

Lab SOP 6 MiSeq i100 Sequencing v1

The MiSeq i100 sequencer uses room temperature reagents and there is no need to wait for reagents to thaw before preparing a sequencing run.

MiSeq i100 Sample Sheet Preparation

1. Navigate to Illumina's Basespace online.
 - [Dashboard - BaseSpace Sequence Hub](#)
2. Click the "Runs" tab, select "New Run" and then "Run Planning".
3. On the "Create a Run" page enter a run name, run description (optional), under Instrument Platform select "MiSeq i100 Series", for Secondary Analysis select "On Instrument (Local)".
4. For Read Lengths, ensure that the reads match the appropriate kit and Index PCR primer you are sequencing with.
 - ~~For a 300-cycle kit, Read 1 and Read 2 will be 151 and 151.~~
 - For a 600-cycle kit, Read 1 and Read 2 will be 301 and 301.
 - For an Illumina DNA-RNA UD Index Set (A/B/C/D Tagmentation), Index 1 and Index 2 will be 10 each.
5. Sample Container ID is optional. Select "Next".
6. On the Configuration 1 page, for Application select "DRAGEN BCL Convert - X.X.X"
7. X.X.X is the current version of software on the MiSeq i100 (e.g., 4.3.13).
8. For Library Prep Kit select appropriate library preparation (e.g., "Illumina DNA Prep").
9. For Index Adaptor Kit select appropriate index adaptor kit that was used on this run (e.g., Illumina DNA-RNA UD Indexes Set A/B/C/D Tagmentation). Select "Next".
10. On the Configuration 1: Dragen BCL Convert – X.X.X page, review that the correct Library Prep Kit, Index Adapter Kit and Override Cycles say the correct information.
11. Upload a csv file of samples using the template provided by Basespace with "1" entered in each row of samples for Barcode Mismatches.
12. For FASTQ File Compression Format select "gzip". Select "Next".
13. On the Run Review page, ensure information is correct before exporting sample sheet onto approved external hard drive.

Qubit Library and PhiX

14. Vortex and centrifuge pooled library (5 µL from each sample), PhiX community, and Qubit HS standards.
15. In appropriate size tube, make a master mix by combining 199 µL of buffer solution and 1 µL of HS Concentrate multiplied per sample needing to Qubit.

16. Add 198 µL of Qubit MM to each Qubit tube needed for PhiX and pooled library.
17. Add 190 µL of Qubit MM to each Qubit tube needed for Standards.
18. Add 2 µL of Library and PhiX to appropriate tubes.
19. Add 10 µL of each Qubit HS standard to appropriate tubes.
20. Vortex each Qubit assay tube and let incubate at room temperature for 2 minutes.
21. On the Qubit, select dsDNA, then dsDNA High Sensitivity, then read standard.
22. Follow instructions to read standard 1 and standard 2.
23. Place PhiX and pooled library tube(s) into the Qubit individually and select read sample.
24. Using the link below, convert the ng/µL reading to nM. When converting the library, use 350 bp as library size. When converting the PhiX, use 500 bp as library size.
 - <https://support.illumina.com/help/nanomolar-conversion/nanomolar-conversion.htm>
25. To convert from nM to pM, multiply the value by 1000.

Diluting Library & PhiX to 10x Starting Concentration

Since the KLD denaturation buffer from the kit will be added last, the final loading concentration will first have to be a 10x starting concentration. For example, if the final loading concentration will be 60 pM, the pooled library and PhiX must first be diluted to 10x that concentration (600 pM) using the RSB in the sequencing kit. This SOP will use a final loading concentration of 60 pM as its example concentration hereafter.

26. Using the equation below, determine the volume of pooled library that will be diluted with RSB by inputting the pM reading from the Qubit for X.

$$\text{Volume used for Pooled Library } (\mu\text{L}) = \frac{600 \text{ pM} * 30 \mu\text{L}}{X \text{ pM}}$$

27. Using the equation below, determine the volume of PhiX that will be diluted with RSB by inputting the pM reading from the Qubit for X.

$$\text{Volume used for PhiX } (\mu\text{L}) = \frac{600 \text{ pM} * 30 \mu\text{L}}{X \text{ pM}}$$

28. Determine the amount of RSB that will be added to dilution calculated in step 1, using the equation below for the pooled library.

$$\text{Volume of RSB } (\mu\text{L}) = 30 \mu\text{L} - \text{Volume used for dilution of pooled library}$$

29. Determine the amount of RSB that will be added to dilution calculated in step 2, using the equation below for PhiX.

$$\text{Volume of RSB } (\mu\text{L}) = 30 \mu\text{L} - \text{Volume used for dilution of PhiX}$$

30. Dispense calculated volumes of RSB into separate tubes for the pooled library and PhiX.

31. Dispense calculated volumes of PhiX and pooled library into their respective tubes containing RSB Tween20.
32. Vortex and briefly centrifuge tubes.
33. To diversify the library, spike the pooled library (prepared above) with the diluted PhiX (prepared above) in a new 1.5 mL tube (e.g., a spike of 40% adds 12 μ L of diluted PhiX to 18 μ L of the combined library). Note: optimal PhiX spike is still being determined based on run results.
34. After combining, vortex and briefly centrifuge.
35. In the same 1.5 mL tube, to dilute to your final loading concentration, add 270 μ L of KLD denaturation buffer to the PhiX spiked library.
36. After combining, vortex and briefly centrifuge.
37. Store mixture on ice until ready for use. Final diluted library solution is stable up to 6 hours when stored on ice or at 4°C.

Starting a Run

38. Log into MiSeq i100.
39. Select External Storage.
40. Connect the external hard drive to the blue-colored USB port. Make sure you have an appropriately named folder on the external hard drive for the MiSeq i100 to output the run files to.
41. Select “Add USB storage” then select the matching external hard drive.
42. Under Default folder, select “Add folders” and select your output file previously created. If you have used this external hard drive before, you can skip this step.
43. Click the X in the top right corner.
44. Click the three bars in the top left corner and select “Runs”.
45. Select “Import Sample Sheet” if you have one stored on the external hard drive.
 - If you didn’t set up the sample sheet previously, you can do this on the machine by selecting “Create Run” and importing the filled-out sample template csv from the website and following the earlier mentioned steps the same way.
46. Select “Manual” then “Import sample sheet for local analysis”.
47. Find and select your sample sheet and ensure the correct information is shown below the pathway, then click “Review”.
48. Ensure information is correct with “Sequence indexes first” not checked. Then click “Load consumables”.
49. Open the package on the dry cartridge of your sequencing kit and use a clean pipette tip to pierce and widen the hole on the “Library” well of the flow cell cartridge.
50. Load 250 μ L of spiked library into sequencing kit well labeled “Library”.
51. Place the loaded flow cell cartridge on the top shelf that has come out of the machine. Then click “Next”.

52. Place the opened wet cartridge on the bottom shelf that has tilted out of the machine. Then click “Verify”.
53. Once you are ready, click “Start run”. It will begin pre-run checks and will then tell you when it will be done sequencing.

Ejecting External Hard Drive and Disposing of Waste

54. When the run has completed, you can press “Eject Cartridges” in the lower right and it will allow you to remove the wet and dry cartridges.
55. To dispose of the wet and dry cartridge, remove the outer white casing. Recycle the white casing and throw away the black interior casing.
56. When you are ready to remove the external hard drive, select “Home”.
57. Select “External Hard Drive” and select “Eject” next to the correct one.
58. When prompted to empty waste bottle, select the menu icon in the upper left corner, select “Settings”, and then “Open used reagent door”. Carefully empty the waste down the drain with water, replace the waste bottles and close the door.