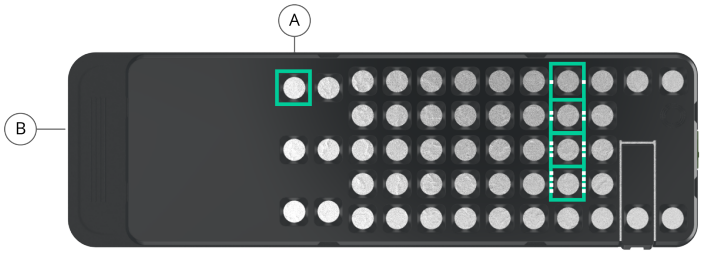
NOTE
If you need to dilute the custom sequencing primers, make sure to use the 5X Primer Annealing Buffer (5X PAB) to arrive at a final buffer concentration of 1X PAB. For example, for diluting a 100 µM primer stock to 1 µM final concentration, mix 1580 µL molecular-grade water, 400 µL 5X PAB, and 20 µL primer stock, and load in the custom sequencing primer well.

See also the video: How to Use Custom Primers

3. Place one tube of Quenching Solution per reagent cartridge in a room temperature water bath for 5 minutes.
4. Dry the tube. Make sure the Quenching Solution has thawed completely; if needed, briefly warm the tube briefly with your hands. Invert the tube 10 times to mix.

NOTE
The Quenching Solution contains DMSO, which has a melting temperature close to room temperature.

5. Puncture the foil of the bottom left well.



A Well for Quenching Solution
B Handle

6. Add the appropriate volume of Quenching Solution into the well. If needed, briefly spin down the content.

G4 F2 or F3 Sequencing Kit	50 cycles	100 cycles	200 cycles	300 cycles
Volume to add	70 µL	90 µL	130 µL	170 µL

7. Mix the solution in the well 10 times with a 10 mL serological pipette, taking care to not introduce bubbles.
8. Dry up liquid on the exterior of the well and gently tap the cartridge on the table to release any bubbles.
9. Set aside until prompted to load reagent cartridges onto the G4 Sequencing Platform.

See also the video: How to Prepare Reagent Cartridges

Set Up a Run

There are a number of different ways you can set up a run. The major differences in run setup are listed below:

- You can load an existing run, see *Load an Existing Run* on page 34.
- You can set up a new run, see *Set Up a New Run* on page 32. When setting up a new run, you can define sample data a few different ways:
  - Upload a sample sheet before the run
  - Define the samples while setting up the run
  - No definition of samples. Note that without index information the G4 Sequencing Platform does not perform on-instrument demultiplexing.

The main run setup options for the G4 Sequencing Platform are illustrated in Figure 15.

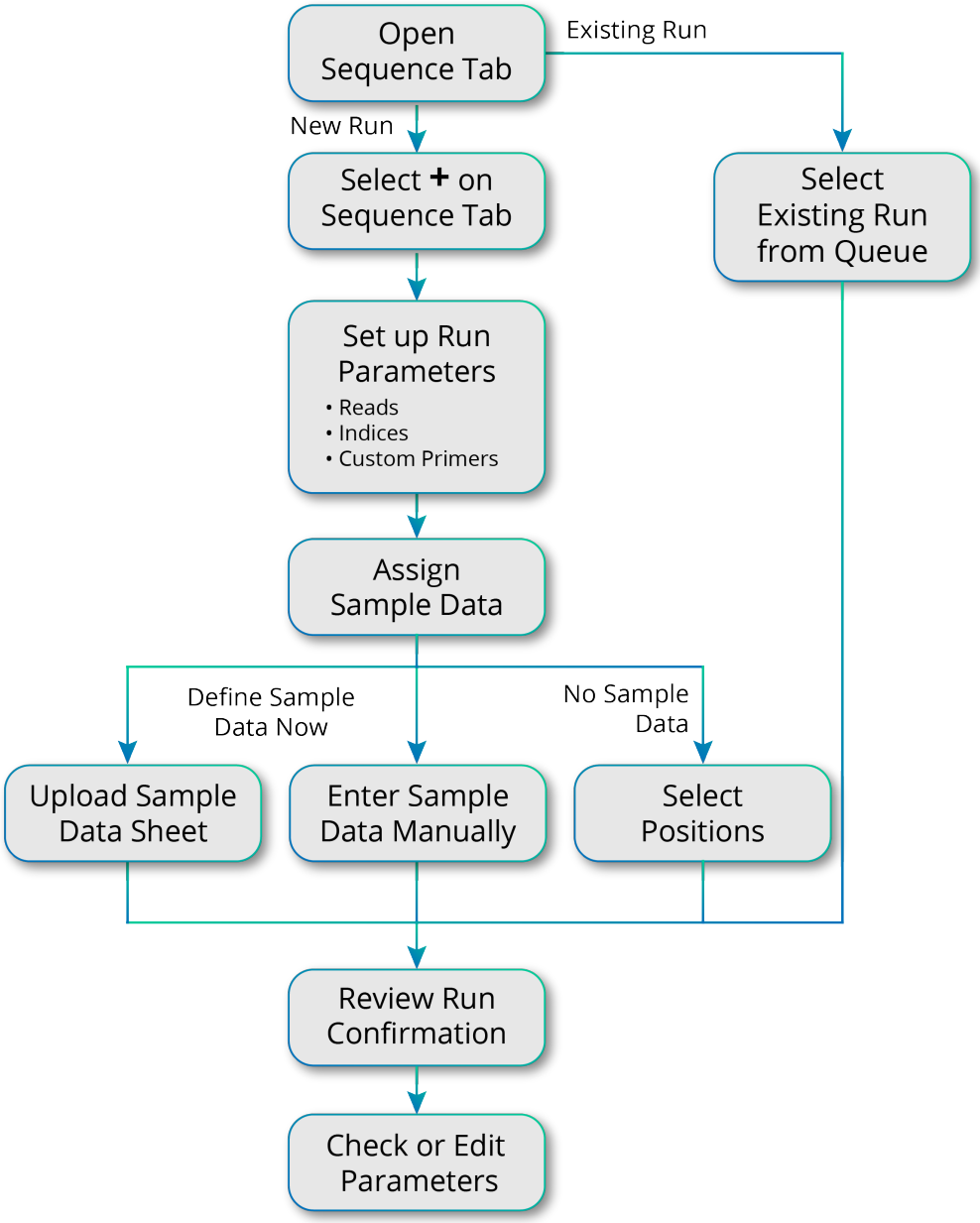


Figure 15 Main run setup options for the G4 Sequencing Platform.

Set Up a New Run

To specify parameters for a new run, use the G4 SeqOS. Tap the Singular Genomics icon on the instrument touchscreen to show the main menu.

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.

Specify Run Parameters

1. Select **Sequence** from the main menu.
2. To open The New Run screen select **+**.
3. Assign a name for the run.
4. Specify the run type: **Single** or **Paired**.
5. Enter the number of cycles for Read 1, and if a Paired run, the number of cycles for Read 2 (maximum 150 cycles per read).
6. Specify if the run requires Index Reads: **None** for no indexing, **1** for a single-indexed run, or **2** for a dual-indexed run.
7. Click Add Custom Primers if you want to use custom sequencing primers for the read or index.
   + Add Custom Primers
8. If the run is indexed, enter the number of cycles for any index reads, 8 to 24 base pairs.
9. Select the **forward arrow** → to proceed.

   NOTE
   Do not enter an index length that is longer than the physical length of the indices. This may interfere with the binding of the sequencing primers in subsequent sequencing steps.

Specify Sample Data

1. Select a category for assigning the sample data: **Now** or **Not Required**.
2. If assigning the sample sheet now:
   a. Select the **upload button** ↑ to open the Sample Data Upload screen.
   b. Browse to a folder or use search to locate the sample sheets for the flow cell.

      If browsing to a network location, specify the permissions required for the folder as needed.
   c. Select the button to the right of each sample sheet file name intended for the flow cell.
   d. Repeat step c until all sample sheets are added.
3. Select the **forward arrow** → to proceed.

   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.

CAUTION
Do not combine lanes with indexed libraries and lanes with non-indexed libraries in the same run. This results in dark clusters for the non-indexed library during the index read and will compromise the output data.

Review Run Setup

1. Review parameters specified for the run.
2. 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.

3. Select the **forward arrow** → to proceed. The instrument automatically prepares for loading.

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 G4 SeqOS 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.

CAUTION
Do not reload a run that has already been started once but not finished, for example due to an issue or changed parameter. In that case, generate a new run.

Load Consumables

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

CAUTION
Make sure not to pinch your fingers when closing doors or drawers of the consumable compartments.

Empty Waste Reservoir

The G4 Sequencing Platform comes with an internal waste reservoir, but you can also order an external waste reservoir (Contact Singular Genomics, see *Customer Care* on page 72). The external waste reservoir holds more waste and is needed for Max Read sequencing. 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 35.

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. Be aware reservoir may be full.

WARNING
Make sure to 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.

WARNING
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.

See also the video: How to Dispose of Waste on the G4 Sequencing Platform

Empty External Waste Reservoir

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

      WARNING
      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.

   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 G4 Sequencing Platform 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 64 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.

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

3. Carefully remove each new flow cell from the packaging and remove the seal tape covering the inlet and outlet ports.
4. Hold the 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 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 are often hard to see. Running a gloved finger carefully at the rear makes it easier to detect.

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.

   See the *Handling and Loading Flow Cells* video, also available here: https://singulargenomics.com/videos/, under *G4 Support*.

   NOTE
   If the G4 Sequencing Platform 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 64 for more information.

7. Select the **forward arrow** → to proceed. The instrument software confirms that each flow cell is fully engaged. A prompt appears if reseating is necessary.

8. 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 65. Select the **forward arrow** → to proceed.
9. 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 8

See also the video: Handling and Loading Flow Cells

Load Reagent Cartridges

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

CAUTION
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 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 G4 Sequencing Platform 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 64 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 20).
4. Empty the remaining contents of the reagent cartridge into the appropriate hazardous waste container.

WARNING
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.

See also the video: How to Dispose of a Used Reagent Cartridge

Load Sample Cartridges

1. Remove used sample cartridges from the previous run.

2. Lightly tap each sample cartridge before loading to remove bubbles from the sample wells.
3. Load each sample cartridge into the slot that corresponds with the flow cell position associated with that sample as recorded in the sample sheet. Ensure that the front edge of the sample cartridge rests behind the front lip of the slot. Select the **forward arrow** → to proceed.

   NOTE
   If the G4 Sequencing Platform 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 64 for more information.
4. Close the Reagent Compartment door.
5. Select the **forward arrow** → to proceed. The Run Summary screen opens.

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 67.

   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 58 for more information.
4. When the system check is successfully completed, 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 Run Progress

Preliminary real-time run metrics begin to appear after base calling is complete for the first insert read. Periodic updates are available at intervals thereafter. The following metrics are available:

Metric	Description
Clusters PF	Number of index reads or insert reads passing the quality filter. For an explanation of the filters used, see *Filtering* on page 41.
Yield	Amount of data produced for a specific sample measured in Gbp.
≥Q30	Percentage of bases with quality scores greater than or equal to Q30. For an explanation of quality scores, see *Quality Scores* on page 41.
% Cluster PF	Percentage of clusters passing filter. For an explanation of the filters used, see *Filtering* on page 41.

NOTE
Accuracy estimates for short reads (< 40 bp) may be imprecise because there is a greater probability of misalignment for short reads.

Metrics continue to change during the run. Final full run metrics are calculated when the run is finished (see *View Results* on page 42).

NOTE
You can also monitor run progress from a desktop using Singular Connect. See the Singular Connect User Guide for more information.

Post-Run Wash

An automatic post-run wash begins after the last sequencing cycle completes. The post-run wash cleans the lines and removes any contaminants from the previous run using wash reagents. The post-run wash takes approximately 20 minutes. After the post-run wash, the instrument is ready for the next sequencing run.

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 57.

Run Transfer Completed

Run data generated is automatically transferred from the G4 Sequencing Platform to the SCS, and from there to your off-instrument storage, as described in *Off-Instrument Storage* on page 11.

NOTE
Do not start post-processing or customer scripts until the data transfer is complete.

There are two ways to see if the data transfer to your off-instrument storage is complete:

- Look for the presence of the file transfer_complete in the run folder in your off-instrument storage.
- Sign up for email notifications from SCS. SCS can be set up to send email notifications when the data transfer is completed. Contact Singular Genomics Customer Care for more information.

  SCS uses the email address *run-completion@sgconnect.link*, make sure your IT department white lists this email address.

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. Sequencing output is generated in FASTQ files, and is stored in the Run Folder, along with a number of report, log, and metric files.

Quality Scores

The Phred quality score (Q score), a common metric, measures base calling accuracy and is a standard for assessing data quality in sequencing technologies. During sequencing, each base called in a read is assigned a Q score. Higher Q scores indicate a smaller probability of error. For example, a Q score of 30 (Q30) indicates the probability of an incorrect base call is 1 in 1000 or a base call accuracy of 99.9%.

Sequencing on the G4 Sequencing Platform has been designed to deliver a base call accuracy of Q30 for at least 85% of bases depending on application and sample quality.

Filtering

The Q-scores are used to filter out low-quality reads and mask low quality base calls with an N. This filter is automatically applied to reads of 35 bases or more and no filter is applied to reads with fewer than 35 bases. The following filtering methods are used:

Read	Filter	Description
Insert	Mean QS Filter < Q30	Remove insert read if the mean Q-score for the first 30 cycles is lower than Q30. Note that for Max Read runs, the insert is removed if the mean Q-score for the first 30 cycles (or full read if it is shorter than 30 cycles) is lower than Q28.
Insert	Quality Score Base Filter < Q10	Mask all base calls that have a Q-score below Q10 with an N.
Index	Mean QS Filter < Q17	Remove index read if the mean Q-score for all cycles is lower than Q17.

By default, reads are only reported in the final FASTQ files if all of the reads of one cluster pass the Mean QS Filter. For example, indexed paired reads only get reported if both index reads have a mean Q-score over Q17, and both insert reads a mean Q-score over Q30 for the first 30 cycles.

For customizing N-masking threshold, see *Custom Demultiplexing Settings in the Sample Sheet* on page 27.

FASTQ Files

The results of a sequencing run are recorded in FASTQ files. A FASTQ file is a text-based file format that contains information about the read, sequencing data from clusters that pass filter, and a quality value for each base in the sequence.

One FASTQ file is generated for each lane of the flow cell and for each read in the sequencing run. For example, a single-read run on one four-lane flow cell generates four FASTQ files. A paired-read run on the same flow cell generates eight FASTQ files.

For indexed libraries, a demultiplexing step first assigns clusters to a sample based on the index sequences reported in the sample sheet. After demultiplexing, FASTQ files are then generated for each sample in a lane.

FASTQ files use a compressed format with the extension **.fastq.gz* and can be several megabytes or gigabytes in size. They are used as input for performing downstream analysis, such as alignment and assembly.

View Results

The Results Screen provides access to metrics, starting when the first read of sequencing is complete. A summary of metrics per flow cell are displayed on the flow cell tabs of the Results Screen.

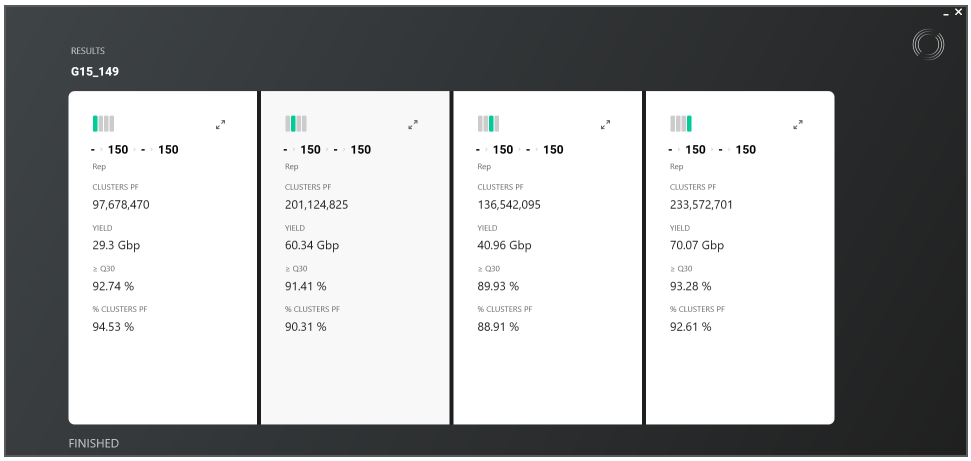


Figure 16 Results Screen

Clicking on a flow cell tab allows access to Results tabs with more detailed information.

NOTE
You can also view results from a desktop using Singular Connect. See the Singular Connect User Guide for more information.

Results Tabs

The Results Tabs allow access to metrics by read, index, lane, and sample on four tabs for each flow cell. Click the chevrons ( ) to browse through the flow cells. Click the paired/unpaired toggle (Paired Unpaired) to see the metrics from paired or unpaired data, when available. Note that until analysis is complete, SeqOS displays only throughput and Q30 metrics from unpaired data.

Figure 17 Results Header

NOTE
The results reported for a Max Read run include rounds, which represent the sequencing reads for one fraction of a combined library. The metrics reported in each Max Read round are similar to a standard run. For more information, see the *Max Read Kit User Guide*, available from https://singulargenomics.com/g4/support/.

Results Summary Tab

The Summary Tab displays metrics by read and, if used, index, for one flow cell. For Max Read runs the metrics are displayed by round as well. The Summary Tab also provides an overview of the consumables used.

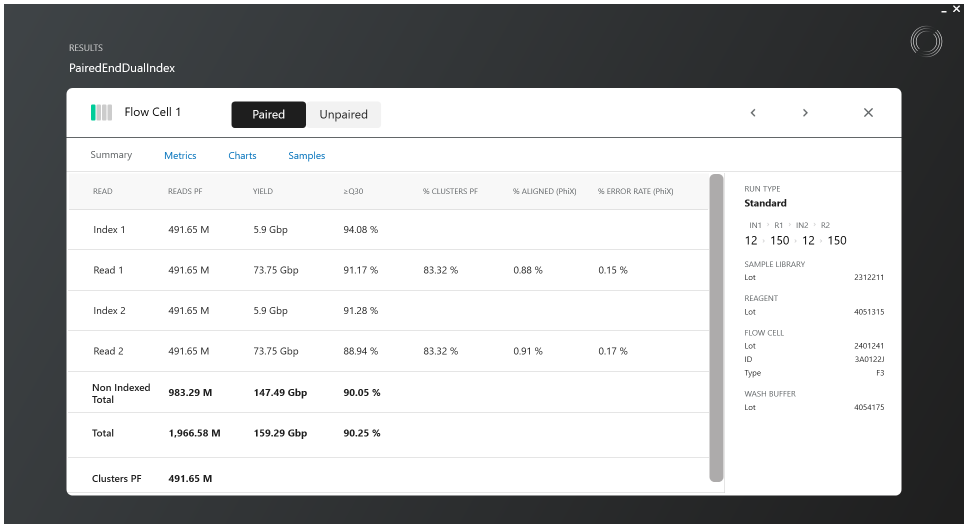


Figure 18 Results Summary Tab

Results Lane Metrics Tab

The Lane Metrics Tab displays detailed metrics by lane for one flow cell.

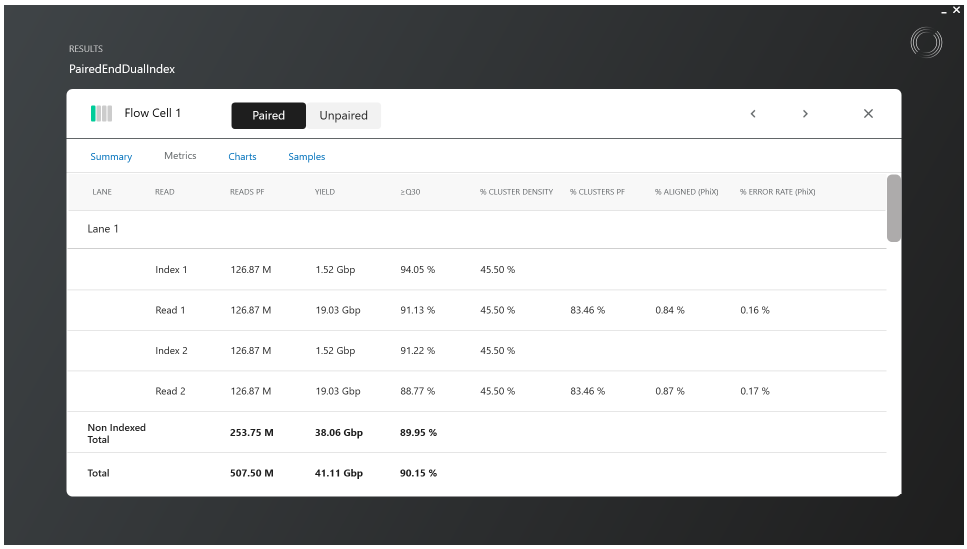


Figure 19 Results Lane Metrics Tab

Results Charts Tab

The Charts Tab displays the Q-score by Cycle Graph by read and lane for one flow cell.

NOTE
During the run, the Graph shows Q-scores from unpaired data. After analysis is complete, the Q-scores are from paired data.

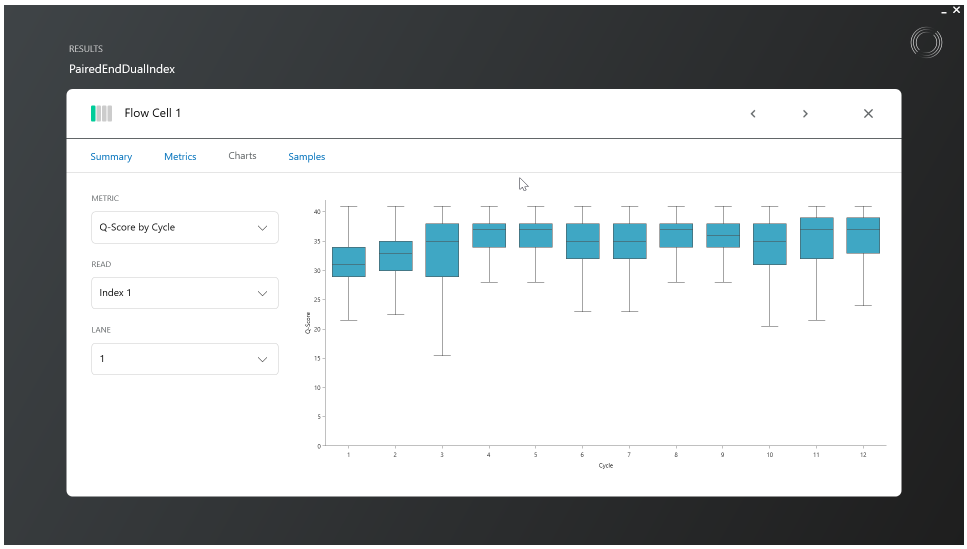


Figure 20 Results Charts Tab

Results Samples Tab

The Samples Tab provides information by sample present on one lane of one flow cell.

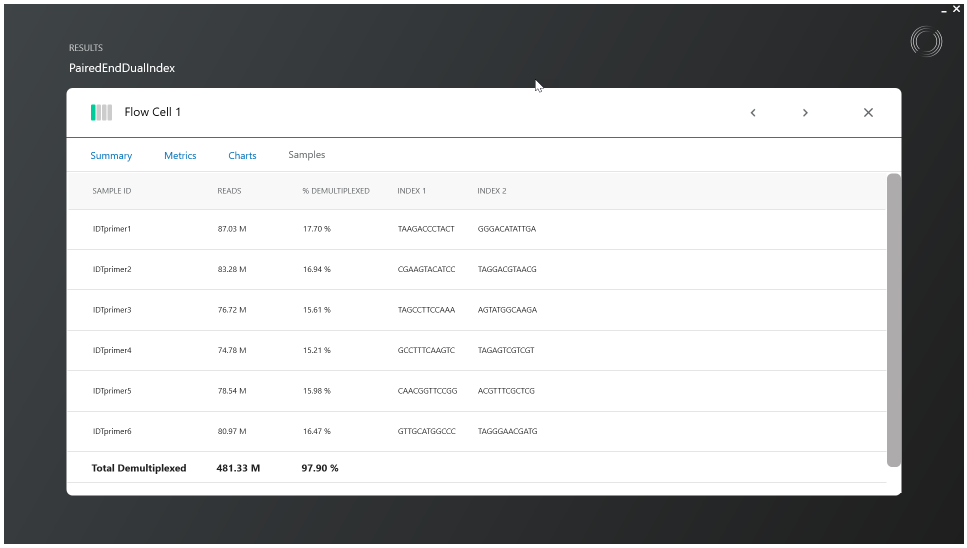


Figure 21 Results Samples Tab

Results Metrics

The following metrics are displayed on the Results Screen and Tabs:

Metric	Results Screen	Summary Tab	Metrics Tab	Description
Reads PF	y	y	y	Number of index reads or insert reads passing the quality filter. For an explanation of the filters used, see *Filtering* on page 41.
Yield	y	y	y	Amount of data produced for a specific sample measured in Gbp.
≥Q30	y	y	y	Percentage of bases with quality scores greater than or equal to Q30. For an explanation of quality scores, see *Quality Scores* on page 41.
% Cluster Density	n	n	y	Number of detected clusters divided by number of wells, expressed as percentage.
% Cluster PF	y	y	y	Percentage of clusters passing filter. For an explanation of the filters used, see *Filtering* on page 41.
% Aligned (PhiX)	-	-	y	The percentage of reads that align to a PhiX control.
% Error rate (PhiX)	y	y	y	Estimated percentage of miscalled bases based on PhiX control.

Run Folder

When the sequencing run has finished, the G4 Sequencing Platform writes a number of output files to the Run Folder. The Run Folder is saved on the instrument computer and in a remote location, specified during run setup (*Review Run Setup* on page 33).

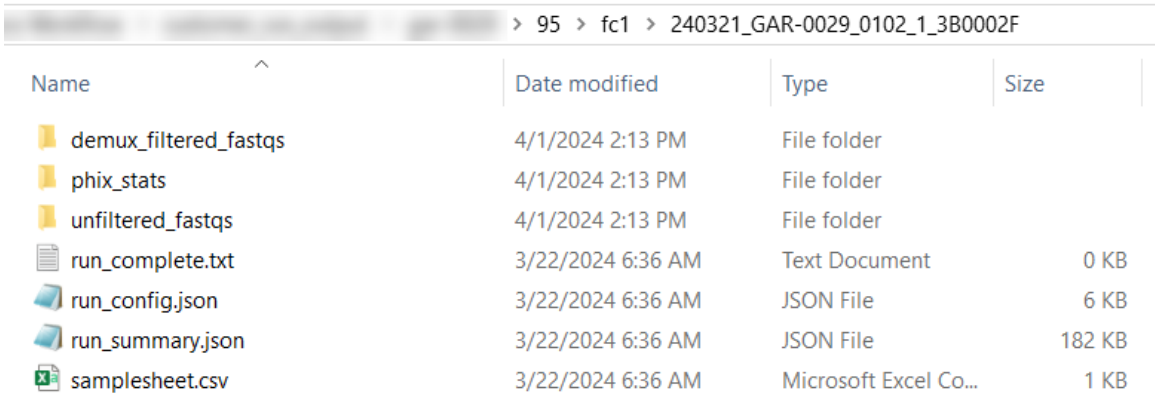


Figure 22 Run Folder after demultiplexing

The Run Folder has the following naming convention, with the different elements described in Table 1:

```
<Date>_<InstrumentSerialNumber>_<RunNumber>_<FlowCellPosition>_
<FlowCellSerialNumber><RunConfigID>
```

Table 1 Description of Naming Convention Elements for Run Folders

Run Folder Name Element	Description
Date	Date in format YYMMDD.
InstrumentSerialNumber	Serial number of the instrument used.
RunNumber	Number of the run on the particular instrument.
FlowCellPosition	Position of the flow cell on the instrument (1 through 4)
FlowCellSerialNumber	Serial number of the flow cell used.
RunConfigID	Run configuration ID, for troubleshooting purposes.

For example, this is a valid Run Folder name:

```
220622_G4X1234_0057_2_FC000007A
```

The Run Folder contains the following sub-folders and files:

Table 2 Run Folder Contents Description

Name	Purpose	Description
demux_filtered_fastqs	Folder	Contains the FASTQ files after demutliplexing. See *Demultiplexed FASTQ Files* on page 46.
logs	Folder	Contains the log files for the run. See *Run Logs* on page 1.
phix_stats	Folder	Contains the PhiX statistics for the run.
thumbnails	Folder	Contains thumbnails of images of the flow cell during the run.
unfiltered_fastqs	Folder	Contains the FASTQ files with raw sequencing data before any filtering or demultiplexing. See *Unfiltered FASTQ Files* on page 48.
samplesheet.csv	Run parameters file	*Sample Sheet* on page 17*Sample Sheet* on page 17
run_config.json	Run parameters file	Parameters used to start the sequencing run, including date-time stamp, flow cell ID, operator ID , read and index lengths, and instrument serial number.
run_summary.json	Metrics file	File with run summary information.
run_complete.txt	Flag file	Flag generated when all sequencing operations are complete.
transfer_complete.txt	Flag file	Flag generated when copying the files to the remote location is complete.

Demultiplexed FASTQ Files

The demux_filtered_fastqs folder in the Run Folder contains the FASTQ files after filtering and demultiplexing. If a project was provided in the sample sheet, then the FASTQ files are saved in sub-folder

with the project name.

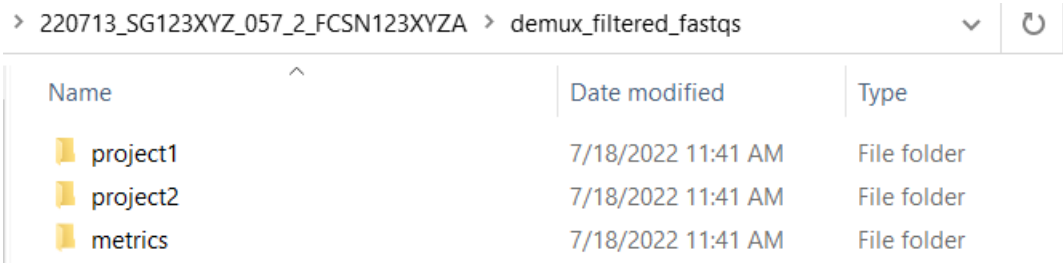


Figure 23 demux_filtered_fastqs folder

The FASTQ files are saved by sample, lane, and read, which is indicated in the filename. For example, **SID123XYZ_rep1**_S1_**L001_R1**.fastq.gz is the FASTQ file for SID123XYZ_rep1 in lane 1, read 1. The S1 indicates that this was the first sample in the sample sheet. If a FASTQ file is compiled from different lanes, the lane designator is set to L000 (for example, SID123XYZ_rep1_S1_**L000**_R1.fastq.gz).

See *FASTQ Files* on page 41 for a description of FASTQ files.

Demultiplexing Metrics

The demux_filtered_fastqs folder in the Run Folder also contains the demultiplexing metric files in the metrics folder.

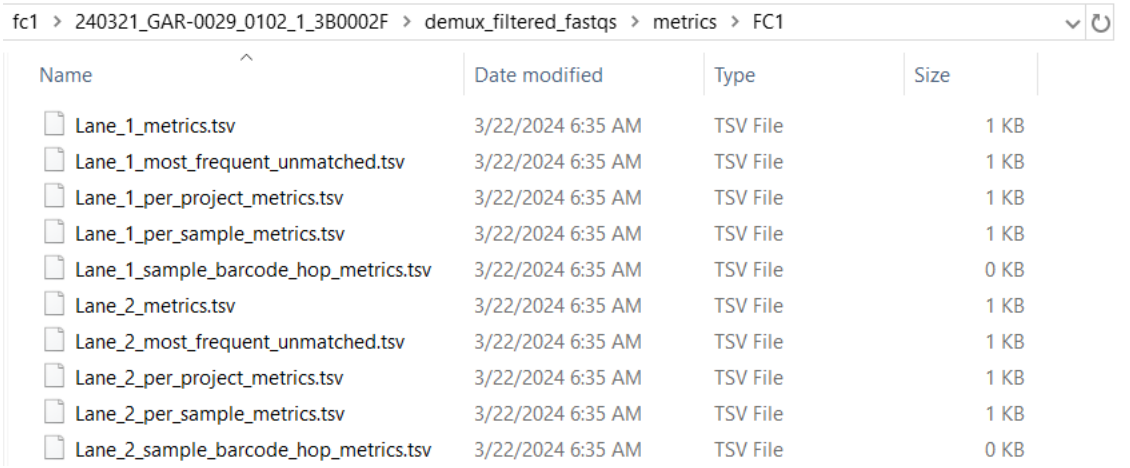


Figure 24 demux_filtered_fastqs folder

The metrics folder contains the following demultiplexing metrics files, saved by flow cell and lane:

File	Description
metrics.tsv, per_project_metrics.tsv, per_sample_metrics.tsv	Demultiplexing metrics by run, project, or sample: • Counts/percentages of demultiplexed reads with edit distance. • Counts/percentages of undetermined indices.
most_frequent_unmatched.tsv	Top undetermined indexes sorted descending by count.
sample_barcode_hop_metrics.tsv	Index hopping report reporting samples with known index1 matched with known index2, and counts.
demux_stdout_stderr_log.txt	Demultiplexing log file.

For more information about metrics, contact Customer Care (see *Customer Care* on page 72

Unfiltered FASTQ Files

The unfiltered_fastqs folder in the Run Folder contains the FASTQ files before filtering or demultiplexing. The FASTQ files are saved by lane and read or index (if used), which is indicated in the filename. For example, Undetermined_S0_**L003_R1**.fastq.gz is the unfiltered FASTQ file for lane 3, read 1, while Undetermined_S0_**L001_I2**.fastq.gz is for lane 1, Index 2.

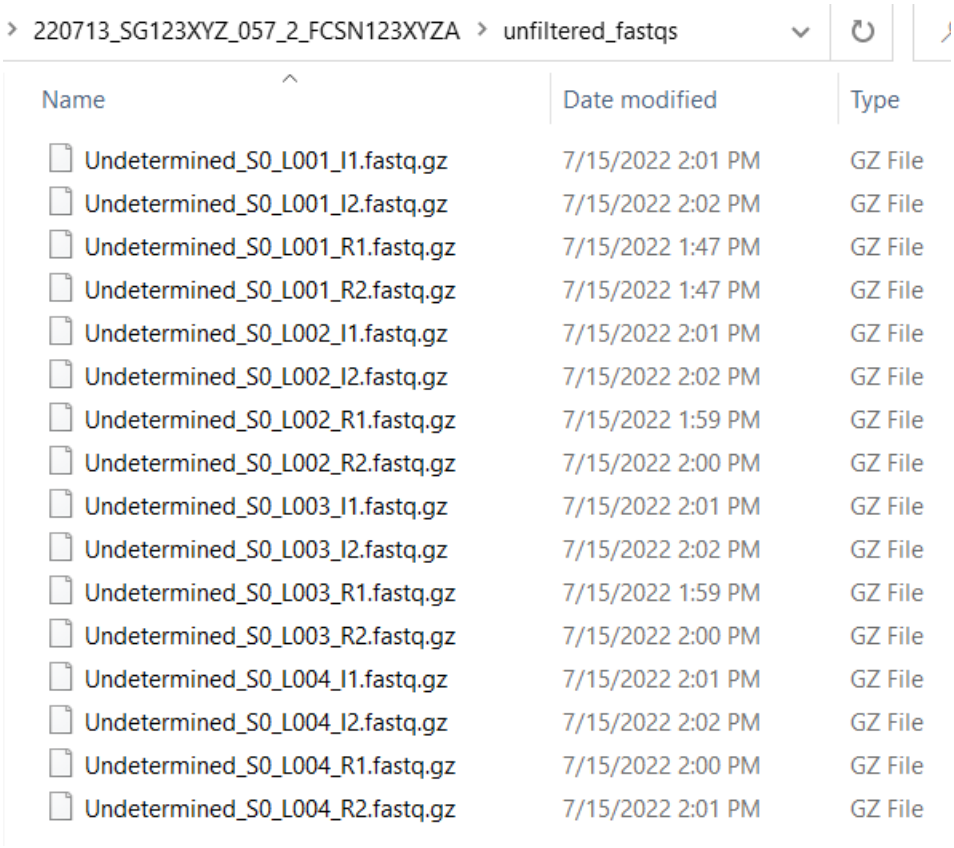


Figure 25 unfiltered_fastqs folder

See *FASTQ Files* on page 41 for a description of FASTQ files.

PhiX Metrics Generation and Location

The insert reads generated in each G4 run are aligned to the PhiX reference genome at cycle 40, and PhiX alignment metric files are saved in the *phix_stats* output folder. Because PhiX alignment starts at cycle 40, only insert reads that are 40 base pairs or longer generate PhiX statistics.

PhiX Alignment Metrics File

The metrics of the PhiX alignment are available in the multiqc_report.html, located in the phix_stats folder, organized by flow cell and lane.

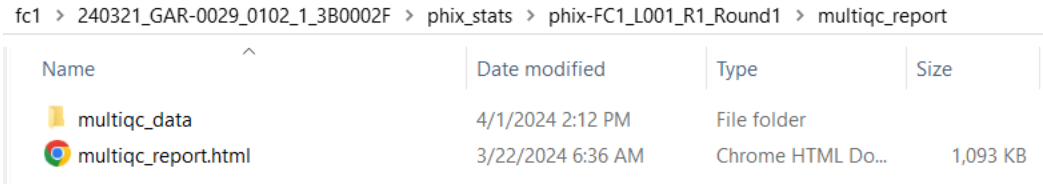


Figure 26 Location of the multiqc_report.html file.

The following metrics are reported:

Metric	Description
Aligned to PhiX	The percentage of reads that align to a PhiX control.
ErrorRateByReadPosition	Estimated percentage of miscalled bases based on PhiX control.
Mean Base Quality by Cycle	Plot showing the mean base quality by cycle. For an explanation of quality scores, see *Quality Scores* on page 41.
Error Rate by Read Position	Error rate by read position. You can also select for specific substitution types.

MAINTENANCE

In order to keep the G4 Sequencing Platform in good working order, you need to perform the maintenance tasks listed below regularly.

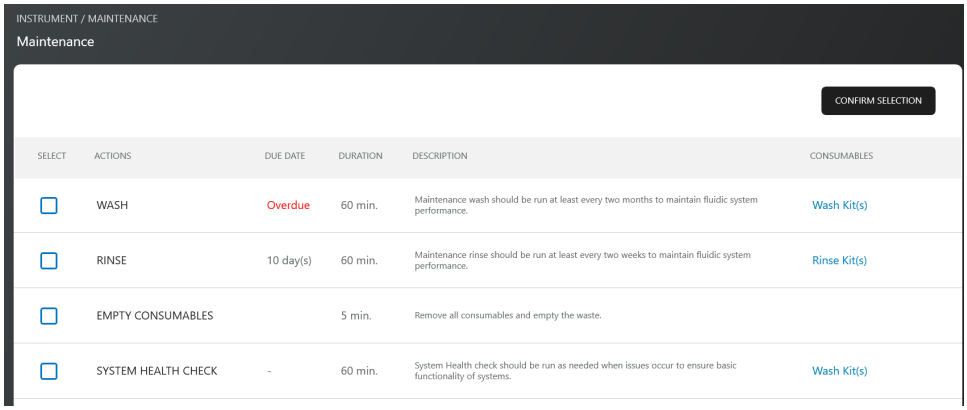


Figure 27 SeqOS Maintenance Screen. Selecting the Wash Kit(s) or Rinse Kit(s) links provides access to guides for loading the reagents.

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 50

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 700,002), which has the following components:

Item	Description	Quantity
Maintenance Sample Cartridge	G4 empty sample cartridge (reusable)	4
Maintenance Reagent Cartridge	G4 empty maintenance reagent cartridge (reusable)	4
Maintenance Wash Cartridge	G4 empty wash cartridge (reusable)	4
Maintenance Flow Cell	G4 maintenance flow cell (reusable)	4

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

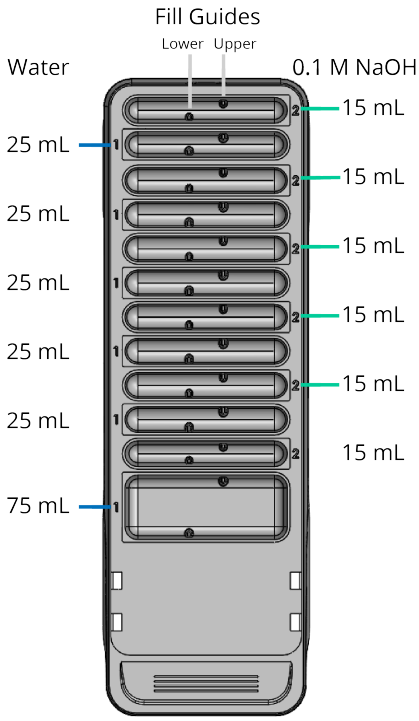
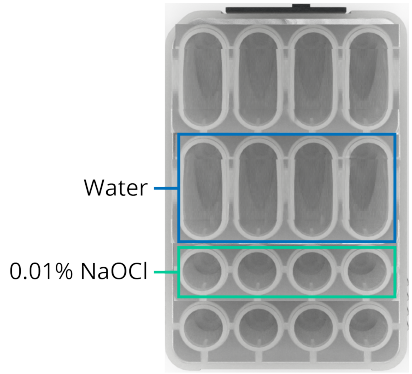
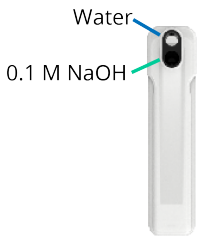
Performing a Maintenance Wash

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 water to remove any dust or particulates that may have accumulated in the cartridges.
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	

4. Start the wash protocol one of these two ways:
   - Select the **Start Wash** in the SeqOS prompt.
   - Go to the **Instrument | Maintenance** section in the G4 SeqOS and select **Wash**.
5. Unselect the positions you do not want to wash (see Figure 28). By default all positions are selected.

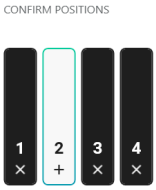


Figure 28 Confirming maintenance wash positions with positions 1, 3, and 4 selected, and position 2 unselected.

6. Load the consumables as prompted.

WARNING
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 as you can injure your hands.

CAUTION
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.

WARNING
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 G4 SeqOS. For an explanation of the flow cell position light colors, see *Flow Cell Compartment* on page 8
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.

CAUTION
Failure to clean and correctly store maintenance cartridges can result in fluidic system damage to the G4 Sequencing Platform.

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 50

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.
- G4 Maintenance Kit (part number 700,002), which has the following components:

Item	Description	Quantity
Maintenance Sample Cartridge	G4 empty sample cartridge (reusable)	4
Maintenance Reagent Cartridge	G4 empty maintenance reagent cartridge (reusable)	4
Maintenance Wash Cartridge	G4 empty wash cartridge (reusable)	4
Maintenance Flow Cell	G4 maintenance flow cell (reusable)	4

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

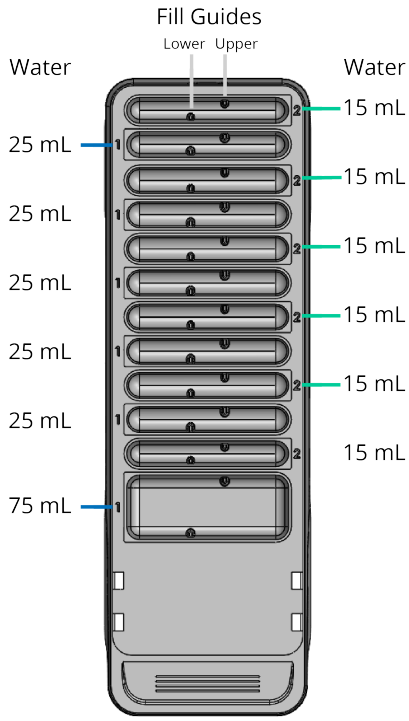
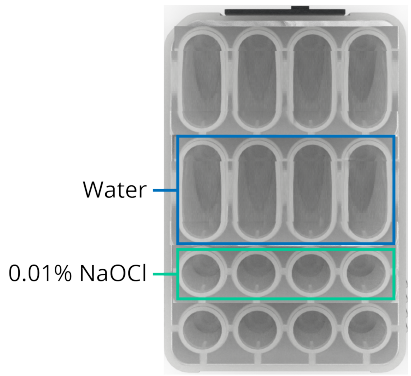
Performing a Maintenance Rinse

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

1. For each position you want to rinse, make fresh 2 mL 0.01% sodium hypochlorite by diluting sodium hypochlorite stock solution in water.

    If you are rinsing multiple positions, multiply volumes by the number of positions.
2. Rinse all wells of the maintenance cartridges with water to remove any dust or particulates that may have accumulated in the cartridges.
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 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	0.01% sodium hypochlorite	0.4 mL per well	

Cartridge	Location (counted from top of picture)	Rinse Reagents	Volume	Image
Maintenance Wash Cartridge	Top (bulk) compartment	Water	200 mL	
	Small rinse well	Water	9 mL	

4. 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 G4 SeqOS and select **Rinse**.
5. Unselect the positions you do not want to rinse (see Figure 29). By default all positions are selected.

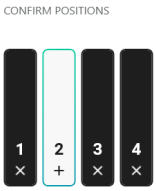


Figure 29 Confirming maintenance rinse positions with positions 1, 3, and 4 selected, and position 2 unselected.

6. Load the consumables as prompted.

WARNING
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 as you can injure your hands.

CAUTION
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.

WARNING
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 G4 SeqOS. For an explanation of the flow cell position light colors, see *Flow Cell Compartment* on page 8
8. 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. Make sure no 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.

CAUTION
Failure to clean and correctly store maintenance cartridges can result in fluidic system damage to the G4 Sequencing Platform.

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 35.

1. Go to the **Instrument** | **Maintenance** section in the G4 SeqOS 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. Be aware reservoir may be full.

WARNING
Make sure to 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.

WARNING
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.

See also the video: How to Dispose of Waste on the G4 Sequencing Platform

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 G4 SeqOS and select **Empty Consumables**.
2. Follow the instructions in the G4 SeqOS.

Computer Maintenance

In order to keep the G4 Sequencing Platform 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 G4 Sequencing Platform 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 G4 local storage once the data has been moved to your off-instrument storage.

NOTE

The G4 Sequencing Platform 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, like a 1X50 run. However, if you plan to do a bigger run, such as a 2X150 run, you should delete older data to make space for the current run.
- If you get a red warning, you should delete older data to make space for the current run before proceeding.

Clean up the G4 local storage the following way:

1. Check if the run has been fully moved to your off-instrument storage location.
2. If your run was an indexed run, check if the demultiplexing process has yielded results as expected. If not, follow the instructions described in *Demultiplexing Off-instrument* on page 63.
3. Click the More Options icon at the end of the line (•••).
4. Select **Delete**.

The G4 SeqOS (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 G4 Sequencing Platform 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 G4 SeqOS 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 G4 Sequencing Platform. These are all managed by Singular Genomics.

CAUTION
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: Alternation or changes to the system configuration, drivers, or other similar changes can directly affect reliability and performance of the G4 Sequencing Platform. 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 G4 Sequencing Platform 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 700,002), which has the following components:

Item	Description	Quantity
Maintenance Sample Cartridge	G4 empty sample cartridge (reusable)	4
Maintenance Reagent Cartridge	G4 empty maintenance reagent cartridge (reusable)	4
Maintenance Wash Cartridge	G4 empty wash cartridge (reusable)	4
Maintenance Flow Cell	G4 maintenance flow cell (reusable)	4

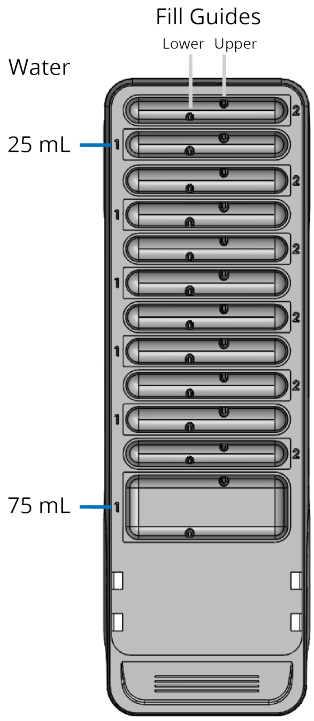
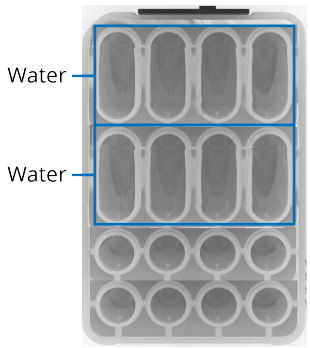
- Instead of the Maintenance Flow Cell, you can also use a regular used flow cell for each position that needs a maintenance wash or rinse.

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	

Cartridge	Location (counted from top of picture)	Reagent	Volume	Image
Maintenance Wash Cartridge	Top (bulk) compartment	Water	200 mL	

3. Go to the **Instrument | Maintenance** section in the G4 SeqOS 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 30). By default all positions are selected.

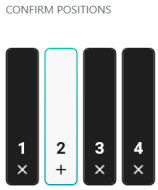


Figure 30 Confirming system health check positions with positions 1, 3, and 4 selected, and position 2 unselected.

5. Load the consumables as prompted.

WARNING
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 as you can injure your hands.

CAUTION
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.

WARNING
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 G4 SeqOS. For an explanation of the flow cell position light colors, see *Flow Cell Compartment* on page 8
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.

CAUTION
Failure to clean and correctly store maintenance cartridges can result in fluidic system damage to the G4 Sequencing Platform.

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.
- Error alerts appear in yellow and indicate that attention is recommended or that you need to acknowledge the alert before proceeding.
- Warning alerts appear in red and indicate that attention is required.

A list of troubleshooting topics is available at:

https://knowledge.singulargenomics.com/

Fixing Improper Demultiplexing

If you made an error in the sample sheet, for example, you entered the wrong index sequences, results will not have been demultiplexed properly. However, you can do the demultiplexing process again with a corrected sample sheet. Use one of the two procedures described in *Demultiplexing Off-instrument* on page 63. This does not take up instrument resources, and does not rely on all sequencing files being present on the instrument computer

Demultiplexing Off-instrument

If you have the unfiltered_fastq files, you can demultiplex your sequencing data using stand-alone demultiplexing software packages. Choose one of these two packages:

- Use the Singular-Demux software if you want fine level control over the various options for demultiplexing your files. The software has additional functionalities for working with your G4 Sequencing Platform FASTQ files.
- Use the Singular-Demux Folder Level Nextflow Workflow if you want an easy way to demultiplex your unfiltered_fastq data directory with your sample sheet using mostly default options.

Perform off-instrument demultiplexing the following way:

1. Correct the sample sheet.
2. Download and use one of these two packages to demultiplex off-instrument:

- Singular-Demux software, available at https://github.com/Singular-Genomics/singular-demux.
- Singular-Demux Folder Level Nextflow Workflow, available at https://github.com/Singular-Genomics/sgdemux_folder_nf2.

3. Follow the instructions available in the repository.

Cannot Select Sample Sheet

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

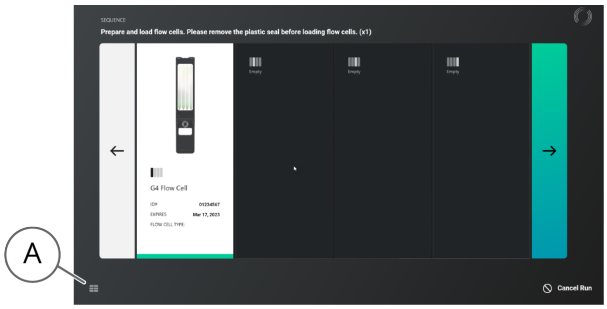
- Make sure the sample sheet is saved in a location SeqOS can access, and navigate to that location.
- Make sure there is no mismatch between the sample sheet parameters and the run parameters. For example, if the run format or custom primer settings are different, SeqOS cannot select the sample sheet.

Correct the sample sheet using the information in *Create a Sample Sheet* on page 25.

Product Identifier Not Recognized

If the G4 Sequencing Platform doesn't 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 31 Position of Product Properties Manual Entry Button.

The *Product Properties Manual Entry* screen opens (Figure 31).

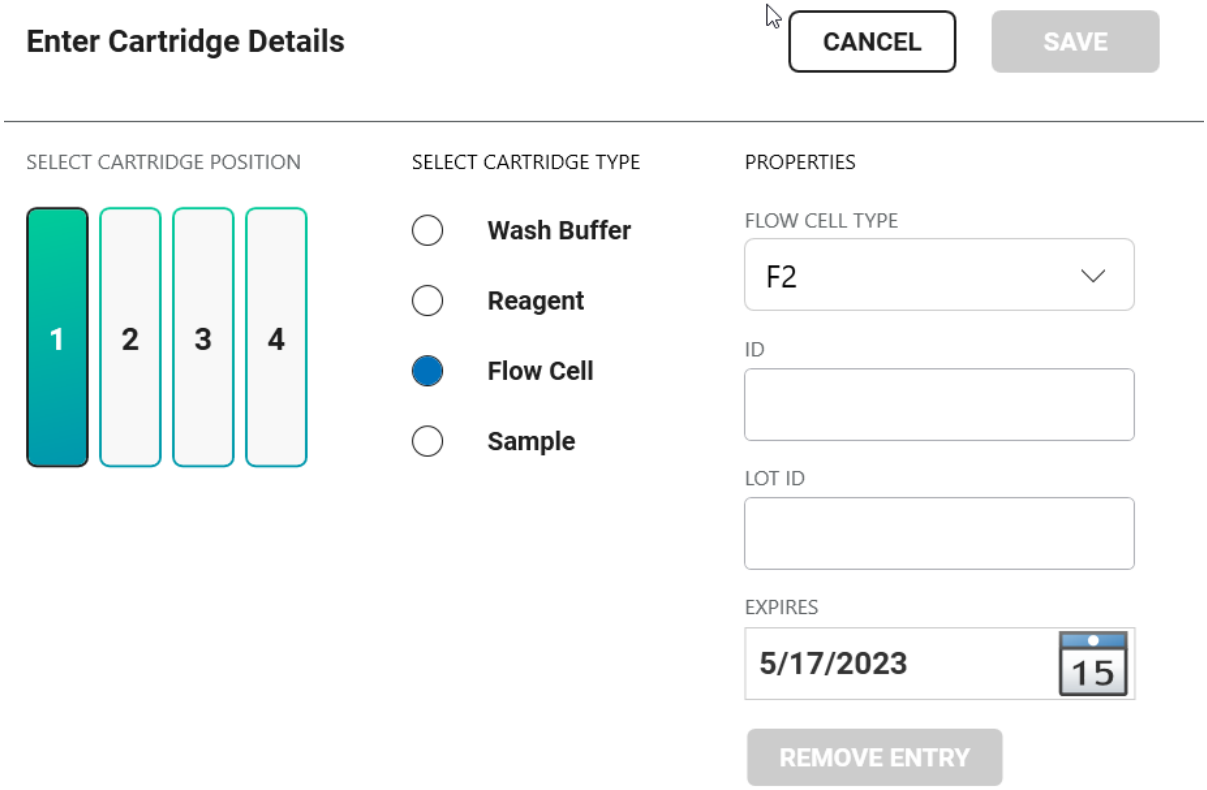


Figure 32 Product Properties Manual Entry Screen.

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.
   - The flow cell ID is also etched on the edge of the flow cell. Flip the flow cell to read the ID.
   - 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:

NOTE
See the *Handling and Loading Flow Cells* video, also available here: https://singulargenomics.com/videos/, under *G4 Support*.

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 33).

   NOTE
   Raised gaskets at the far end of the flow cell position are often hard to see. Running a gloved finger carefully at the rear makes it easier to detect.

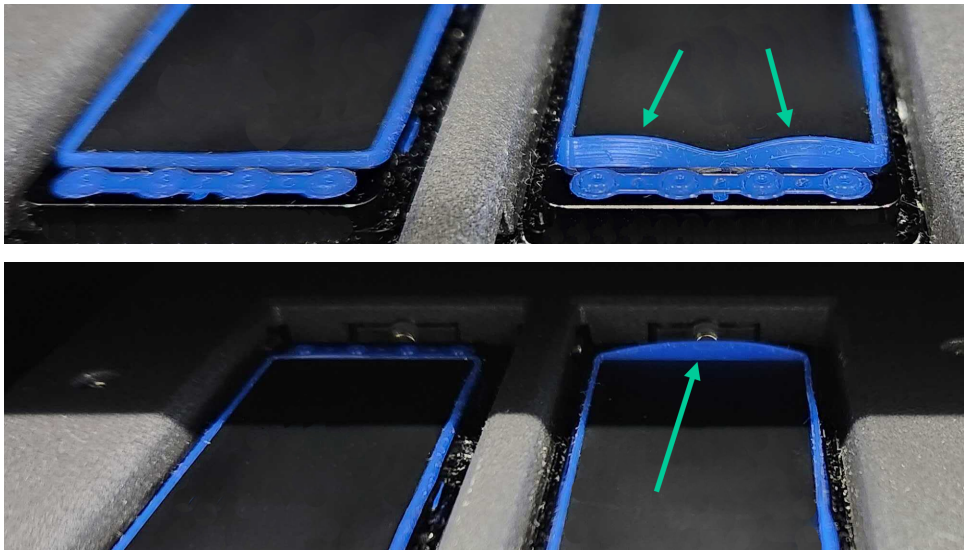


Figure 33 Raised gaskets at the front end and far end of a flow cell position, indicated by arrows.

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.

   NOTE

   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'.

CAUTION
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:

NOTE
See the *Handling and Loading Flow Cells* video, also available here: https://singulargenomics.com/videos/, under *G4 Support*.

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 34).

NOTE
Raised gaskets at the far end of the flow cell position are often hard to see. Running a gloved finger carefully at the rear makes it easier to detect.

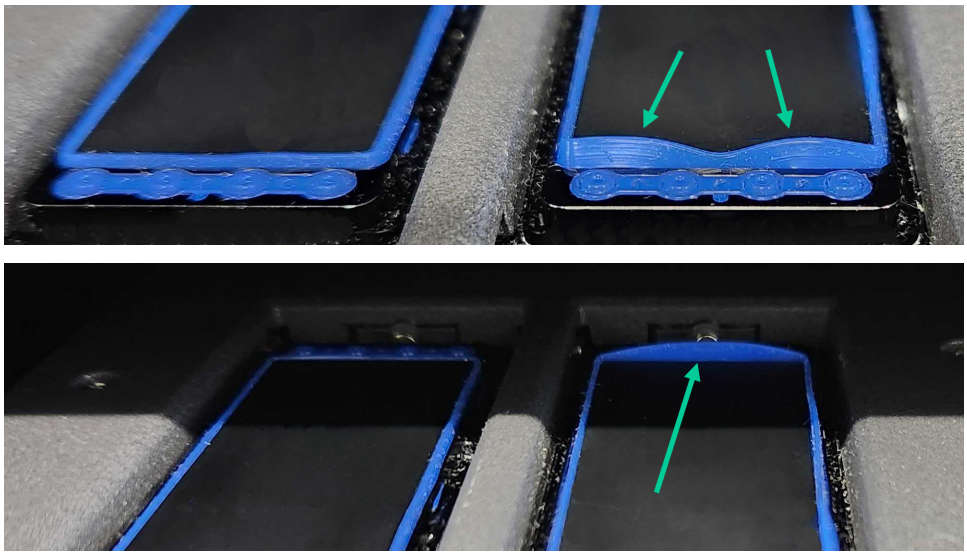


Figure 34 Raised gaskets at the front end and far end of a flow cell position, indicated by arrows.

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.

   NOTE

   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'.

CAUTION
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 G4 Sequencing Platform comes with an internal waste reservoir, but you can also order an external waste reservoir (Contact Singular Genomics, see *Customer Care* on page 72). The external waste reservoir holds more waste and is needed for Max Read sequencing.

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.

If the waste in the external waste container reaches the waste limit (around the 15 Liter mark), empty the waste container by performing the procedure described in *Empty External Waste Reservoir* on page 35.
See also the video: How to Dispose of Waste on the G4 Sequencing Platform

Window Freezes

If you click a button and it looks like nothing is happening, wait for 15 seconds before doing anything else. This will usually result in the window becoming responsive again.

Clicking 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 G4 Secondary 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 *G4 Secondary*.

   The indicator LED adjacent to the power button should turn on.
7. Wait for 7 minutes
8. Power on the G4 Primary Computer by pressing the power button on the front of the computer in the section labeled *G4 Primary*.
9. Wait 2 minutes.
10. Start SeqOS.

If the G4 Primary Computer fails to reboot or has a blank screen, see *Blank Screen After Restart* on page 70.

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 G4 Primary Computer by using the **Shut down** option in the Windows menu.
5. Power off the G4 Secondary Computer the following way:
   a. Press the power button on the front of the computer in the section labeled *G4 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 G4 and SeqOS. Follow these steps when advised by Customer Support:

1. Close SeqOS.
2. Power off the G4 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 G4 Primary Computer by pressing the power button on the front of the computer in the section labeled *G4 Primary*.
8. Wait 2 minutes.
9. Start SeqOS.

Blank Screen After Restart

If the G4 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 G4 Primary Computer by pressing the power button on the front of the computer in the section labeled *G4 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 G4 Secondary Computer by pushing the power button on the front of the computer in the section labeled **G4 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 **G4 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:

1. Power off the Secondary Computer by holding down the power button on the front of the computer in the section labeled **G4 Secondary**.

   Lights should turn off in about 10 seconds.
2. Wait 30 seconds.
3. Press the power button on the front of the computer in the section labeled **G4 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.

Website	singulargenomics.com
Email	care@singulargenomics.com
Telephone	442.SG.CARES(442.742.2737)
Address	Singular Genomics Systems, Inc. 3010 Science Park Rd San Diego, CA 92121 U.S.A.

See also the video: Singular Genomics Support and Customer Care

Additional Resources

Go to https://singulargenomics.com/g4/support/ to find the following additional resources:

Resource	Description
G4 Sequencing Platform Safety and Compliance Guide	Information about operational safety considerations, compliance statements, and instrument labeling.
G4 Sequencing Platform Site Preparation Guide	Information for preparing your site for delivery, installation, and operation of the G4 Sequencing Platform.
G4 Sequencing Kit Overview	Overview of available sequencing kit sizes, kit contents, and recommended use.
G4 Sequencing Platform Quick Reference Card	Instructions for setting up and using Singular Connect.
Singular Connect User Guide	Dynamic Quick Reference Card for running the G4 Sequencing Platform for experienced users.
Loading Concentration Calculator	Calculator that helps you set up denature and dilute steps.
Sample Sheet Generator	Tool that helps you fill out all data, which minimizes errors in the sample sheet.
G4 Best Practices and Quality Control Guide	Best practices for setting up your sequencing experiment on the G4 Sequencing Platform.
Adapters and Indices for the G4 Sequencing Platform Reference Guide	Instructions for using Singular adapters with library prep protocols.
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.

Resource	Description
Training videos	Visual instructions for run setup and instrument operation.
Singular Connect User Guide	Instructions and description for remote monitoring tool Singular Connect.

Revision History

Document #	Revision	Release Date	Description of Change
600001	Rev. 5	June 2024	• Added descriptions of SCS and SCN. • Rewrote Off-Instrument Storage and moved it to the SCS section. • Added references to Singular Connect. • Updated sample sheet and sample sheet template locations and upload procedure. • Updated instructions to load 300 µL diluted library per lane into the sample cartridge. • Added *Run Transfer Completed* topic. • Added *Results Tabs* topic. • Added *Results Charts Tabs* topic describing Q30 vs Cycle plot. • Added *Results Samples Tabs* topic. • Added description of PhiX percent aligned and PhiX percent error rate per read. • Added description of option to toggle between paired and unpaired data once analysis is complete. • Updated Run Folder structure description and screenshots. • Added *Demultiplexing Metrics* topic. • Added *PhiX Alignment Metrics File* topic. • Added references to *phix_stats* output folder. • Split *Maintenance Wash or Rinse* topic in separate *Maintenance Wash* and *Maintenance Rinse* topics. • Reorganized and consolidated overview of tasks in the *Maintenance* chapter • Added *Remove Empty Consumables* topic. • Added *System Health Check* topic. • Minor edits in the *Maintenance* section to reflect updated instructions, new options, and added UI elements. • Added SCS in restarting instructions. • Added *Restarting SCS and Secondary Computer* topic. • Added statement to remove external, non-standard devices before (re)start. • Removed Later option for sample sheet upload. • Added links to support videos.
600001	Rev. 4	November 2023	• Added references to Max Read Kit User Guide. • Added description of real-time run metrics. • Updated run metrics descriptions. • Added description of customized demultiplexing settings in sample sheet. • Added description of 35 base minimum for filtering and link to customization. • Added external waste reservoir emptying instructions. • Removed requeue demultiplexing on-instrument topic. • Changed wash cartridge loading instructions. • Added examples of and instructions to correct raised gaskets. • Rewrote flow cell loading instructions. • Changed Windows updates procedure. • Added maintenance rinse instructions to prevent salt buildup

Document #	Revision	Release Date	Description of Change
600001	Rev. 3	June 2023	• Added custom index sequencing primer workflow. • Updated Maintenance Wash instructions for new maintenance reagent cartridge. Note that the fill rows for water and NaOH have changed for the maintenance reagent cartridge. • Added Troubleshooting sections Flow Cell Not Engaged, Flow Rate Check Failed, Window Freezes, and Restarting • Minor edits.
600001	Rev. 2	April 2023	• Added Product Identifier descriptions. • Added manual entry of product properties. • Added offline storage settings screens. • Added Later option for sample sheet upload. • Added links to Sample Sheet Generator. • Updated sample sheet format. • Added Empty Waste Reservoir option. • Added off-instrument demux links. • Added Adding Quenching Solution. • Added maintenance rinse instructions • %cluster PF and % Cluster density metrics. • Updated custom primer dilution instructions. • Added F3 Flow Cell. • Minor edits.
600001	Rev. 1	January 2023	Corrected part number in footer.
600001	Rev. 0	October 2022	Initial release.

Singular Genomics Systems, Inc.

Website	singulargenomics.com
Email	care@singulargenomics.com
Telephone	442.SG.CARES(442.742.2737)