

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.

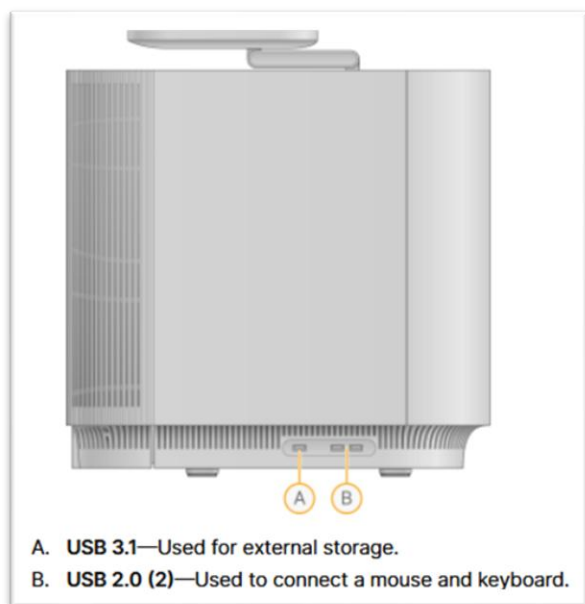
3.1 Sign in

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.



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.

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Review Run

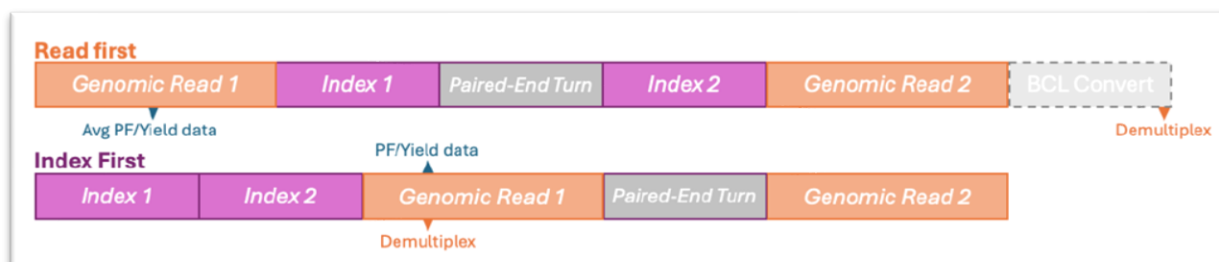
Select workflow, custom primer settings, output folder, and custom recipe options

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

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



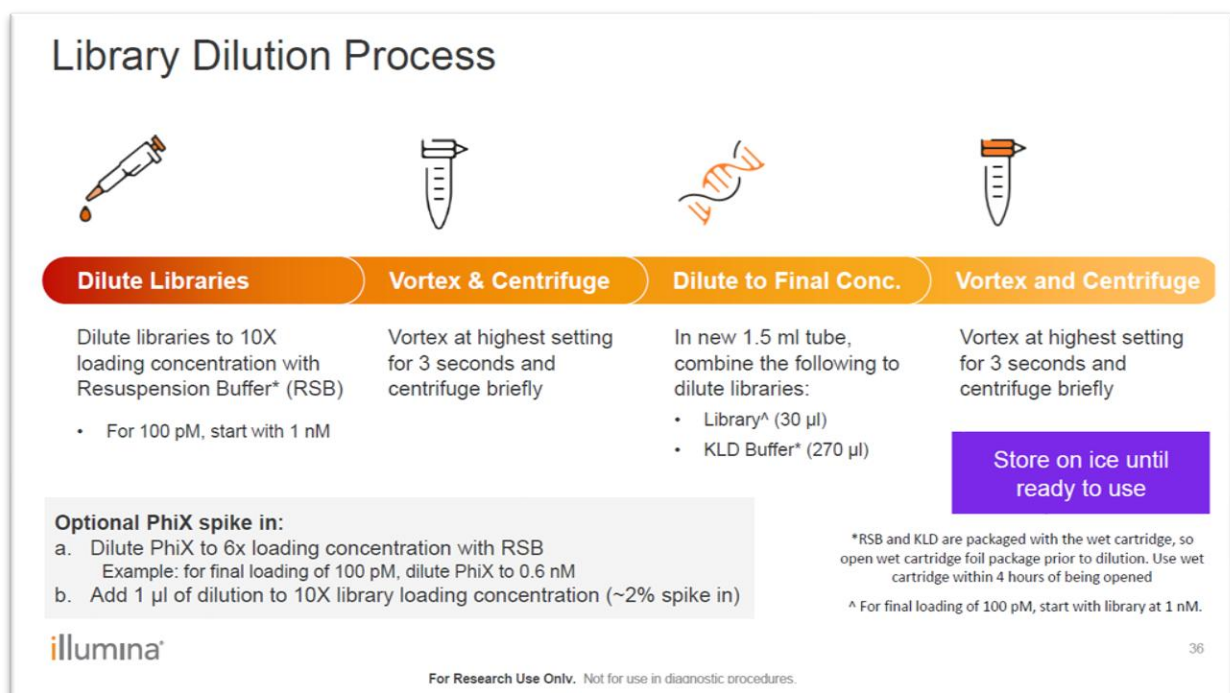
3.4 Prepare Dry Cartridge

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:

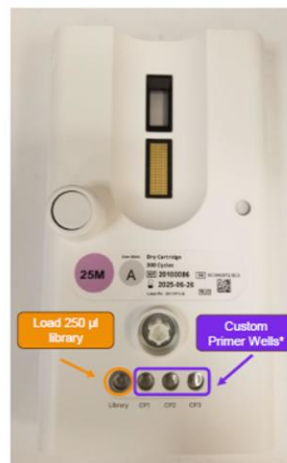
- Dilution of library to 10x loading conc with RSB to a final volume of 30µl.
- Dilution of PhiX to 6x or 10x loading conc with RSB (dilution depends on the desired spike-in percentage).
- Addition of diluted PhiX to your 10x loading conc library dilution (amount added depends on the desired spike-in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



Loading Libraries in Dry Cartridge

1. Put on new pair of powder free gloves
2. Use scissor to open dry cartridge foil package
3. Remove dry cartridge from package by grabbing from sides (avoid touching the flow cell)
4. Pierce foil seal covering port labeled **Library** using a clean pipette tip
5. Pipette 250 μ l of final diluted library solution into sample well

Note: use dry cartridge within 4 hours of opening foil package



*Custom primer wells are only for read first sequencing only

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3.5 Load Consumables

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

MiSeq i100 Series Consumable Components

All consumables shipped and stored at **Ambient** (15°C to 30°C) temperatures



Dry Cartridge

- Contains flow cell and lyophilized reagents
- Loaded with final diluted library



Wet Cartridge

- Contains sequencing reagents and buffers



Resuspension Buffer (RSB) Tube

- Use for initially diluting library
- Red capped tube



Library Denaturation Buffer (KLD) Tube

- Use for final dilution of library
- Blue capped tube

Keep consumables in original packages until ready for use

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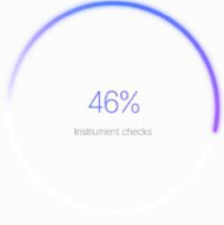
3.6 Pre-Run Checks

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[200060803]

Pre-Run Checks

Performs software system checks, instrument checks and fluidics checks

Pre-run checks



46%

Instrument checks

Cancel checks

→

Checks
complete

- Pre-run checks take around 15 minutes to complete
- Once completed, run will start automatically
- If pre-run checks are canceled and indicates consumables have been pierced, consumables cannot be reused

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3.7 Monitor Run Progress

Expected Run Times:

Clustering: ~2 hours

Each cycle: ~1 min

PE Turnaround: ~1 hour



Run name

SH32_COCATestRun_17JUN2024

Completing today at

20:33

2024-06-17

R2D2

Metrics appear after first 25 cycles of genomic read completes

% >= Q30
-- %

Projected yield
-- Gb

Total reads PF
-- M

Total % demux
-- %

Cancel run

The screenshot shows the NGI OpenLab interface with a large purple circle in the center stating "Sequencing complete" and "20:17 | 2024-06-17". To the left, a box labeled "Run name" contains "SH32_COCAstestRun_17JUN2024", with an arrow pointing to a "Run Details" window. To the right, a list of "Final run metrics are displayed" includes: "% >= Q30: 93.9%", "Total yield: 9.89 Gb", "Total reads PF: 30.97 M", and "Total % demux: 86.53 %". A callout box notes "Only available if Index metrics were provided" with an arrow pointing to the demux metric. At the bottom, there are buttons for "Eject consumables" and "Home", and the text "R2D2". A callout box at the bottom left says "Can view additional run results" with an arrow pointing to the Run Details window.

- 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](#).

The screenshot shows a window titled "Monitoring Run in Control Software" with a sub-header "Run Details". It displays sequencing metrics in a table:

% >= Q30	Yield	Total raw reads	Total reads PF	% reads PF	% occupied	% aligned to control	% error rate % demux
93.88%	9.89 Gbp	30.54 M	30.97 M	76.34%	93.74%	2.49%	0.28% 86.53%

Below this is a section "Metrics by lane" for "Lane 1" with the same metrics. Further down is a section "Demultiplex metrics by sample" with a table of sample IDs and their % demux values. A callout box says "If BCL Convert enabled" with an arrow pointing to the sample table. Another callout box on the right says "May need to scroll down in Run Details to obtain additional metrics and demux info" with an arrow pointing to the bottom of the window. At the bottom of the window are buttons for "Exit", "Delete", and "Delete run data". The Illumina logo is at the bottom left, and the text "For Research Use Only. Not for use in diagnostic procedures" is at the bottom center.

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



3.8 Eject Used Consumables

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



3.9 **Dispose of Used Consumables**

Seal the wells of both cartridges with tape and discard them in the yellow safety bin.

3.10 Empty Waste Bottle

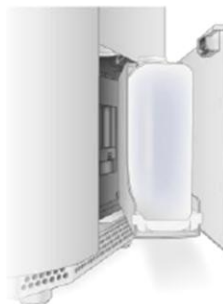
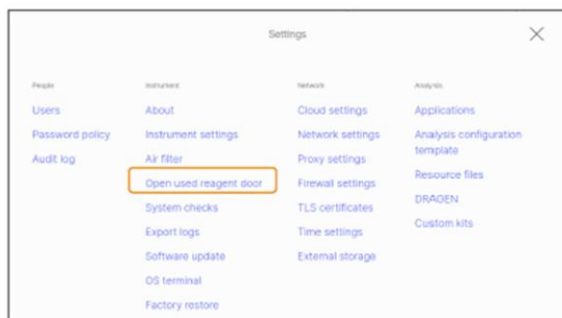
Empty the contents of the waste bottle after each run in the designated container stored on the sink in the NGI OpenLab.



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.

Control Software Settings

- Control software settings will have selection to open waste door to empty waste
- Select Open used reagent door
- Empty waste bottle



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*.

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-](https://support-docs.illumina.com/SHARE/IndexedSeq/Content/SHARE/IndexedSequencing/IndexedSeqIntro.htm)

[docs.illumina.com/SHARE/IndexedSeq/Content/SHARE/IndexedSequencing/IndexedSeqIntro.htm](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>