NGI OpenLab MiSeq i100 Plus Instruction

This document is based on the MiSeq i100 product documentation. It contains hyperlinks (underlined in blue) that refer to relevant sections of the *MiSeq i100 product documentation*. Please read this document and the paragraphs the hyperlinks refer to, before attending the MiSeq i100 Plus introductory course.

If you are using custom primers, please ensure that you review this section in the *MiSeq i100 product documentation* in advance.

Prior to the sequencing course, please consult the Illumina website for detailed guidance on normalizing library concentrations, loading concentration optimization and PhiX spike-in procedures.

1 Consumables and Equipment

Please ensure that you have the recommended consumables before attending the MiSeq i100 Plus introductory course.

2 BaseSpace

An Illumina BaseSpace Sequence Hub (BSSH) account is required to run the MiSeq i100 Plus system in the NGI OpenLab. Please create an account if you do not already have one.

Note! *When creating Sample Sheets, it is important to use the EU site: euc1.sh.basespace.illumina.com. If the Sample Sheet is created in another region, it will not appear as a planned run on the MiSeq i100 Plus.*

2.1 Sample Sheet

When creating your Sample Sheet, please follow the guide on the OpenLab website.

Note! *If you do not choose "Local" as the Secondary analysis option when creating your sample sheet your demultiplexing will not happen on the instrument and the fastq files will not be stored on the USB stick.*

3 Protocol

Please follow the sections below when running your samples on the NGI OpenLab MiSeq i100 Plus.

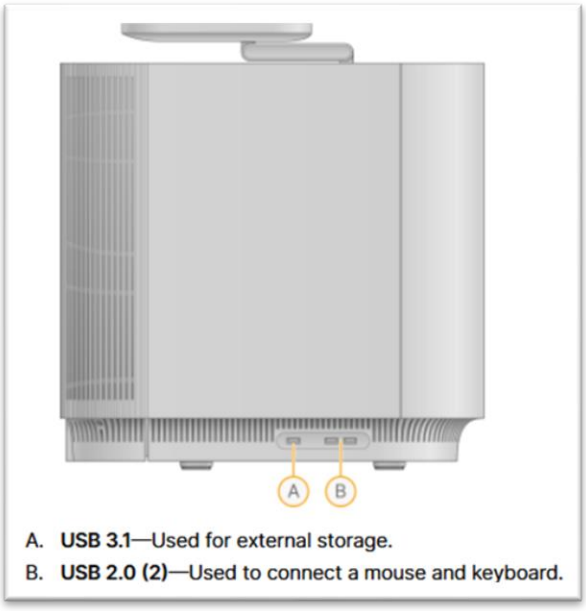
3.1 Sign in

1. Select **Start** to sign in.
2. Sign in with your Illumina BSSH username and password.
3. Select **Next**.

3.2 Add a USB stick

USB sticks with an output folder are provided by NGI OpenLab.

1. Get a clean USB stick from the red box on the shelf in the cabinet above the TapeStation workbench. Connect it to the USB port on the left side of the instrument, see picture below.
2. Press the menu icon in the upper-left corner.
3. Select **Settings**, and then select **External storage**.
4. Select **Add USB storage** – select **Data Traveler 3.0** - do not enter a password.
5. Select **Add** to make the USB available as an output storage volume.
6. Select **Add Folders**.
7. Specify the default output folder location by selecting **Add folder**.
8. Select your USB as server location from the drop-down list.
9. Select **"output"** as the desired default output folder from Available folders.
10. Select **Save** – select **Save**.
11. Press **X** in the upper right corner to exit and go back to the start screen.



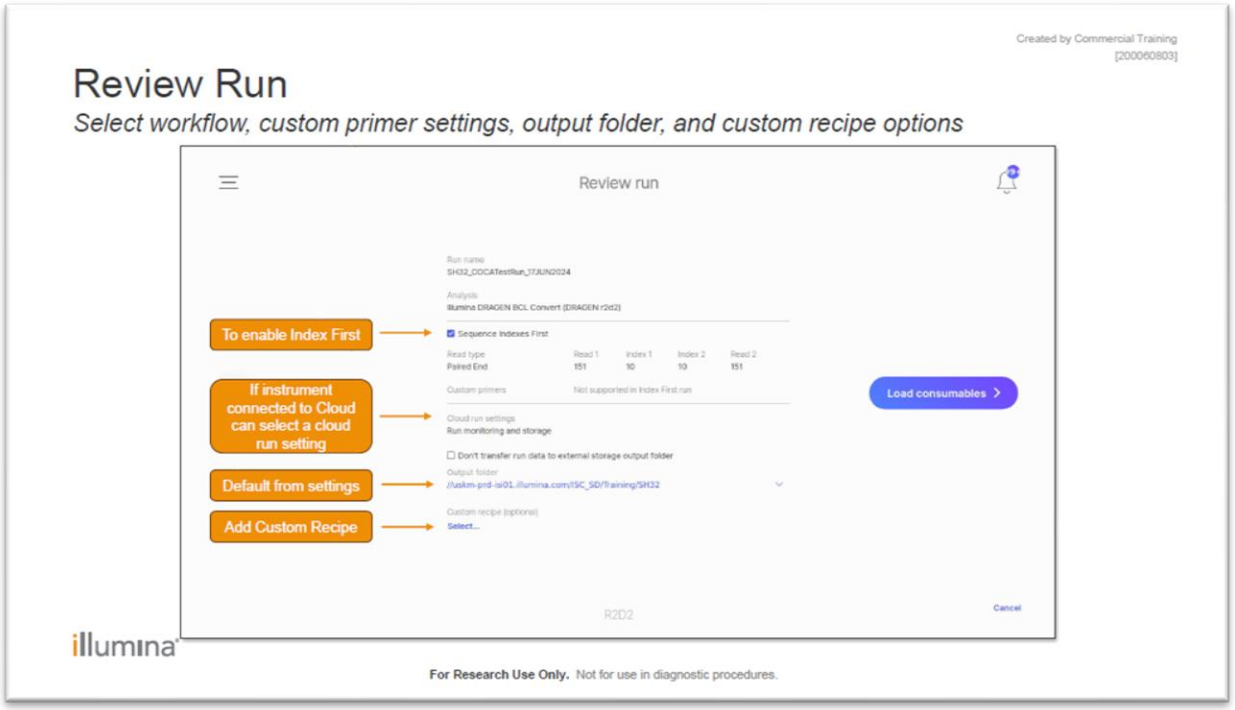
A. **USB 3.1**—Used for external storage.
B. **USB 2.0 (2)**—Used to connect a mouse and keyboard.

Note! *Private USB sticks must not be inserted into the NGI OpenLab MiSeq i100 Plus.*

3.3 Start a Planned Run

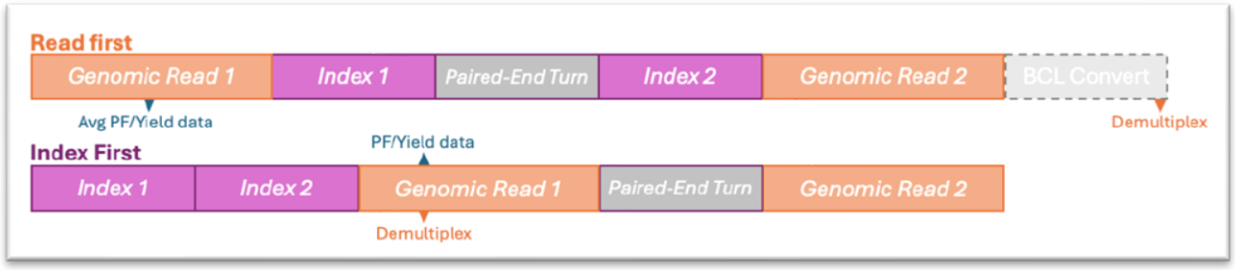
- Press **Start.**
- Select a run from the list of planned runs.
- Select **Review**, and then review your run information. Configure the following optional run settings:

• If Read First sequencing is required, deselect the **Sequence Indexes First** checkbox, see additional information below.
• If using custom primers, select the appropriate custom primers checkboxes. Refer to *Custom Primers* for more information.
• Set Cloud run setting to Run monitoring.



- After reviewing the run information, **refer to section Prepare Dry Cartridge <u>before</u> pressing Load Consumables**.

Note! *Using Sequence Indexes First will change the read order and is therefore not recommended, see picture below.*



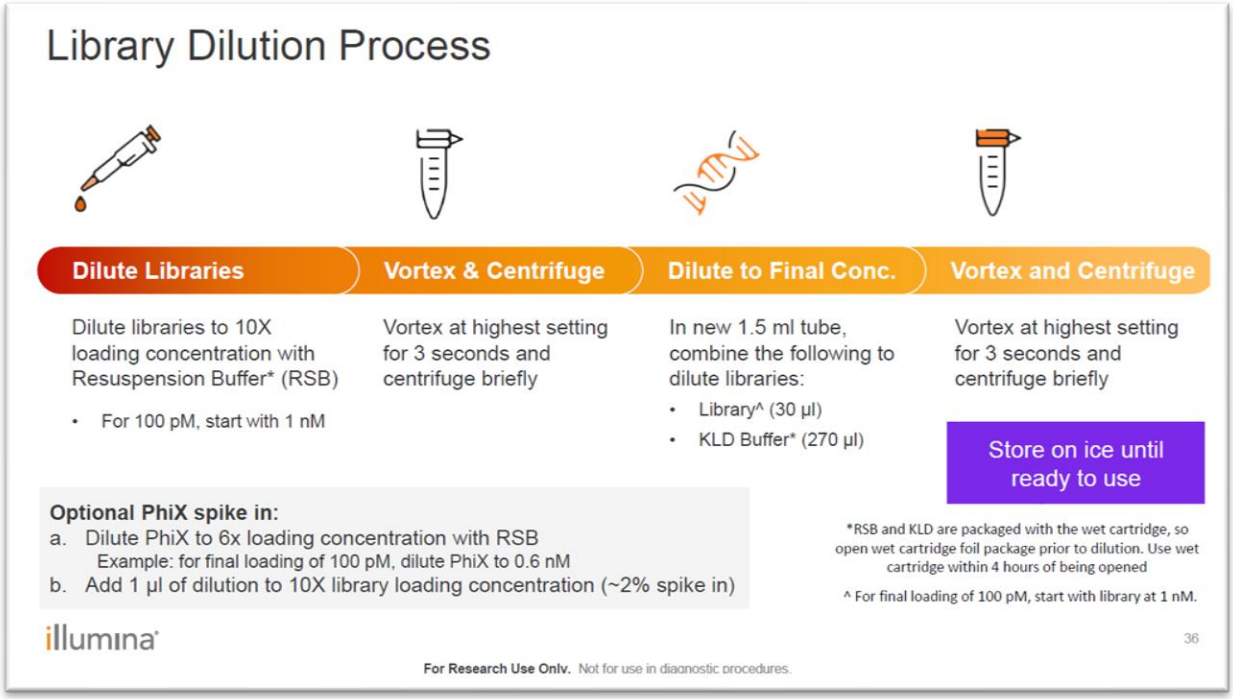
3.4 Prepare Dry Cartridge

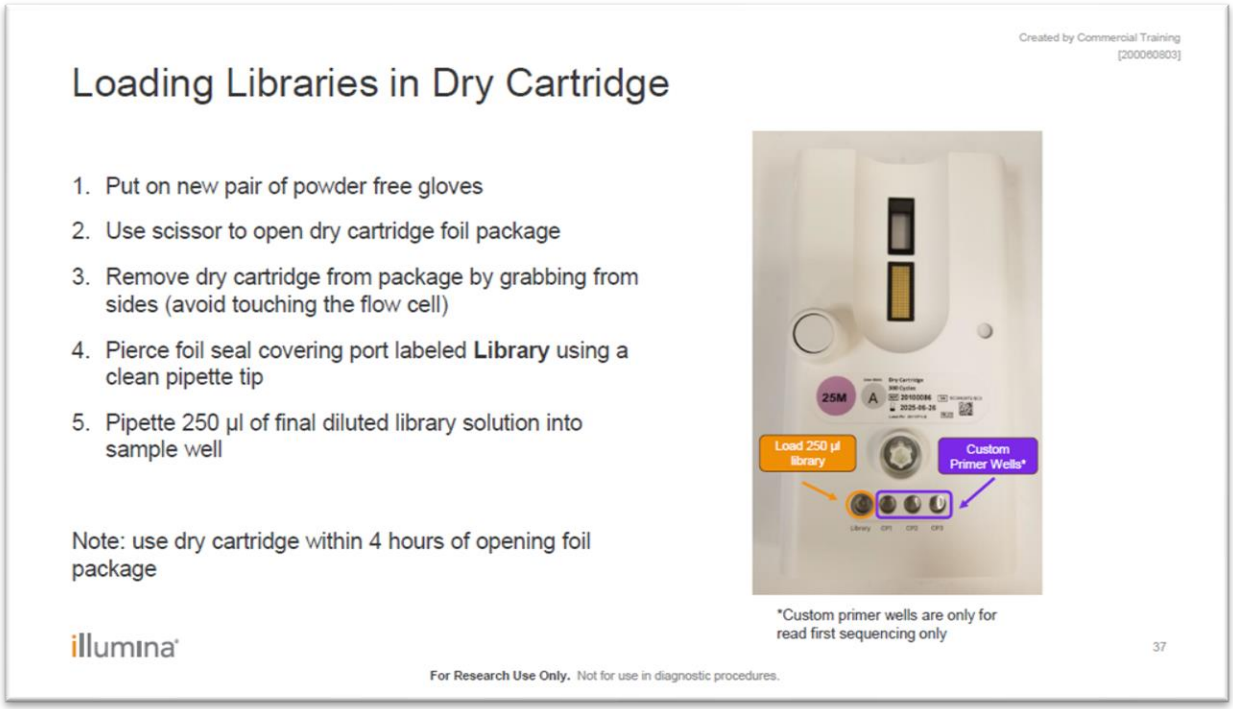
PhiX and ice are provided by NGI OpenLab.

See Loading Concentration Optimization Recommendations for help with calculating the library pool concentration. For help with how to spike in PhiX see How to Spike in PhiX for the MiSeq i100 Series.

When diluting the libraries and PhiX, follow the instructions under Prepare Dry Cartridge In short, the procedure includes the following steps:

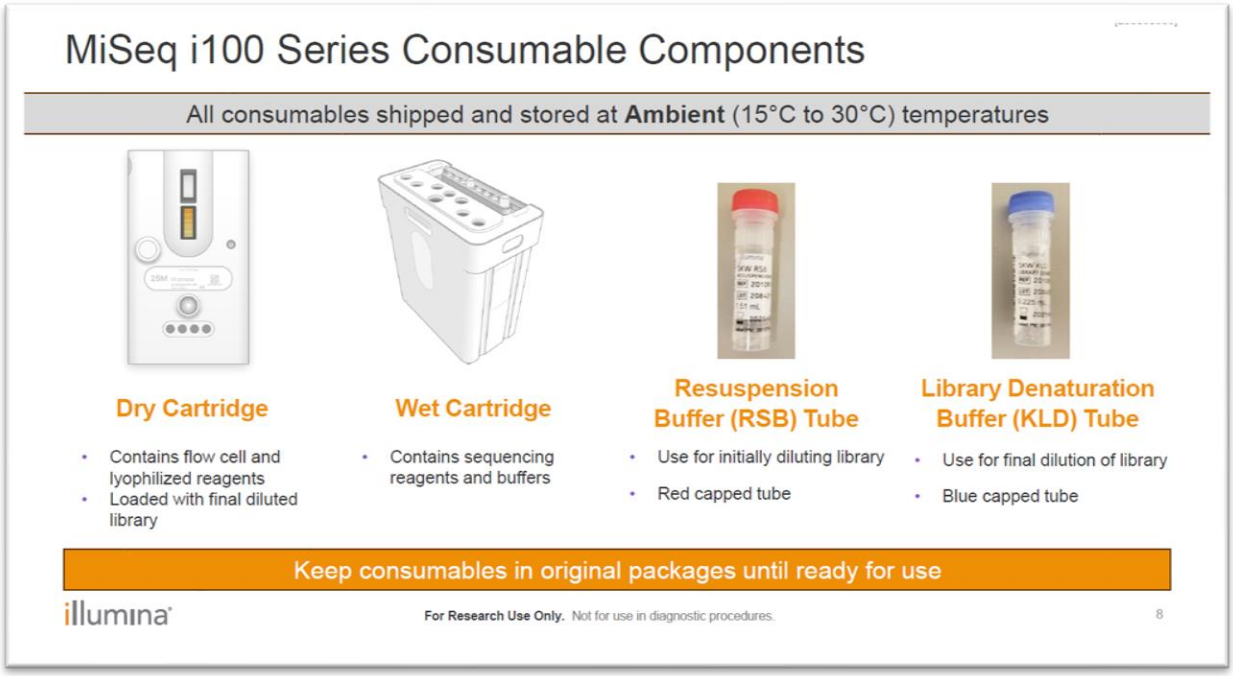
- Dilution of library to 10x loading conc with RSB to a final volume of 30µl.
- Dilution of PhiX to 6x or 10x loading conc with RSB (dilution depends on the desired spike-in percentage).
- Addition of diluted PhiX to your 10x loading conc library dilution (amount added depends on the desired spike-in percentage. If the spike-in is >2% remove corresponding volume before adding PhiX).
- Addition of 270µl KLD to the 30µl Diluted library/PhiX mix.



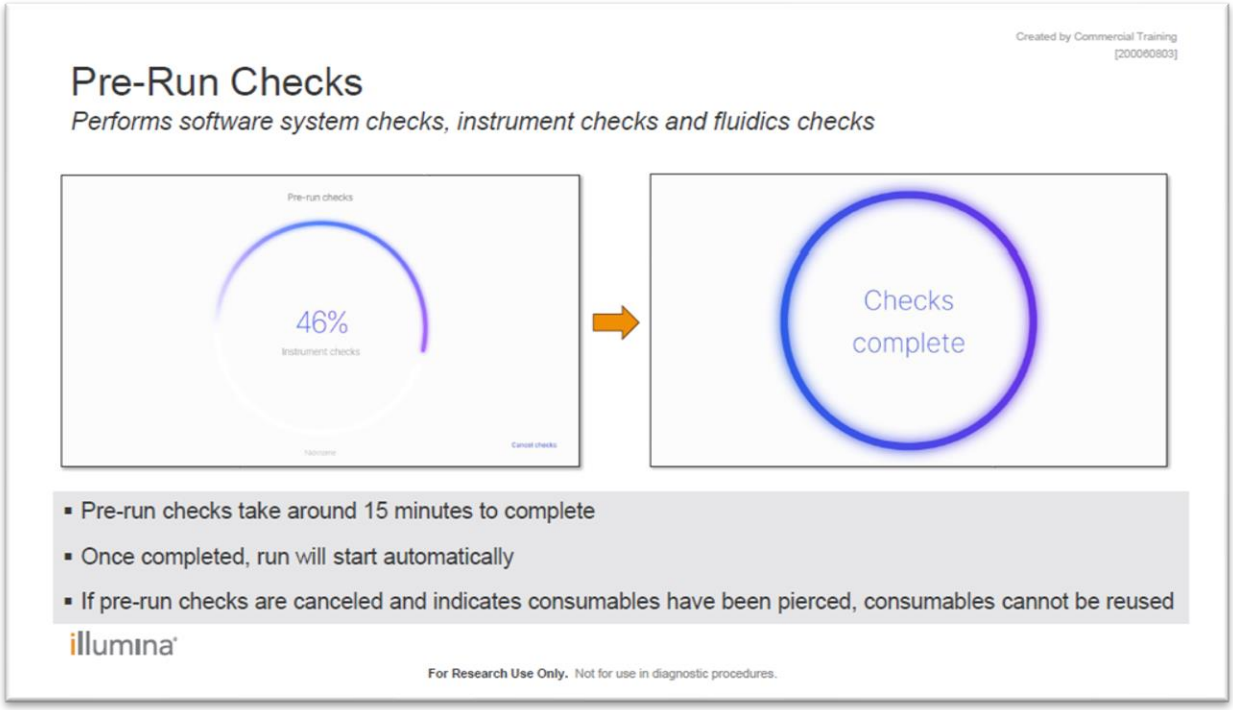


3.5 Load Consumables

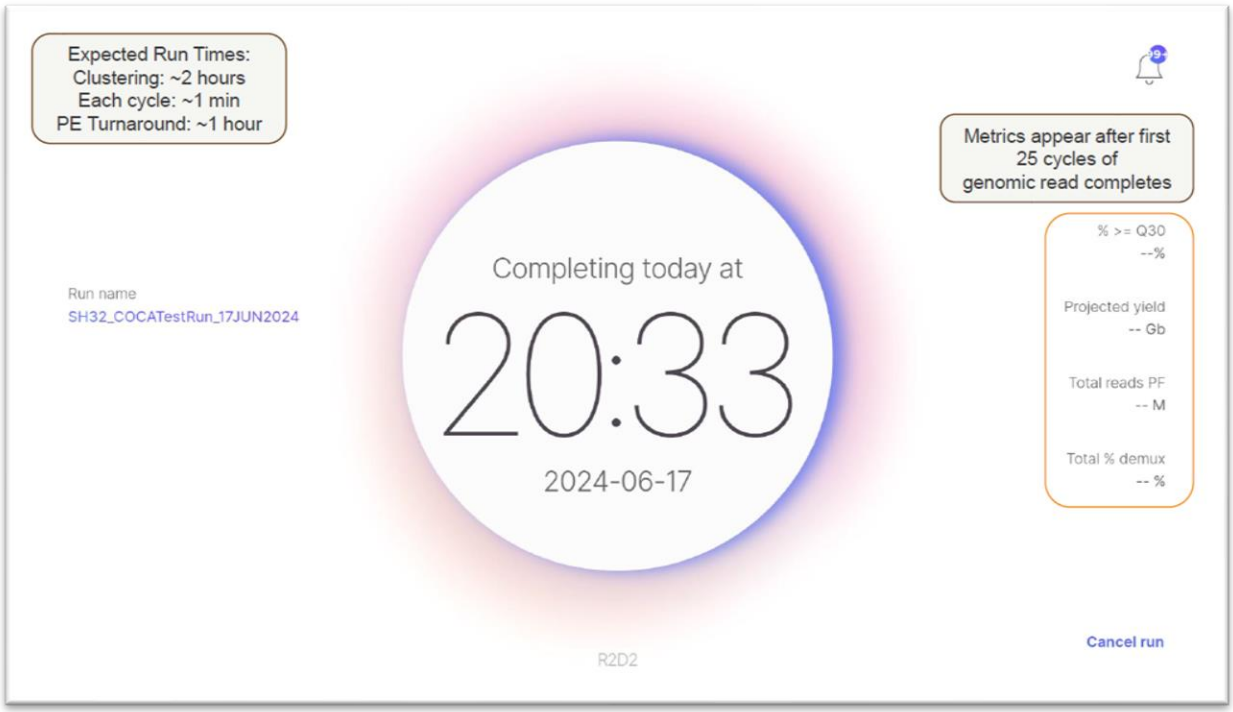
Press **Load Consumables** and follow the loading guide on the screen.

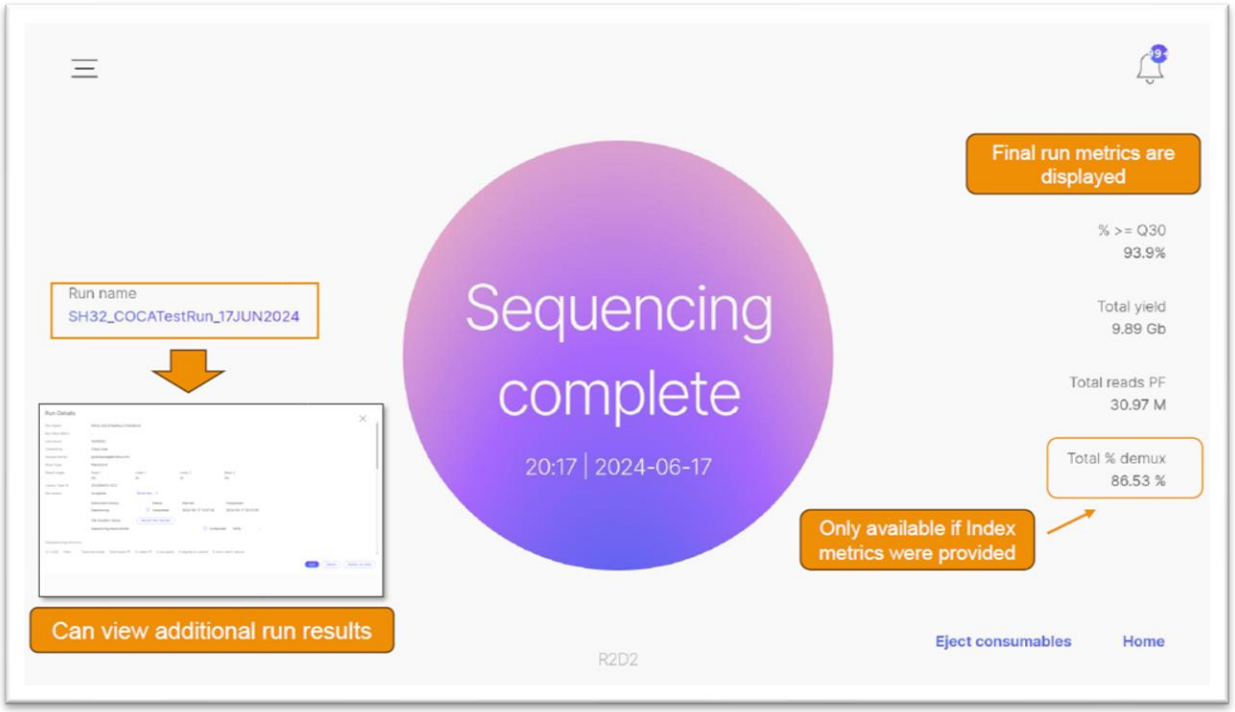


3.6 Pre-Run Checks

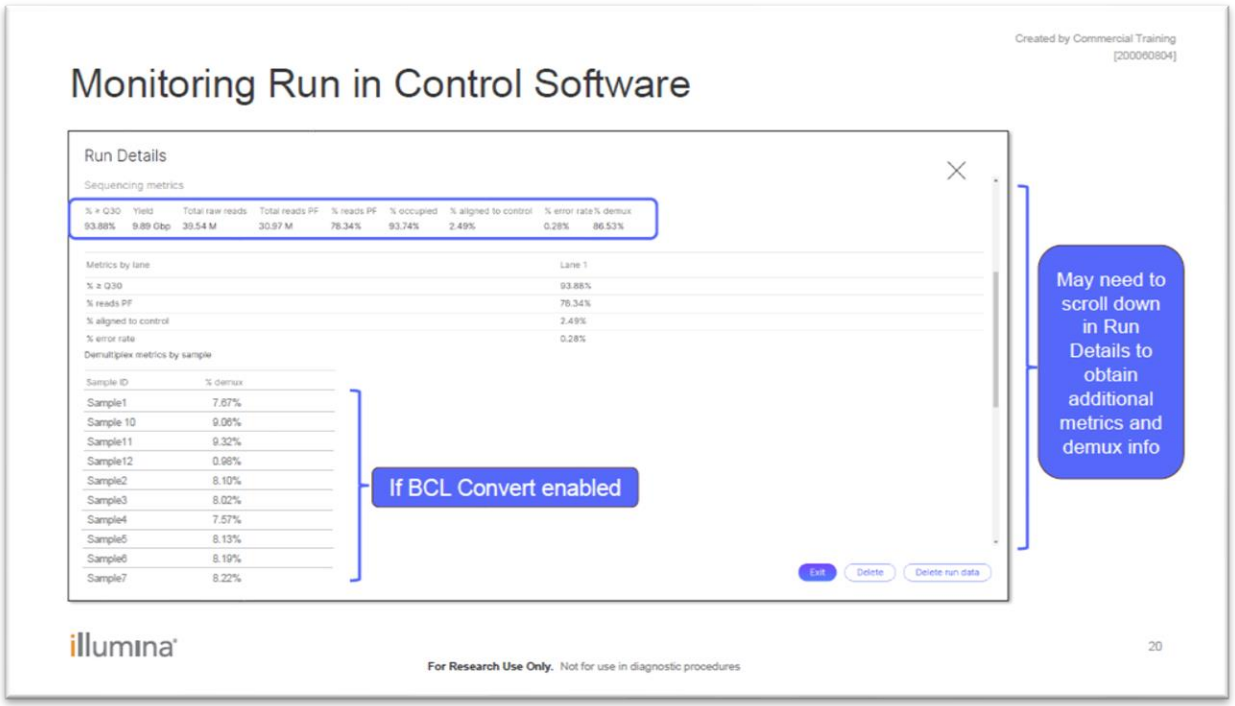


3.7 Monitor Run Progress





- Once the Sequencing run is complete, press the screen and login in again. Then press "**Run name**" to view the run details. Expected results can be found in [MiSeq i100 Series Specification].



- If the samples were not properly demultiplexed, we recommend you to use the following [script for demultiplexing].

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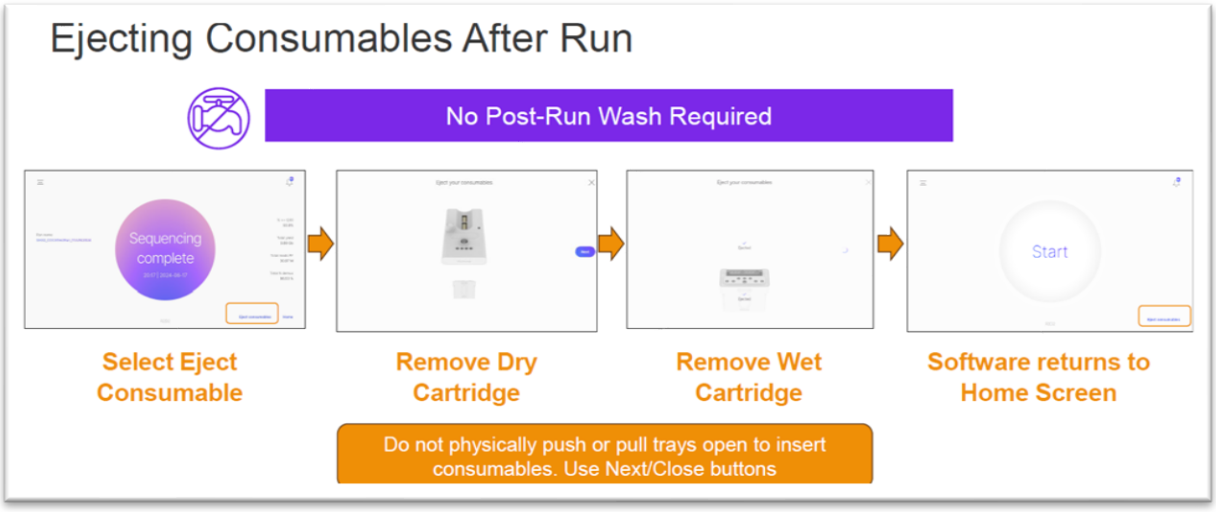
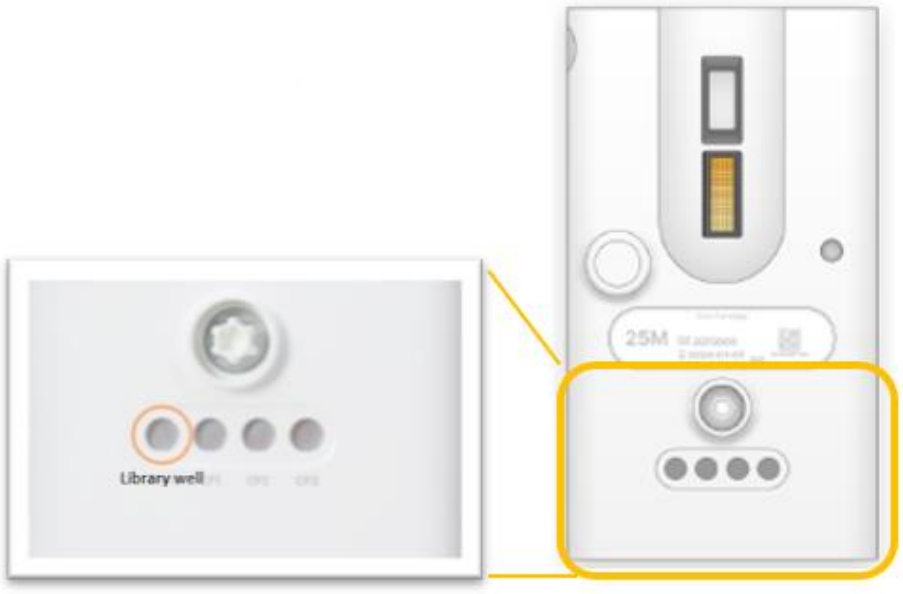
3.8 Eject Used Consumables

Press **Eject consumables** and follow the guide on the screen.



3.9 Dispose of Used Consumables

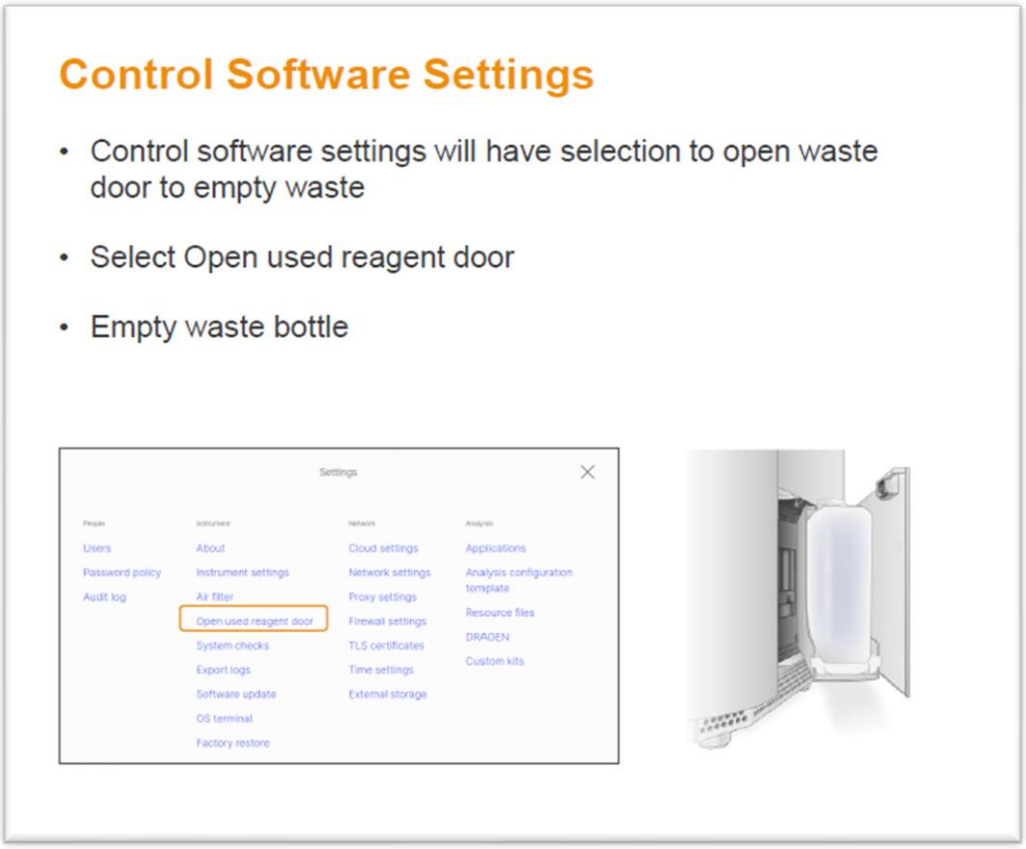
Seal the dry cartridge Library well (see picture below) with tape and discard both cartridges in the yellow safety bin.



3.10 Empty Waste Bottle

Empty the contents of the waste bottle after each run in the designated container stored on the sink in the NGI OpenLab.

Note! *This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Ventilation should be appropriate for handling of hazardous materials in reagents. 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. For additional environmental, health, and safety information, refer to the SDS at* support.illumina.com/sds.html.



Note! *Close the door by pressing gently*.

4 Retrieve data

The raw data is stored on to the USB stick. To remove the USB stick, go to the menu icon, select **Settings** and **External storage**. Press **Eject** in the actions column of the server to safely remove the USB.

5 Return the USB stick and delete raw data from the instrument computer.

Once you have copied the raw data to your private computer, please return the used USB stick to the red box just inside the door of the NGI OpenLab. Delete your raw data from the instrument hard drive by navigating to *Completed Runs*. Choose the correct run and select *Delete Run*.

If you are lacking information regarding sequencing on the MiSeq i100 Plus system, please consult the links provided under the Illumina Knowledge section (below) and the NGI OpenLab website.

6 Illumina knowledge:

Reference Material MiSeq i100 Series:
https://knowledge.illumina.com/instrumentation/miseq-i100-series/instrumentation-miseq-i100-series-reference_material-list

Spiking custom primers into the Illumina sequencing primers for MiSeq i100 Series:
https://knowledge.illumina.com/instrumentation/miseq-i100-series/instrumentation-miseq-i100-series-reference_material-list/000009551

Two Channel Chemistry and Imaging on the MiSeq i100 Series:
https://knowledge.illumina.com/instrumentation/miseq-i100-series/instrumentation-miseq-i100-series-reference_material-list/000009348

Considerations for Index color balancing on the MiSeq i100 Series:
https://knowledge.illumina.com/instrumentation/miseq-i100-series/instrumentation-miseq-i100-series-reference_material-list/000009405

Indexed Sequencing: https://support-docs.illumina.com/SHARE/IndexedSeq/Content/SHARE/IndexedSequencing/IndexedSeqIntro.htm

Why is allowing mismatches when demultiplexing desirable?
https://knowledge.illumina.com/software/general/software-general-reference_material-list/000007484

7 Support

Troubleshooting: https://knowledge.illumina.com/instrumentation/miseq-i100-series/instrumentation-miseq-i100-series-troubleshooting-list

Technical Support: https://emea.illumina.com/company/contact-us.html#/united-kingdom/technical-support