GeneLab Standard Operating Procedure: Setting up NovaSeq 6000 and iSeq 100 sequencers

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Version 1.0

Document Revisions

Document Number	Revision Number	Date	Description of Changes
GL-SOP-3.1	1.0	May 2020	Original document

Scope and Purpose

This procedure describes the workflows and parameters to follow in order to set up NovaSeq 6000 and/or iSeq 100 sequencers.

➔ If setting up iSeq 100 sequencer refer to procedure A.

➔ If setting up NovaSeq 6000 sequencer refer to procedure B.

This SOP only describes the set-up of the sequencer, for all the other procedural details; make sure to read the most updated System Guides and the proper SOP's.

Procedure A: Reagents

1. iSeq 100 i1 Cartridge (Stored at -20°C)
2. iSeq 100 i1 Flow Cell (Stored at 4°C)
3. Consumables required for library dilution and spike in

Procedure A: Setting up iSeq 100 sequencer

1. Thaw the iSeq cartridge and the flow cell according to the System Guide

Method	Thaw Time	Instruction
20°C to 25°C water bath	6 hours, not exceeding 18 hours	• Use 6 L (1.5 gal) water per cartridge. • Set a temperature-controlled water bath to 25°C ***or*** mix hot and cold water to achieve 20°C to 25°C. • Face the bag label up, submerge the cartridge completely, and apply ~2 kg (4.5 lbs) weight to prevent floating. • Do not stack cartridges in the water bath unless it is temperature-controlled.
2°C to 8°C refrigerator	36 hours, not exceeding 72 hours	Position the cartridge so that the label faces up and air can circulate on all sides, including the bottom.
Room-temperature air (20°C to 25°C)	9 hours, not exceeding 18 hours	Position the cartridge so that the label faces up and air can circulate on all sides, including the bottom.

Flow cell should be at room temperature at the time of sequencer loading.

2. Prepare SampleSheet file. Make sure to use the right configuration of i5 index (Index2).
3. Dilute the multiplexed library and spike the PhiX control according to the experimental design, dilute the spiked pool to loading concentration.
4. Load the library pool onto the cartridge following the System Guide.
5. Set up the sequencing run.

Local Run Manager mode:

a. Open iSeq Control Software.
b. Select Local Run Manager → Open Local Run Manager.
c. Create a sequencing run by Importing a sample sheet.
d. Save run.
e. In the control software, select “Sequence”. The Software will open the sequencer door and eject the tray.
f. Place the sequencing cartridge with the flow cell and diluted, spiked library onto the tray.
g. Select Close Door, the tray will retract and the door will close.
h. If does not appear automatically, select the previously created run from the “Run Name” list. Try Refreshing if the run is missing.

i. Make sure the read length parameters for Read 1, Index 1, Index 2, Read 2 fit with the planned experimental design:

Kit	Number of Cycles	Requested read lenght	Adapters used	Read 1 Setting	Index 1(i7) Setting	Index 2(i5) Setting	Read 2 Setting
i1	300	PE150	Illumina UDI	151	8	8	151
	300	PE150	IDT UDI + UMI	148	17	8	148
	300	PE100	Illumina UDI	101	8	8	101
	300	PE100	IDT UDI + UMI	99	17	8	99
	300	PE50	Illumina UDI	51	8	8	51
	300	PE50	IDT UDI + UMI	49	17	8	49

j. Make sure to record/take an image of the run set up window.

k. Select Start Run.

l. When run is finished, dispose of the used reagents according to the safety regulations.

Manual mode (without SampleSheet and Local Run Manager):

a. Open iSeq Control Software.

b. In the control software, select “Sequence”. The Software will open the sequencer door and eject the tray.

c. Place the sequencing cartridge with the flow cell and diluted, spiked library onto the tray.

d. Select Close Door, the tray will retract and the door will close.

e. In the “Run Name” field, enter a unique name, make sure to include the date, the project and any other information that might be useful in identifying this run.

f. Select “Single Read/Dual Read” option.

g. Enter the number of cycles to perform in each read following the planned experimental design.

Kit	Number of Cycles	Requested read length	Adapters used	Read 1 Setting	Index 1(i7) Setting	Index 2(i5) Setting	Read 2 Setting
i1	300	PE150	Illumina UDI	151	8	8	151
	300	PE150	IDT UDI + UMI	148	17	8	148
	300	PE100	Illumina UDI	101	8	8	101
	300	PE100	IDT UDI + UMI	99	17	8	99
	300	PE50	Illumina UDI	51	8	8	51
	300	PE50	IDT UDI + UMI	49	17	8	49

h. Make sure to record/take an image of the run set up window.

i. Select Start Run.

j. When run is finished, dispose of the used reagents according to the safety regulations.

Procedure B: Reagents

1. NovaSeq 6000 SBS Cartridge v1 (Stored at -20°C)
2. NovaSeq 6000 Cluster Cartridge v1 (Stored at -20°C)
3. NovaSeq 6000 Buffer Cartridge v1 (Stored at RT)
4. NovaSeq 6000 Flow Cell v1 (Stored at 4°C)
5. Consumables required for library dilution and spike in

- If using version 1.5 of sequencing reagents, longer read1 and read 2 is possible.

Procedure B: Setting up NovaSeq 6000 sequencer

1. Thaw the two NovaSeq cartridges and the flow cell according to the System Guide:

Thaw in a room temperature water bath (19°C to 25°C).
Submerge about halfway.

Use the following table to determine thaw duration.

CAUTION

Using hot water for thawing reagents may cause reduced data quality or run failure.

Cartridge	Duration of Thaw
SP, S1, and S2 SBS cartridge	4 hours
SP, S1, and S2 cluster cartridge	Up to 2 hours
S4 SBS cartridge	4 hours
S4 cluster cartridge	Up to 4 hours

Set the flow cell package aside at room temperature for 10-15 minutes to allow it to reach room temperature.

2. Empty used reagent bottles from the NovaSeq, dispose according to laboratory safety regulations.
3. Change gloves after handling waste.
4. Prepare SampleSheet file. Make sure to use the right configuration of i5 index (Index2).
5. Dilute the multiplexed library and spike the PhiX control according to the experimental design, denature and dilute the spiked pool according to the guide.
6. Inspect the SBS and the Cluster cartridges, invert 10 times and tap the bottom on the bench.
7. Load the library into the library tube and insert into position #8 of the cluster cartridge.
8. In the control software, select “Sequence” and then select a single or dual flow cell run
   The flow cell door will open.
9. With new powder free gloves, unpack the flow cell and inspect for defects.
10. Align the flow cell over the four raised clamps and place it on the flow cell stage.
11. Select “Close Flow Cell Door.”
12. Load the SBS, Cluster and Buffer Cartridges.
13. Select Checkbox acknowledging that the used reagent bottles have been emptied.

14. Select Run Setup button to enter run parameters.
15. Select workflow type (Xp/Standard).
16. In the “Run Name” field enter a unique name, make sure to include the date, the project and any other information that might be useful in identifying this run.
17. Enter the number of cycles to perform in each read following the planned experimental design and the reagents used to make the libraries and sequence:

Kit	Number of Cycles	Requested read length	Adapters used	Read 1 Setting	Index 1(i7) Setting	Index 2(i5) Setting	Read 2 Setting
SP	100	PE50	Illumina UDI	51	8	8	51
		PE50	IDT UDI + UMI	49	17	8	49
	200	PE100	Illumina UDI	101	8	8	101
		PE100	IDT UDI + UMI	99	17	8	99
	300	PE150	Illumina UDI	151	8	8	151
		PE150	IDT UDI + UMI	149	17	8	149
	500	PE250	Illumina UDI	251	8	8	251
		PE250	IDT UDI + UMI	247	17	8	247
		PE250	Nextera Flex DNA	249	10	10	249

S1	100	PE50	Illumina UDI	51	8	8	51
		PE50	IDT UDI + UMI	49	17	8	49
	200	PE100	Illumina UDI	101	8	8	101
		PE100	IDT UDI + UMI	99	17	8	99
	300	PE150	Illumina UDI	151	8	8	151
		PE150	IDT UDI + UMI	149	17	8	149
S2	100	PE50	Illumina UDI	51	8	8	51
		PE50	IDT UDI + UMI	49	17	8	49
	200	PE100	Illumina UDI	101	8	8	101
		PE100	IDT UDI + UMI	99	17	8	99
	300	PE150	Illumina UDI	151	8	8	151
		PE150	IDT UDI + UMI	149	17	8	149
S4	200	PE100	Illumina UDI	101	8	8	101
		PE100	IDT UDI + UMI	99	17	8	99
	300	PE150	Illumina UDI	151	8	8	151
		PE150	IDT UDI + UMI	149	17	8	149

10X Single Cell

Kit	Number of Cycles	Requested read length	Adapters used	Read 1 Setting	Index 1(i7) Setting	Index 2(i5) Setting	Read 2 Setting
Any	200	N/A	ST	28	8	0	91

Spatial Transcriptomics

Kit	Number of Cycles	Requested read length	Adapters used	Read 1 Setting	Index 1(i7) Setting	Index 2(i5) Setting	Read 2 Setting
Any	200	N/A	ST	28	10	10	121

18. Select output folder.
19. Confirm the run parameters on the Review screen.
20. Make sure to record/take an image of the run set up window.
21. Select “Start Run.”