

## 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](http://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.

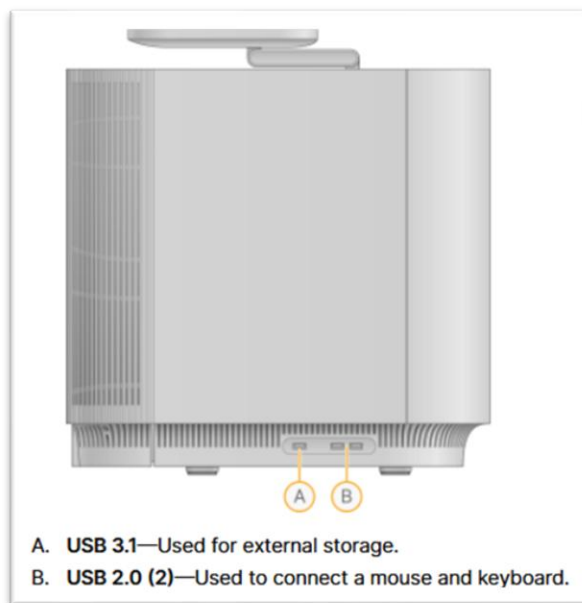
#### 3.1 Sign in

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**.
5. Select **Data Traveler 3.0** and press **Add** (do not enter a “USB password”).
6. Select **Add Folders** to add a default folder for data storage.
7. Choose **Add folder** to specify the default data storage.
8. Select **DataTraveler 3.0** as Server location from the drop-down list.
9. Under Available folders, select **output** and press **Save**.
10. Finally, select **Save** and click the **X** in the upper-right corner to exit and return 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.

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- If using custom primers, select the appropriate custom primers checkboxes. Refer to [Custom Primers](#) for more information.
- Set Cloud run setting to Run monitoring.

Created by Commercial Training [200060803]

### Review Run

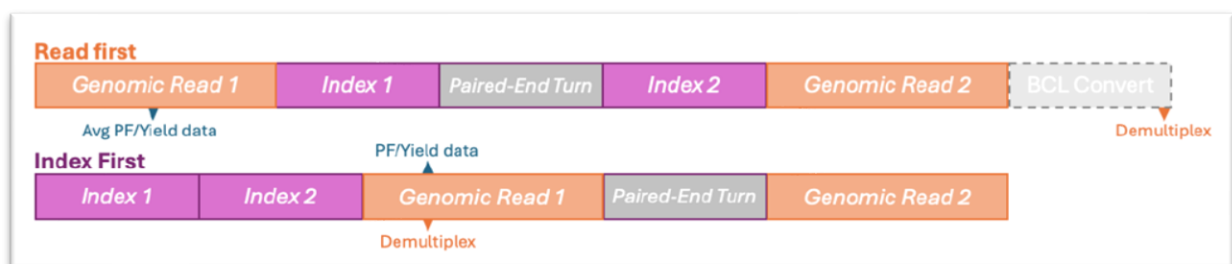
Select workflow, custom primer settings, output folder, and custom recipe options

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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.

### Library Dilution Process

Dilute Libraries	Vortex & Centrifuge	Dilute to Final Conc.	Vortex and Centrifuge
<p>Dilute libraries to 10X loading concentration with Resuspension Buffer* (RSB)</p> <ul style="list-style-type: none"><li>• For 100 pM, start with 1 nM</li></ul>	<p>Vortex at highest setting for 3 seconds and centrifuge briefly</p>	<p>In new 1.5 ml tube, combine the following to dilute libraries:</p> <ul style="list-style-type: none"><li>• Library<sup>^</sup> (30 µl)</li><li>• KLD Buffer* (270 µl)</li></ul>	<p>Vortex at highest setting for 3 seconds and centrifuge briefly</p> <p><b>Store on ice until ready to use</b></p>

**Optional PhiX spike in:**

- Dilute PhiX to 6x loading concentration with RSB  
Example: for final loading of 100 pM, dilute PhiX to 0.6 nM
- Add 1 µl of dilution to 10X library loading concentration (~2% spike in)

\*RSB and KLD are packaged with the wet cartridge, so open wet cartridge foil package prior to dilution. Use wet cartridge within 4 hours of being opened

<sup>^</sup> For final loading of 100 pM, start with library at 1 nM.

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## 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  $\mu$ 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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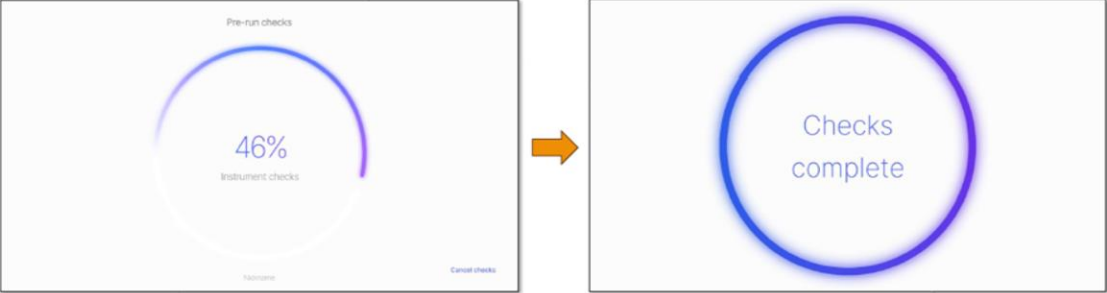
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## 3.6 Pre-Run Checks

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### Pre-Run Checks

*Performs software system checks, instrument checks and fluidics checks*



- 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\_COCA TestRun\_17JUN2024

Completing today at  
**20:33**  
2024-06-17

Metrics appear after first 25 cycles of genomic read completes

- % >= Q30 --%
- Projected yield -- Gb
- Total reads PF -- M
- Total % demux -- %

R2D2

Cancel run

The screenshot shows a central purple circle with the text "Sequencing complete" and the timestamp "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 box labeled "Final run metrics are displayed" lists: "% >= Q30: 93.9%", "Total yield: 9.89 Gb", "Total reads PF: 30.97 M", and "Total % demux: 86.53%". A note below this says "Only available if Index metrics were provided". At the bottom, a button says "Can view additional run results". The interface also includes a menu icon, a notification bell, and buttons for "Eject consumables" and "Home".

- 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 is titled "Monitoring Run in Control Software" and includes the text "Created by Commercial Training [20000804]". It displays a "Run Details" window with the following data:

Sequencing metrics							
% >= Q30	Yield	Total raw reads	Total reads PF	% reads PF	% occupied	% aligned to control	% error rate % demux
93.88%	9.89 Gbp	39.54 M	30.97 M	78.34%	93.74%	2.49%	0.28% 86.53%

Below this, "Metrics by lane" for Lane 1 shows: % >= Q30 (93.88%), % reads PF (78.34%), % aligned to control (2.49%), and % error rate (0.28%).

The "Demultiplex metrics by sample" table is shown below:

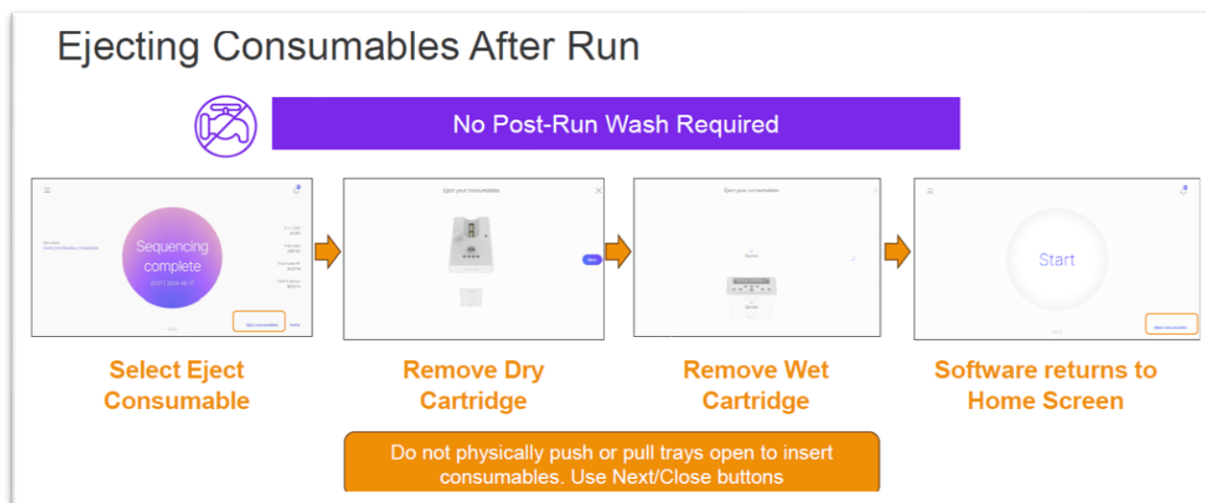
Sample ID	% demux
Sample1	7.67%
Sample 10	9.06%
Sample11	9.32%
Sample12	0.96%
Sample2	8.10%
Sample3	8.02%
Sample4	7.57%
Sample5	8.13%
Sample6	8.19%
Sample7	8.22%

Annotations include: "If BCL Convert enabled" pointing to the demultiplexing table, and "May need to scroll down in Run Details to obtain additional metrics and demux info" pointing to the bottom of the window. The interface also features "Exit", "Delete", and "Delete run data" buttons.

- 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

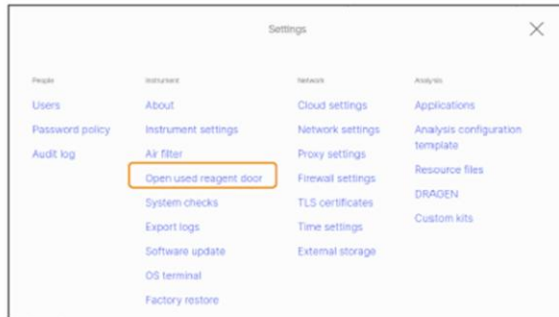
Empty the contents of the waste bottle after each run in the designated container stored on the sink in the NGI OpenLab.



**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](http://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](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](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](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](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](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>