Illumina Novaseq X Sequencing

Version Number: 1.2
Production Start Date: 9/10/24
Version 1.0 Date:

Summary

The prepared libraries were quantified using KAPA Biosystem's next-generation sequencing library qPCR kit and run on a Roche LightCycler 480 real-time PCR instrument. Sequencing was performed on the Illumina NovaSeqX sequencer using NovaSeq X Series Reagent Kit.

Materials and Reagents

Materials/Reagents/Equipment	Vendor	Product Number
Easy open 1.7ml microfuge tubes	ISC Bioexpress	C-3293-2
Microfuge tubes with silicone O rings	VWR	89004-298
Pipet Tips, 2-20 ul , LTS, Filter, Low Retention, Red	Rainin	RT-L10FLR
Pipet Tips, 200 ul, LTS, Filter, Green	Rainin	RT-L200F
Pipet Tips, 1000 ul, LTS, Filter, Green	Rainin	RT-L1000F
Pipet Tips Serological, 50ml, Sterile, Individually Wrapped, Corning	Fisher	1367811F
Pipet Tips Serological, 10ml, Sterile, Individually Wrapped, Corning	Fisher	1367811E
Towels, Brown, WypAll L20, Quarter Fold, 2-Ply		7999-52901
Alcohol wipes, Individual		19-014-854
Kimwipes, Large 14X16		799930393

Kimwipes,Small, 4.4X8.4		799930392
Tubes., 15mL, Conical, Sarstedt	Phoenix	SS-4002
Reagents		
NovaSeq X series10B Reagent Kit (300 cycles)	Illumina	20028312
TailorMix Dual Indexed PhiX Control Library (Nondenatured)	SeqMatic LLC	TM-502-ND
PhiX Control v3	Illumina	FC-110- 3001
1N NaOH	Alfa	AA35629-K2
Tris-HCl Buffer, 10mM (pH 8.5)	Bio-World	42020414-1
UltraPure 1M Tris-HCI, pH 8.0	Fisher	15568025
Tween 20, Fisher BioReagents, Poly Bottle; 500mL	Fisher	BP337-500
spectrophotometric-grade isopropyl alcohol (70%), 100 ml bottle		
Contec Polynit Heatseal wipes	VWR	catalog #68310-176
Sodium Hypochlorite Solution (5% Available Chlorine)	VWR	JT9416-1
Equipment		
NovaSeq X Sequencer	Illumina	
Vortex Genie 2	VWR	G-560
Galaxy MiniStar	VWR	521-2844
Air filter	Illumina	20073109
Open-side Cart		

EH&S

Personal Protective Equipment (PPE)

Any JGI employee performing this procedure MUST wear safety glasses, a lab coat and powder free gloves.

Satellite Accumulation Areas (SAA)

SAAs for various hazardous chemicals are located throughout the lab. ALL reagents used in these kits and in this SOP MUST be disposed of in the appropriate SAA. If you are not aware of where the SAAs are located, or how to dispose of the above reagents properly, please contact your supervisor or Area Safety Lead (ASL).

NOTE: *All reagents/stock solutions should be prepared prior to the start of the procedure. Samples must be loaded within* ***1 hr*** *after denaturation*

Sequencing Consumables

NovaSeq X Series Reagent Kits are available in below configurations:

- 1.5B – 100, 200, 300 cycles
- 10B – 100, 200, 300 cycles
- 25B – 300 cycles

Each Component uses RFID (Radio Frequency Identification), which is read by the instrument for accurate consumable tracking and compatibility

Note: If the RFID of any component is not read by the instrument, please contact Illumina Tech support immediately.

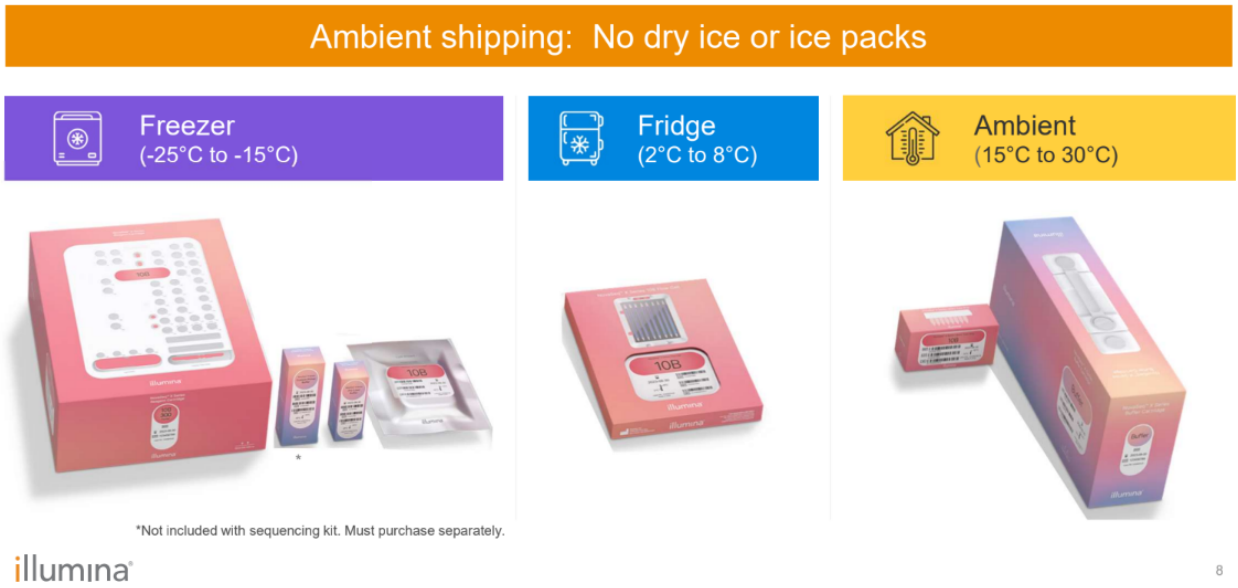
Below are the NovaSeq X Series Reagent Kit (300 cycle) consumables:

- Reagent cartridge
- Buffer cartridge
- Flow cell
- Library tube strip
- Lyo insert
- Pre-load buffer
- Custom primers buffer (only when custom primers are used)

Reagent/Stock Preparation

Note: When the kit is received, visually inspect each component and promptly store components at the indicated temperature to ensure proper performance. All kit components are shipped at room temperature.

Novaseq X Reagent Kit Storage:



Procedure

NOTE: *All reagents/stock solutions should be prepared prior to the start of the procedure. Samples must be loaded within* ***1 hr*** *after denaturation*

1 **Preparation** **Checklist:**

Note: The procedure in this step is not strictly sequential. Some of them can be performed in a different order or concurrently.
For example, the maintenance wash requirement (Step 1.5) can be reviewed and performed whenever the instrument is idle, or run data can be deleted (Step 1.6) as soon as the data transfer completes.

1.1 Confirm all reagents and consumables are available otherwise order from JGI Store; prepare to thaw the sequencing reagent cartridge. See Step 3 for thawing instructions.
- 1.1.1 Record the lot number of each reagent in the Google Sheets and LIMS record.
- 1.1.2 **[Dual flow cells run] Label "A" or "B" on each set of reagents/consumables and adhere with the label for recordkeeping AND loading the reagents accordingly on the instrument. PAY CLOSE ATTENTION to the side selection from this point and on. Any discrepancies will trigger a cascade of corrective actions in order to deliver unambiguous results to the users.**
- 1.1.3 Log in the LIMS and begin the NovaSeq X sequencing job.
- 1.1.4 Ensure all library pools scheduled to be sequenced are found in the queue.
- 1.1.5 Ensure the run mode (10B or 25B) of each pool is assigned correctly.
- 1.1.6 Download and fill out the Dilution Sheet.
- 1.1.7 Upload the completed Dilution Sheet to the LIMS.
- 1.1.8 Proofread all entries and close the job in order to generate the Sample Sheet It may take up to 15 minutes to be found in /staging/Sample_Sheets.

1.2 Confirm the instrument is idle before proceeding.
- 1.2.1 Sign in the Control Software by choosing "Sign in to local instrument". The default screen is for basespace login. The Control Software is automatically logged out after 30 minutes of inactivity.
- 1.2.2 If idle, the screen would either display "Start", "Wash Complete" if the previous activity is the maintenance wash, or "Run complete" in the previous run's status..

1.3 After signing in, three possible actions to be taken in the Control Software:
- 1.3.1 Select "Start" (or "Start a new run" if the screen displays a complete run) to reveal the options "Sequencing" and "Wash".
- 1.3.2 Select the instrument icon (semicircle at the bottom center) to open the navigation menu.
- 1.3.3 Exit from the current screen (e.g. previous run status) by clicking the "X" or "<" button at the bottom..

1.4 Select "Sequencing" and check if the maintenance wash is due. The due date is displayed below "Wash" for each side 7 days after the previous wash. If needed, select "Wash" and prepare the maintenance wash (Step 10). Overdue maintenance wash will prevent starting a new run. **Be aware that the maintenance wash takes 3 hours to complete.**

1.5 Delete run data to free up enough disk space for the upcoming run (the local disk space can hold about 4 flow cells of run data).
- 1.5.1 Select the instrument icon to expand the navigation menu.

1.5.2 Select Runs.
1.5.3 Select "Completed runs" near the top.
1.5.4 Select the run by clicking the run name; there would be a "Transfer complete" timestamp displayed if the data has been uploaded to the network folder. **DO NOT delete if the data transfer isn't complete**.
1.5.5 Press "Delete run data" (lower right) to purge just the run folder but leave the run history in the Control Software.
1.5.6 Press "Exit" to go back to the previous screen.
1.5.7 Repeat 1.5.1 to 1.5.5 to clear more disk space, if needed.

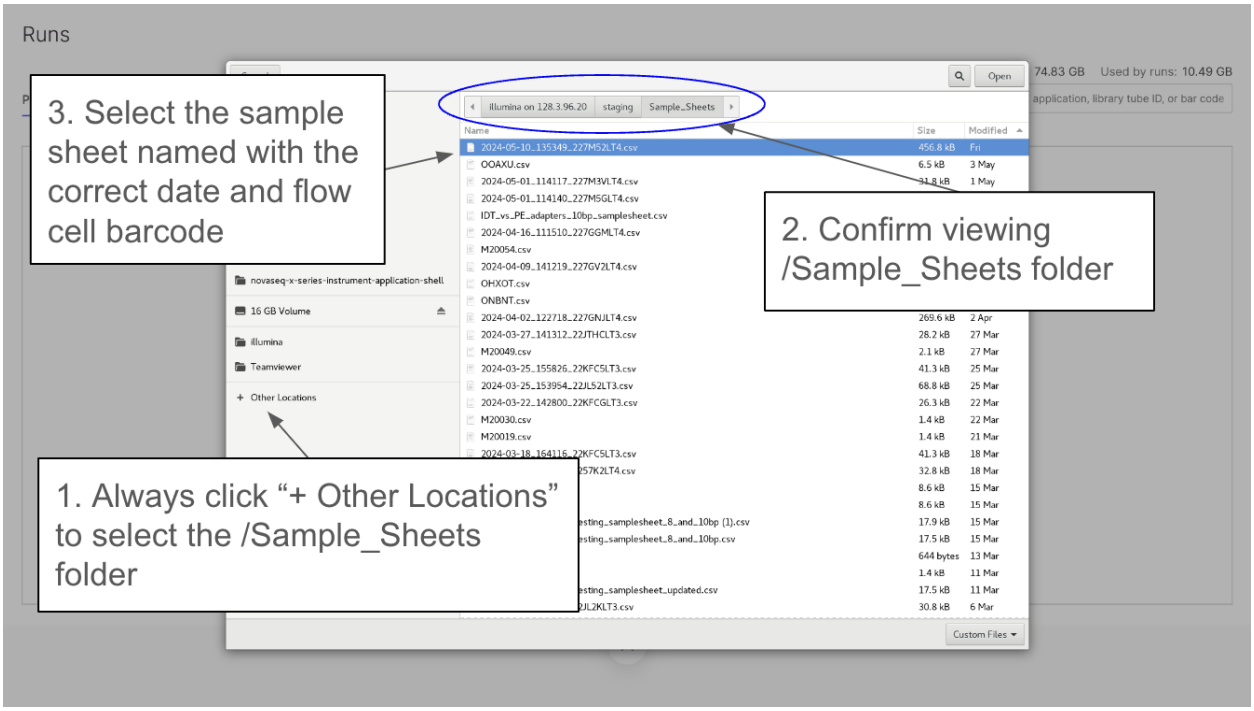
1.6 Rule out sample sheet error and potential fluidics issue.
1.6.1 Import the sample sheet to ensure no format error (see Step 2).
1.6.2 Exit the Runs panel and select "Start" in the center of the screen.
1.6.3 Select "Sequencing"; the imported run should be available for selection.
1.6.4 Assign the imported run to either side and proceed to "Load consumables".
1.6.5 Visually inspect the sequencing reagent cartridge for any spillage/overflow or the wash cartridge for underconsumption of the wash buffer (leftover <1 cm in depth).
1.6.6 Empty and discard the waste appropriately.

1.7 After restarting the instrument, the network drive may be disconnected. Refer to Step 8 for instructions to remount the network drive.

2 Planning a Sequencing Run:

Note: Importing the sample sheet requires that the instrument is connected to the network drive. See Step 8 for instructions to mount the network drive.

2.1 Create a Planned Run
2.1.1 Select the instrument icon at the bottom center to expand the navigation menu.
2.1.2 Select Runs.
2.1.3 Select Import Sample Sheet in the Planned tab.
2.1.3.1 In the popped up Files window, select "+ Other Locations" .
2.1.3.2 Locate the folder illumina/staging/Sample_Sheets.
2.1.3.3 Select the sample sheet with the proper date and flow cell barcode, and then click Open on the upper right corner.
2.1.3.4 Click Save if no error is found.



3 Thaw Consumables:

Use the following instructions to thaw consumables prior to sequencing:

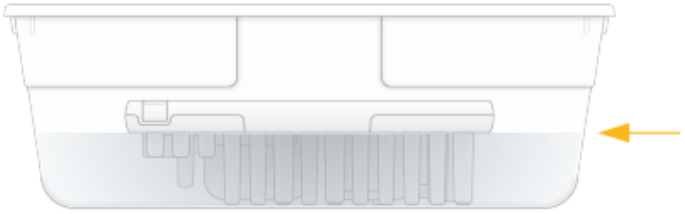
3.1 Thaw Reagent Cartridge: In a Controlled Water Bath

3.1.1 Use the following instructions to thaw the reagent cartridge in a room temperature water bath (15°C to 30°C).

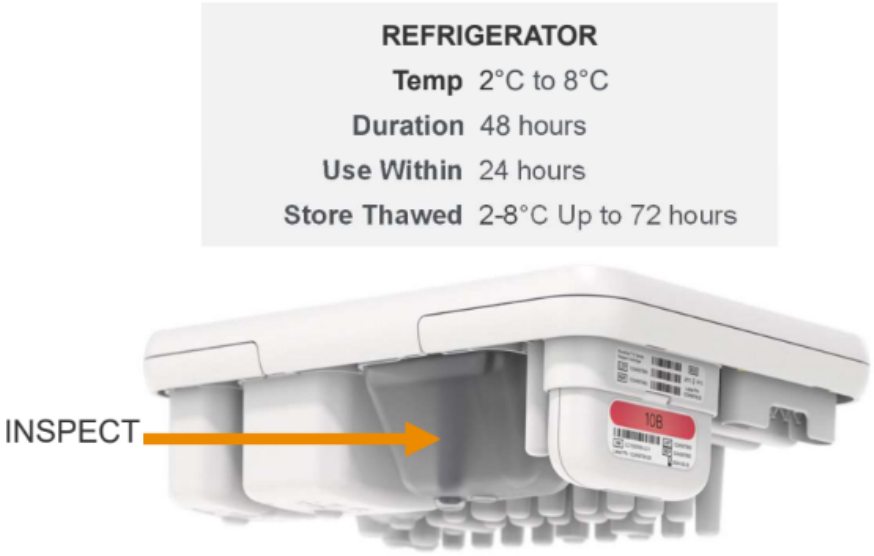
3.1.2 Put on a new pair of powder-free gloves and remove the cartridge from -25°C to -15°C storage.

3.1.3 Remove the cartridge from the box, and then remove from the bag.

3.1.4 Submerge the reagent cartridge in a room temperature water bath until the water reaches the bottom of the cartridge cover.
NOTE: Inserting the library tube strip or lyo insert into the reagent cartridge while thawing might cause reduced data quality or run failure.
NOTE: Using hot water for thawing reagents might cause reduced data quality or run failure.



3.1.5 Thaw for 4 hours. Do not exceed 24 hours.
3.1.6 Inspect the position #12 well on the underside of the cartridge to make sure that the contents are free of ice, which indicates that the reagents are thawed.
3.1.7 Thoroughly dry the cartridge using paper towels. Dry between wells beneath the cartridge so that all water is removed.
3.1.8 Invert or gently tap the bottom of the cartridge on the bench to remove excess water.
3.1.9 Inspect the foil seals for water
3.1.10 If water is still present, blot dry with a lint-free tissue.
3.1.11 Gently tap the bottom of the cartridge on the bench to reduce air bubbles.
3.1.12 If reagents cannot be loaded into the instrument within 24 hours, store at 2°C to 8°C for up to 72 hours.
Alternatively, the Reagent Cartridge can be thawed in a 2 to 8°C refrigerator up to 72 hours.
Do not insert the library tube strip or lyo insert into the reagent cartridge while thawing.



3.2 **Thaw Lyo Insert**
3.2.1 Remove the lyo insert from -25°C to -15°C storage.
3.2.2 Thaw at room temperature for 10 minutes.
3.2.3 If lyo insert cannot be loaded within 24 hours, return to -25°C to -15°C storage.

3.3 **Thaw Pre-load and Custom Primer Buffers**
3.3.1 Remove pre-load and custom primer buffers insert from -25°C to -15°C storage.
3.3.2 Thaw at room temperature for 10 minutes, and then invert five times.
3.3.3 If pre-load and custom primer buffers cannot be loaded within 8 hours, return to -25°C to -15°C storage.

3.4 **Thaw Flow Cell**
3.4.1 Remove a new flow cell package from 2°C to 8°C storage.

3.4.2 Set the sealed flow cell package aside for 10–15 minutes to allow the flow cell to reach room temperature.

3.4.3 Leave the flow cell in the package until use. Use the flow cell within 2 hours of removing it from storage. If flow cell cannot be used within 2 hours, return to 2°C to 8°C storage and use within 24 hours.

4 Prepare Samples:

4.1 Check samples for needed volume and thaw at 4℃

4.2 Remove dual index PhiX from -20℃ and thaw at 4℃

4.3 Make a fresh batch of 0.2N NaOH as below.

	25B single flow cell	25B dual flow cell	10B single flow cell	10B dual flow cell
2N NaOH	12 uL	24 uL	8 uL	16 uL
Nuclease free water	108 uL	216 uL	72 uL	144 uL

4.4 Acquire 8 or 16 (dual flow cells) 1.5mL microcentrifuge tubes; label each tube with the lane number and “A” or “B”.

4.5 Pair up the labeled new tube with the library pool according to the dilution sheet.

4.6 Confirm pool name and LIMS ID of each pool is correctly paired with a tube labeled with its designated side and lane number.

4.7 Keep all tubes closed at all times unless adding a sample/reagent to that tube

4.8 Move tubes either horizontally or vertically once a step has been completed to differentiate which tube is missing which reagent in the event you are interrupted.

5 Denature & Dilute Libraries:

Note: Use Low Retention tips for any reagents that involve DNA.

5.1 Dilute stock dual index PhiX (SeqMatic TM-502-ND) to the loading concentration (≤500pM) according to the dilution worksheet if the diluted PhiX is not readily available.

5.1.1 After opening a new lot of SeqMatic PhiX, thaw and spin down 3 tubes. Combine them into one tube.

5.1.2 Dilute 1uL of the combined PhiX in 9uL of 10mM Tris-HCl pH 8.5 or RSB; use the Qubit HS DNA Assay Kit to measure the concentration of 1uL of the 1:10 dilution.

5.1.3 Back-calculate the actual concentration (convert from ng/uL to nM) based on fragment size 530bp.

5.1.4 Use the measured concentration for dilution.

5.2 Dispense the calculated volume of each component on the dilution worksheet to the appropriate tube.

5.2.1 Add Resuspension Buffer or 10mM Tris-HCl pH8.5.

5.2.2 Add the diluted PhiX

5.2.3 Add the specific library pool; confirm pool name and LIMS ID matching the side/lane number labeled on the tube.

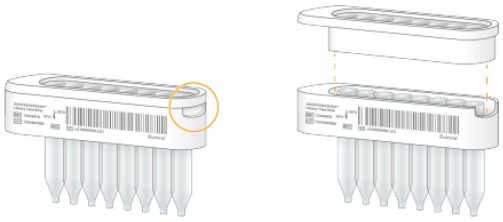
5.3 Add the specified amount of 0.2N NaOH to each tube.
5.4 Vortex and spin down
5.5 Incubate at room temperature for 5 minutes (at this time take out the flow cell from 4˚C and keep in room temp).
5.6 Add the specified amount of Pre-load Buffer to each tube.
5.7 Vortex and briefly centrifuge and place on ice until transferred to the library tube strip.

6 Load Lyo Insert and Library Tube Strip:

Before sequencing, load the lyo insert and library tube strip into the reagent cartridge as follows:

6.1 Use the custom rack to hold the library tube strip.
6.2 Uncap the library tube strip. Do not pierce the library tube strip foils. **[Dual flow cells run] LABEL "A" or "B" on the library tube strip and transfer the denatured library pools accordingly. DO NOT PROCEED until the library tube strip is labeled.**
6.3 Lane numbers are pre-printed on the library strip tube; with the numbers facing you: **[1.5B, 10B]** Dispense 165 µl denatured library with PhiX into each sample tube. **[25B]** Dispense 275 uL denatured library with PhiX into each sample tube.
6.4 **[1.5B, 10B]** Add 165 uL Pre-load Buffer to any unused sample tubes. **[25B]** Add 275 uL Pre-load Buffer to any unused sample tubes.
6.5 After dispensing libraries, cap the library tube strip. Make sure that no air gaps are present at the bottom of the tubes.
6.6 Insert the library tube strip into the reagent cartridge and press down. An audible click indicates that the library tube strip is in place.
6.7 Insert the lyo insert into the reagent cartridge and press down. An audible click indicates that the lyo insert is in place.

Figure 11 Uncap Library Tube Strip



7 Initiating a Sequencing Run:

7.1 Review Step 1 and then load the reagents and buffers.
7.1.1 Select START..
7.1.2 Select Sequencing.
7.1.3 Choose a side (A, B, or A+B for dual flow cells).
7.1.4 Assign the planned run. **[Dual flow cells run] Be cautious to assign each planned run accordingly to its designated side.**
7.1.5 Select Next.
7.1.6 The run design, i.e. number of cycles in each read, will be displayed according to the sample sheet. Confirm the number of cycles is correct and the output folder is

assigned to the network folder (/Illumina/staging/novaseqx/ for production run). Note: if the network folder is not available, refer to Step 9 to set up the default output folder.

7.1.7 Click “Load consumables”.

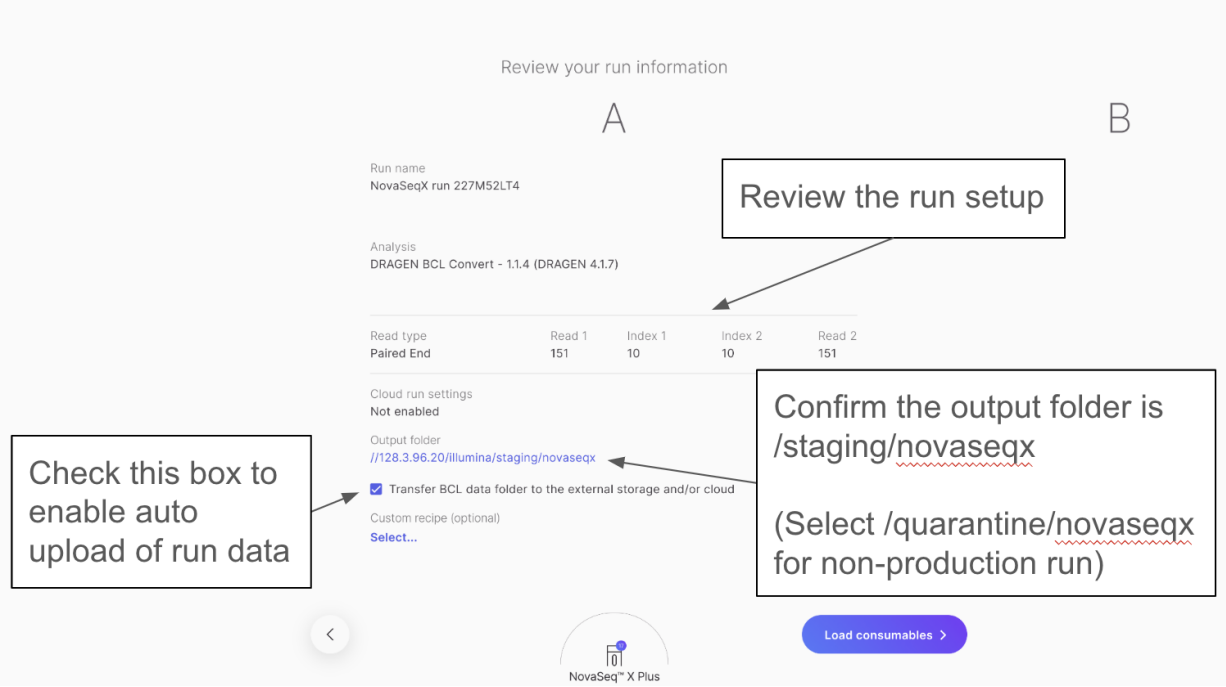
7.1.8 Select “Load reagents and buffers” to unlock the door(s).

7.1.9 Open the lower drawer. ALWAYS empty the small waste bottle, which holds the formamide waste, and discard the waste to the designated SAA. Empty the large waste bottle at the sink after confirming the waste has a pH at 6-9.

7.1.10 Open the upper drawer to load/replace the wash buffer and sequencing reagent cartridge.

7.1.11 Close the door(s) by pressing the inner center of each door where the latch is.

7.1.12 Once the door(s) is closed, it will be locked. Click “Review” and then “Verify reagents”.



7.2 Load the Flow Cell:

7.2.1 Make sure that the flow cell has reached room temperature before loading.

7.2.2 On the load consumables screen, select Load flow cells. After selecting, the display monitor raises and the flow cell door opens. The flow cell light indicates the instrument side sequencing is being performed on. Wait until the flow cell stage has fully extended before proceeding.

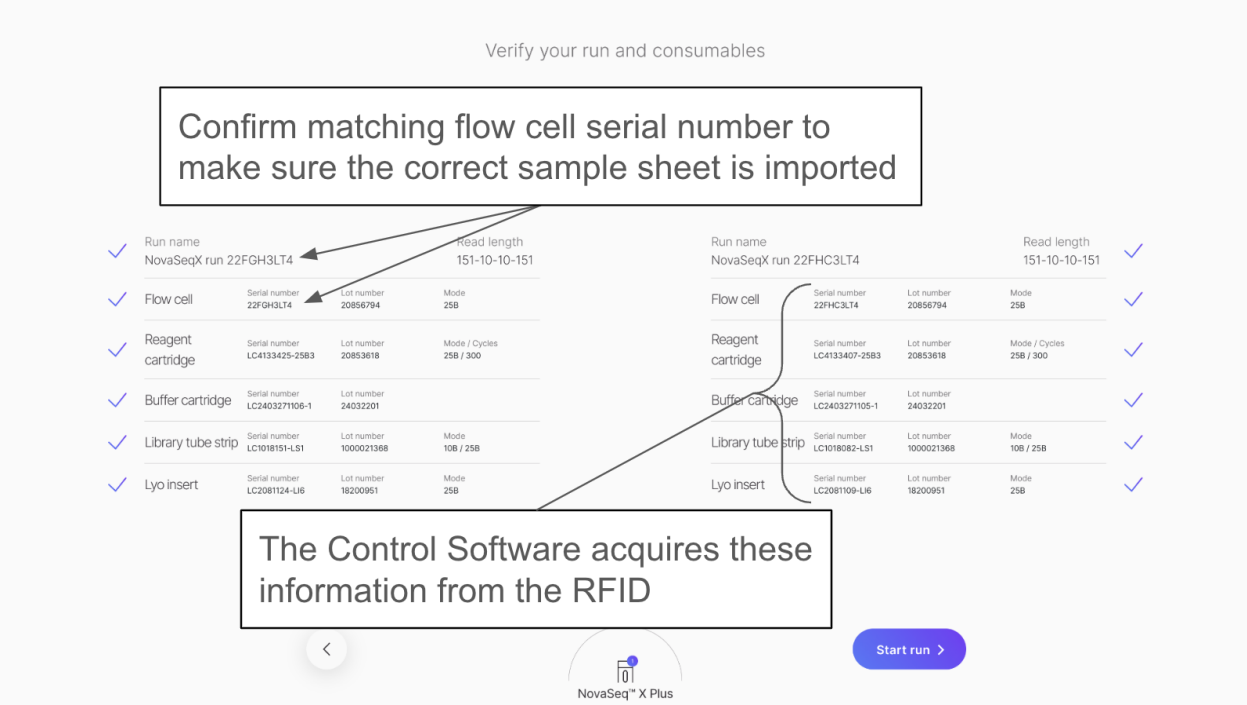
7.2.3 Remove and discard the used flow cell in accordance with the applicable standards for your region. The flow cell is not recyclable.

7.2.4 Inspect the flow cell stage for any contaminants (eg, particulates, lint, or dried reagent). If contaminants are visible, clean the flow cell stage as follows.

7.2.4.1 Wet a polynit heatseal wipe with isopropyl alcohol (70%).

7.2.4.2 Gently clean the applicable surface. Wipe in a lengthwise direction only. Unless you find contaminants on the manifolds, avoid touching them when wiping the flow cell stage.
7.2.4.3 Repeat steps a and b until surfaces are clear of all contaminants.
7.2.4.4 Dry with a new polynit heatseal wipe or an unused side of the used wipe to avoid contamination.

7.2.5 Put on a new pair of powder-free gloves to avoid contaminating the glass surface of the flow cell.
7.2.6 With the flow cell foil package over a flat surface, peel open the foil from the corner tab.
7.2.7 Remove the flow cell from the package. Grasp the flow cell by the sides to avoid touching the glass or the underside gaskets.
7.2.8 Inspect the flow cell for any contaminants (eg, particulates, lint, or dried reagent). If contaminants are visible, clean the flow cell as follows:
7.2.8.1 Wet a polynit heatseal wipe with isopropyl alcohol (70%).
7.2.8.2 Gently clean the applicable surface. Wipe in a lengthwise direction only.
7.2.8.3 Repeat steps a and b until surfaces are clear of all contaminants.
7.2.8.4 Dry with a new polynit heatseal wipe or an unused side of the used wipe to avoid contamination.
7.2.9 Discard the package appropriately.
7.2.10 Place the flow cell in the flow cell stage so that the top surface faces upward.
7.2.11 After flow cells are loaded, select Close flow cell door.
7.2.12 After the flow cell is confirmed, load the reagent and buffer cartridges if not yet done.
7.2.13 Click “Review” in the lower right corner.
7.2.14 Confirm the flow cell barcode in the run name matches the serial number obtained from the RFID. Unmatched flow cell barcodes indicate barcodes uploaded in LIMS are mixed up, the wrong sample sheet is imported, or the flow cell is placed on the opposite side.
7.2.15 Click “Start run”; the run will begin after completing the pre-run check.



8 Mounting the network drive

Note: After power cycling the instrument, the network drive is no longer connected. Before loading a production run, ensure that the network drive is mounted in order to import the sample sheet.

8.1 Click the semicircle at the center bottom of the Control Software to expand the menu.

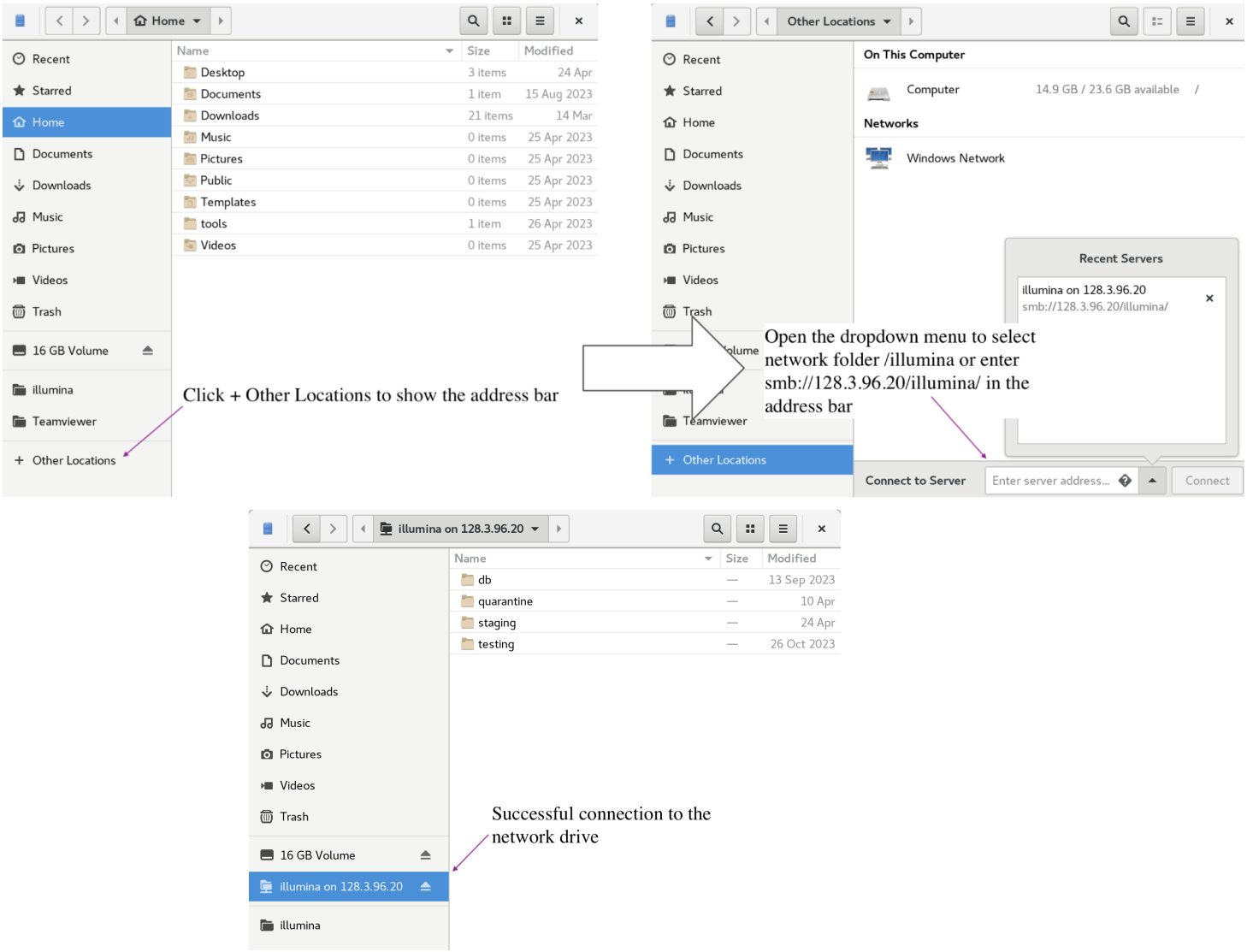
8.2 Select Settings → Minimize software (or press the Windows button on the keyboard and select a different desktop on the right pane).

8.3 Click Activities on the upper left corner to show the mini dock (or swipe the touchscreen to the right), and then click the "9 dots" icon to expand the choices where Files will be found. (Files is equivalent to Explorer on Windows or Finder on Mac)

8.4 In a Files window, on the left pane, select "+ Other Locations" at the bottom of the list.

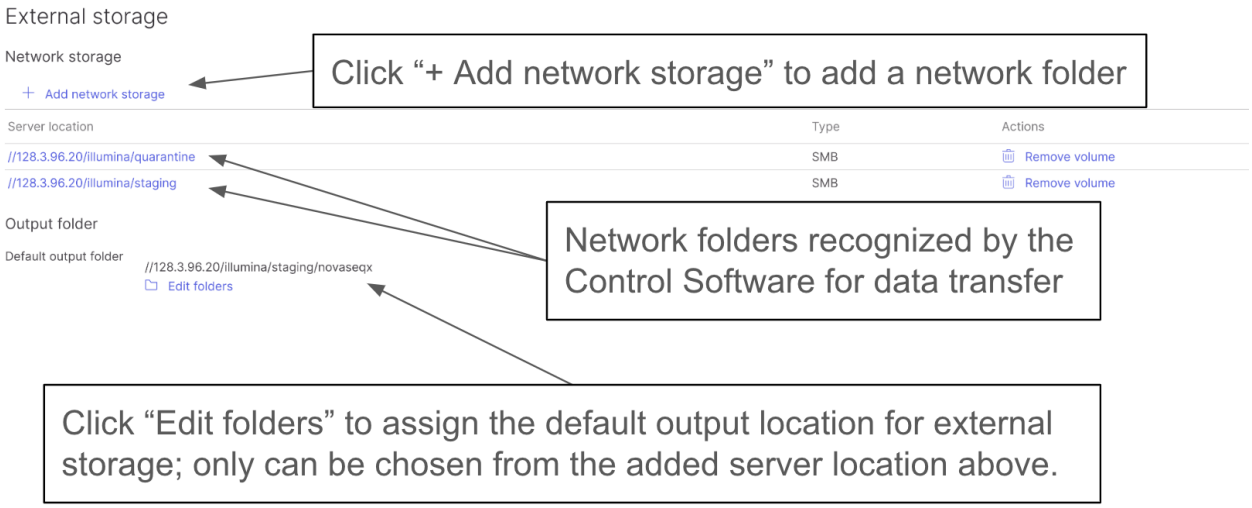
8.5 On the lower right corner, open the drop down menu and select **smb://128.3.96.20/illumina** or enter the URL if it's not available for choosing, and then click Connect.

8.6 Enter the AD credentials (same username/password on all Illumina sequencer)
Address: //128.3.96.20/illumina/staging
Domain: lbl
Username: sbsuser
Password: look up in LastPass or ask IT



9 Set Up External Storage and the Default Output Folder

9.1 Select the instrument icon to expand the navigation menu.
9.2 Select Settings → External storage.
9.3 Select "+ Add network storage".
9.4 In the next screen, fill out the AD credentials (see Step 8.6). Note: Type is "SMB".
9.5 Highlight the folder and click Open.
9.6 Repeat 9.3 to 9.5 for the non-production folder (replace "staging" with "quarantine").
9.7 Assign the default output folder to //128.3.96.20/illumina/staging/novaseqx.



10 Biweekly Maintenance

Note: The Control Software schedules a 14-day countdown for maintenance wash regardless of usage of the instrument within the 14 days. Overdue maintenance wash will block new run setup until it is complete.

10.1 For each side of maintenance wash, prepare 0.12% Sodium Hypochlorite (NaOCl) by mixing 1.2mL of 5% NaOCl with 48mL of deionized water.

10.2 Each side requires 380mL of 0.05% Tween 20. Prepare when needed by adding 1mL of stock Tween 20 to 2L of deionized water.

10.3 Select Wash in the Control Software by either:

10.3.1 Select Start and then Wash, or

10.3.2 In the navigation menu, select Settings → Maintenance wash.

10.4 Select "Load wash cartridges" to unlock the door (takes a few minutes).

10.5 Gently pour 49mL 0.12% NaOCl and 380mL of 0.05% Tween 20 to the labeled compartment in a wash cartridge.

10.6 When the door(s) is unlocked, open the top drawer to load the wash cartridge and leave the used Wash Buffer inside.

10.7 Open the bottom drawer and empty the waste.

10.8 Close the door(s) and click "Verify wash reagents".

10.9 Leave the used flow cell(s) inside; DO NOT click "Load wash flow cell" unless the Wash Flow Cell is available. Once the flow cell door opens, the used flow cell is no longer eligible for wash.

10.10 Click "Start wash" after all consumables are loaded.

10.11 Power cycle the instrument without flipping the switch in the back.

10.11.1 Select Settings in the navigation menu.

10.11.2 Select Shutdown and then click "Yes" if there's no alert. The shutdown process takes >10 minutes.
DO NOT PROCEED IF the instrument is running the analysis of the previous run.

10.11.3 Wait till the instrument is off (the monitor screen is dark and the power button on the right is flashing). Press the power button to turn on the instrument.
10.11.4 Log in the Linux OS.
10.11.5 Click Activities on the upper left corner and open the Control Software. The instrument will then initialize, which takes ~20 minutes.

Instrument Maintenance

Instrument

Biweekly maintenance (Wash & power cycle) by JGI operator
Preventive maintenance scheduled and arranged by the manufacturer

Troubleshooting

Review the common scenarios below when issues arise. If the issue is not one of the below, contact Illumina Technical Support by calling (800)809-4566 or email to techsupport@illumina.com. Illumina Application Scientists may require remote access to the instrument via the software TeamViewer.

1. Press the Windows button on the keyboard or "Activity" on the upper corner of the screen.
2. In the search bar on the top of the screen, enter "teamviewer".
3. The search result will guide you to where TeamViewer is located. Double click the TeamViewer icon to launch the application.
4. Share the ID and password with Illumina.

Sample sheet error (unsuccessful import).
Solution: The most common cause of error is ineligible special characters used in the sample sheet. The Control Software only accepts alphanumerics, hyphen, and underscore with a max length of 100. Another common cause is the barcode mismatch override. In the most recent sample sheet, the override section is removed, and barcode mismatch is only denoted individually. Contact supervisor and data scientist to edit the sample sheet.

Insufficient disk space after run data is deleted (disk space error during Pre-run Check).
Solution: Go to Files. Check the illumina/mnt/runs LOCAL folder to make sure the deleted run folders are no longer present. Restart the Control Software to refresh the available disk space. Start the run again.

The Control Software cannot reach the output folder (connection error at run setup).
Solution: Make sure the network drive is mounted. Go to Settings → External storage. Select "Remove volume" to remove the network folder and add the same network folder again (Step 9).

Unable to start the run within one hour after the library pools are denatured.
Solution: When ready to load, transfer the library pools to clearly labeled tubes. Heat shock the samples at 96˚C for 2 minutes and then put on ice for 5 minutes. Quick spin the tubes. Dispense the denatured library back to the Library Strip Tubes in the correct order. If the instrument requires extra time to be serviced, store the entire Library Strip Tubes at -20˚C.

SOP Approval

DEPARTMENT	APPROVED BY	DATE
Lab Supervisor		
Research & Development		
Instrumentation		
QC		
Purchasing		
EH & S		
Informatics		
Seq Assessment & Analysis		
Dept Head of Prod Seq		

Appendix

Figures
Tables
Diagrams
Attachments
Contact information for vendors or manufacturers that you want included in the SOP

ADDENDUM TRACKING

4/22/25:

Control Software upgraded from version 1.2.2 to 1.3.1 by Illumina Field Service Engineer.

Added stock PhiX concentration measurement in Step 5.1.

8/31/24:

Added the following procedures:

- Preparation Checklist
- Mounting the Network Drive
- Set Up External Storage and the Default Output Folder
- Biweekly Maintenance

Updated the following procedure:

- Step 2 – Planning a Sequencing Run
  - Changed to create a Planned Run by importing the sample sheet.
- Step 4 – Prepare Samples
  - Replaced a new table of 0.2N NaOH recipe that accommodates the larger volume of 25B.
- Step 5 – Denature & Dilute Libraries
  - Added 10mM Tris-HCl pH 8.5 as an alternative to Resuspension Buffer.
  - Clarified the reagent name to be Pre-Load Buffer.

- Step 6 – Load Lyo Insert and Library Tube Strip
  - Added loading instructions for 25B run mode.
  - Changed the loading volume from 160 to 165 uL for 10B run mode.
- Step 7 – Initiating a Sequencing Run
  - Revise Step 7.1 to include detailed instructions of loading the reagents/buffers.

5/27/24:

Control Software upgraded from version 1.1 to 1.2.2 by Illumina Field Service Engineer.

AUDIT TRACKING