NEXTSEQ 550

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References

- NextSeq 550 System:
  - https://support.illumina.com/sequencing/sequencing_instruments/nextseq-550/documentation.html
  - https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/nextseq-550dx/nextseq-550dx-instrument-ref-guide-1000000009513-07.pdf
- NextSeq 500 and 550 System Denature and Dilute Libraries Guide
  - https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/nextseq/15048776_18_nextseq-500-550-denature-dilute-libraries-guide.pdf
- PhiX control:
  - https://www.illumina.com/content/dam/illumina-support/documents/products/technotes/technote_phixcontrolv3.pdf

before running

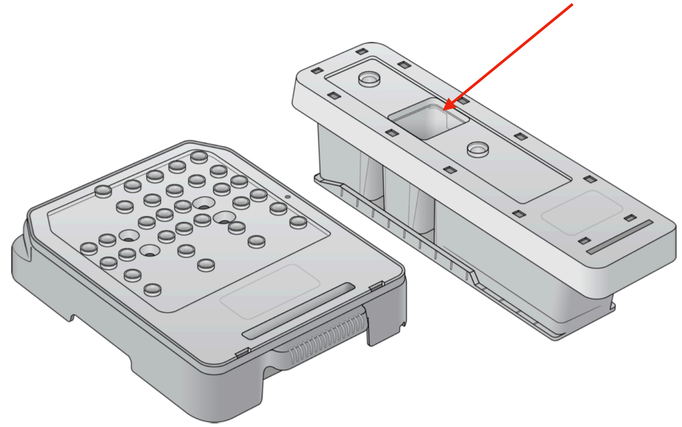
- pre thaw the reagent cartridge, HT1 buffer (put in 4C fridge overnight)
- before running, place flow cell, reagent cartridge at RT for at least 30min

- you need to also prepare: wash solution (125ml 0.05% Tween20 wash)
  - you could also use the wash solution next to the machine, but make sure there are enough

pre step: Wash

- if the previous run on the machine is less than 2 weeks, then this step is not necessary;
- if the previous run is more than 2 weeks ago, the machine will prompt you to do a wash step:
  - select “**quick wash**”, then follow the instructions on screen
  - prepare 125ml 0.05% Tween20 wash (usually they have a bottle of wash solution on the side, but you could also bring your own)
  - load the wash solution to the buffer wash cartridge
  - place the buffer wash cartridge (with wash solution), reagent wash cartridge, and the empty waste collecting cartridge to place
  - click **start**, it will take ~20min.

wash buffer load here



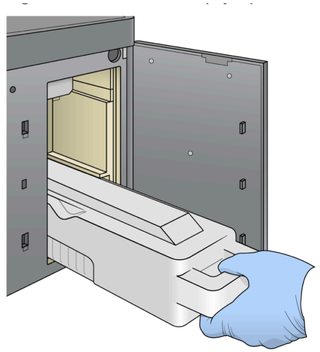


Figure 16 Load Reagent Cartridge

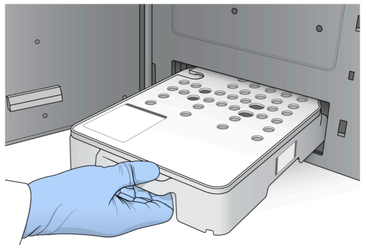
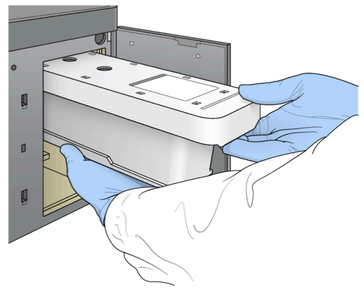


Figure 15 Load the Buffer Cartridge



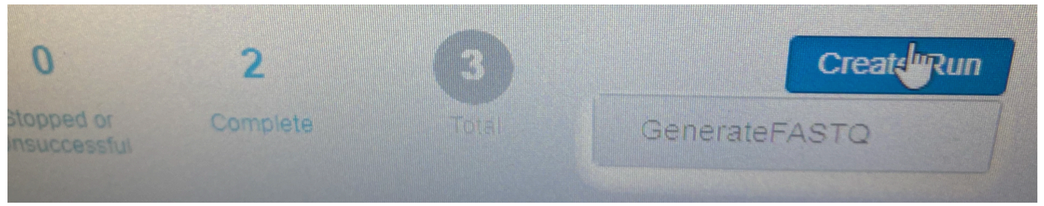
step1: create your run on “Local Run Manager” software

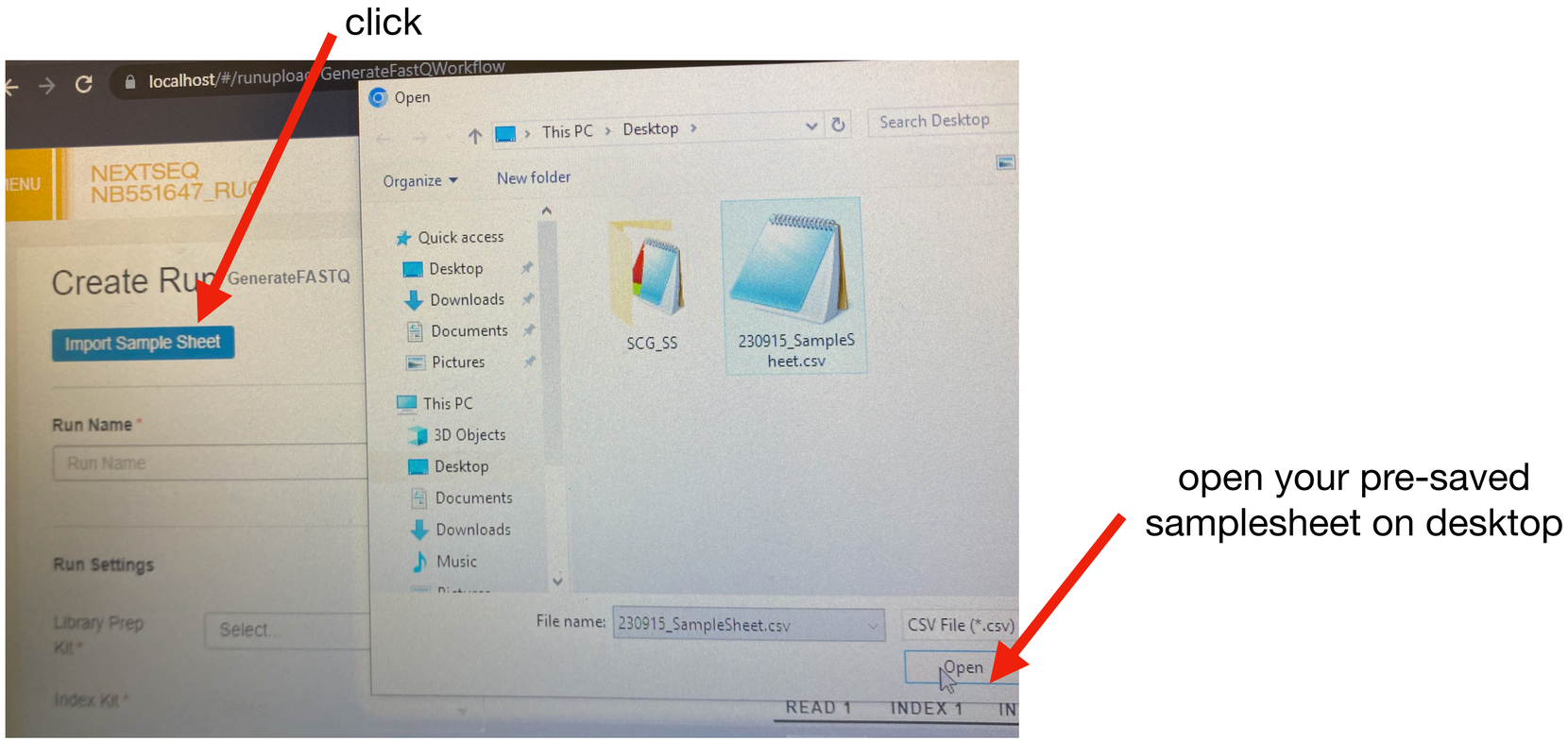
- prepare a sample sheet in .csv format (refer to the sample sheet template), save to your flash drive, plug-in and copy-paste to the desktop

- open “local run manager” software

- log in (the sheet with login name and password info are on the wall to the left)

- click: Create Run—GenerateFASTQ

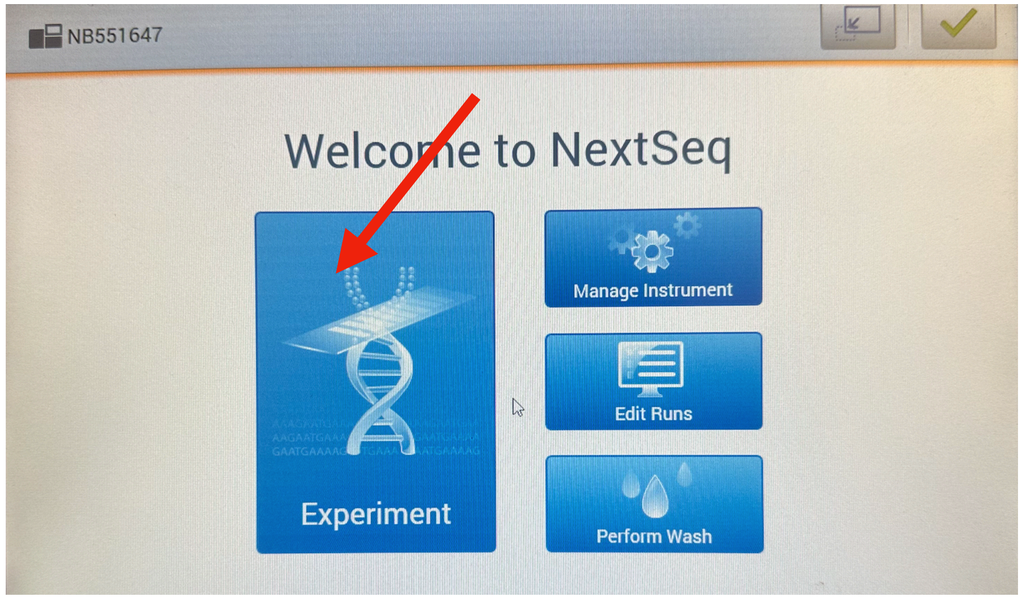


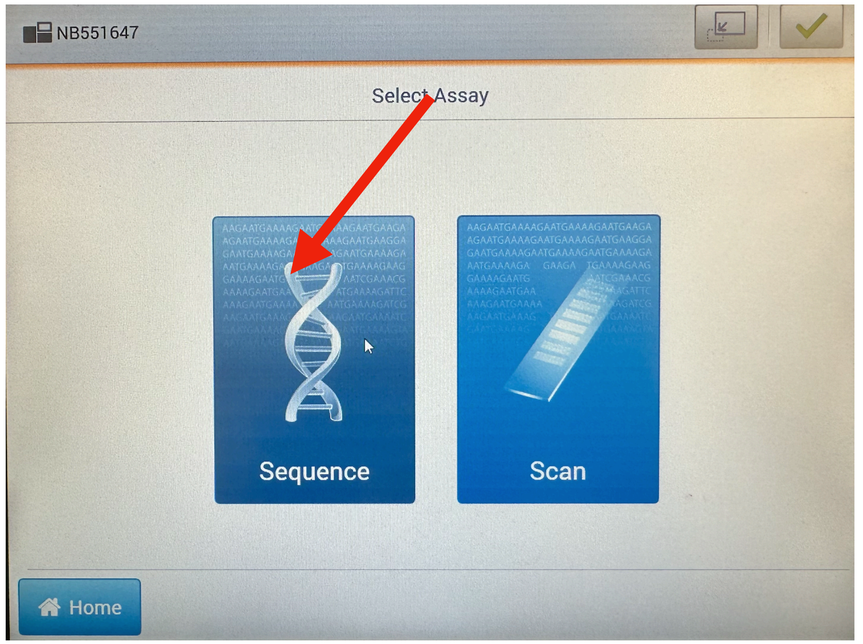


- you could select “Import Sample Sheet” to directly upload your pre-generated sample sheet.csv to the software; or
- you could manually type in the information
- once you make sure every info is there, you click “save run” then minimize the software window

step2: set-up a sequencing run

- 2.1: Select Run
- in the home screen, select: “Experiment” —“Sequence”

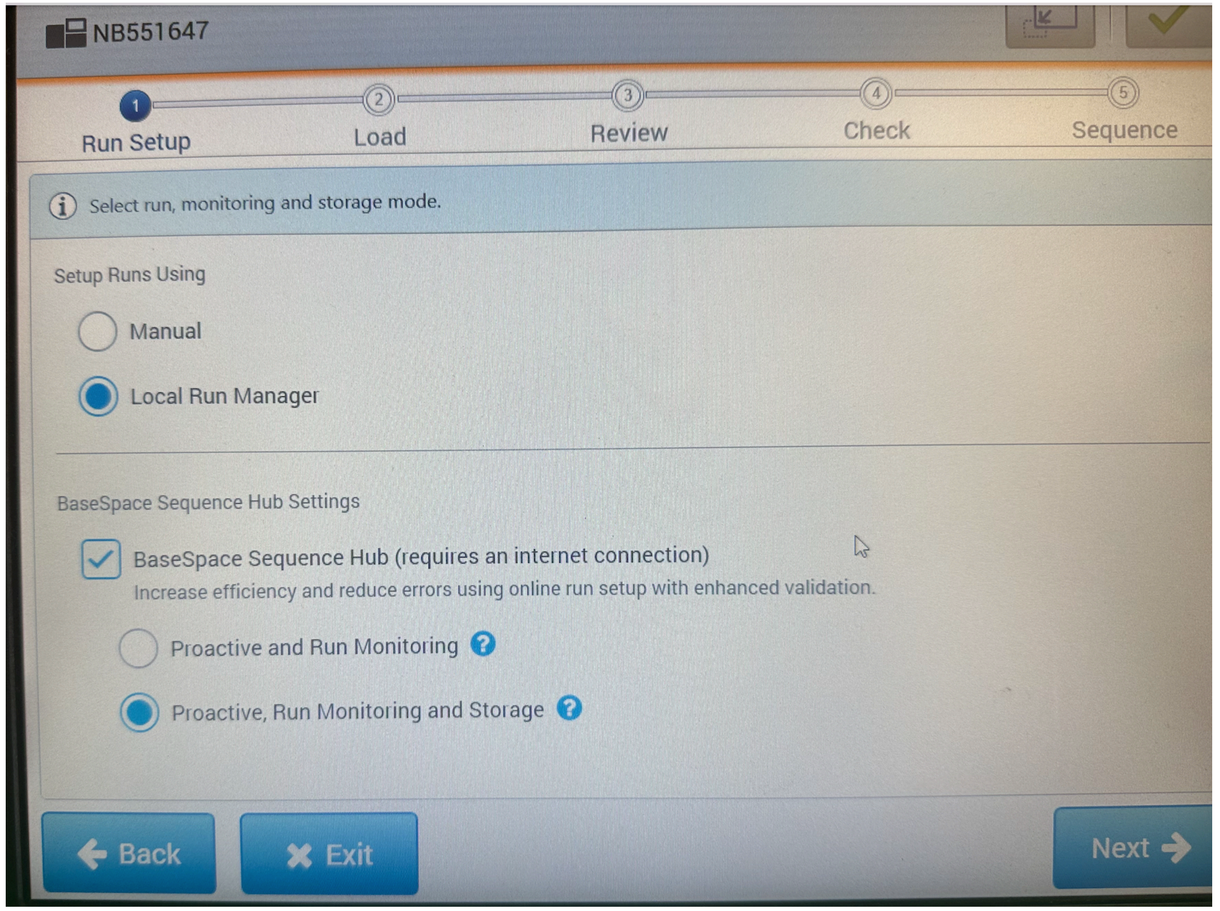


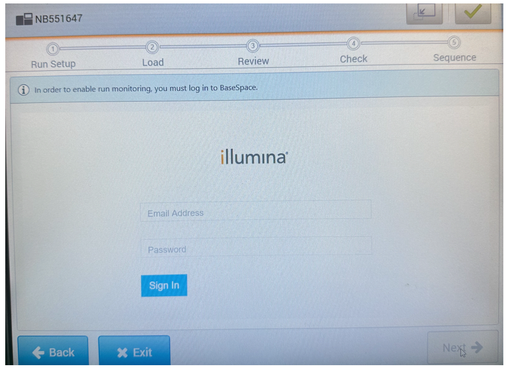


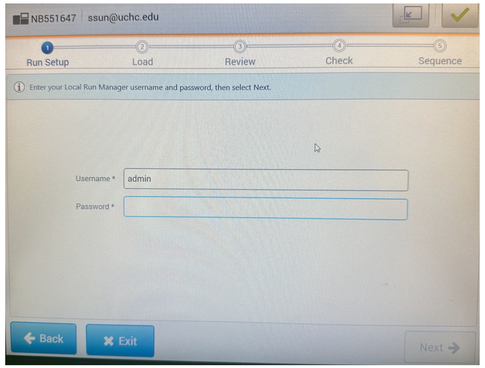
- select a run from the list — select “local Run Manager”, check BaseSpace and “Proactive, Run monitoring and Storage”

- you will need to log in to your personal illumina account at this point

- you will then input the username and password of “Local Run Manager”

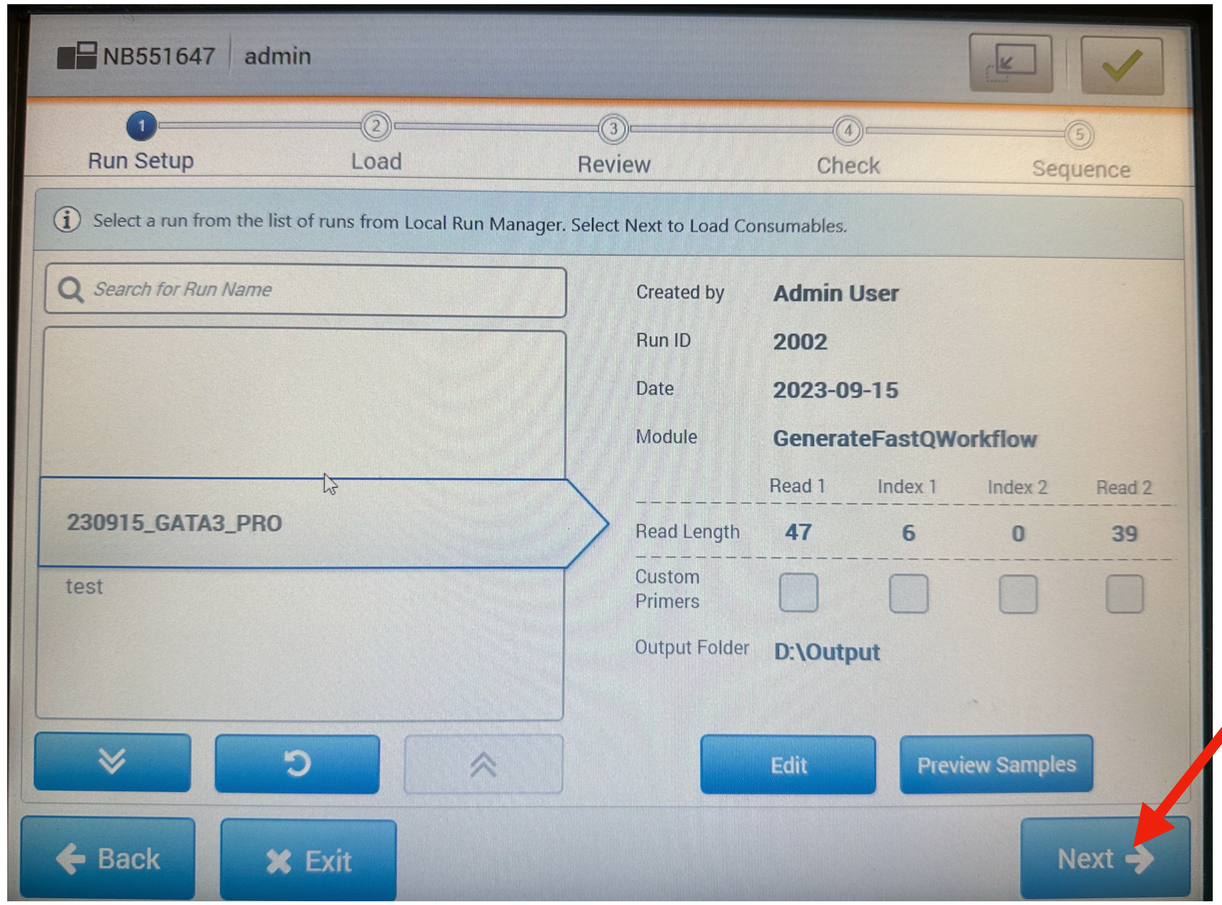






- after log-in to “local run manager”, you are able to extract the previously created run to the NextSeq RUO software.

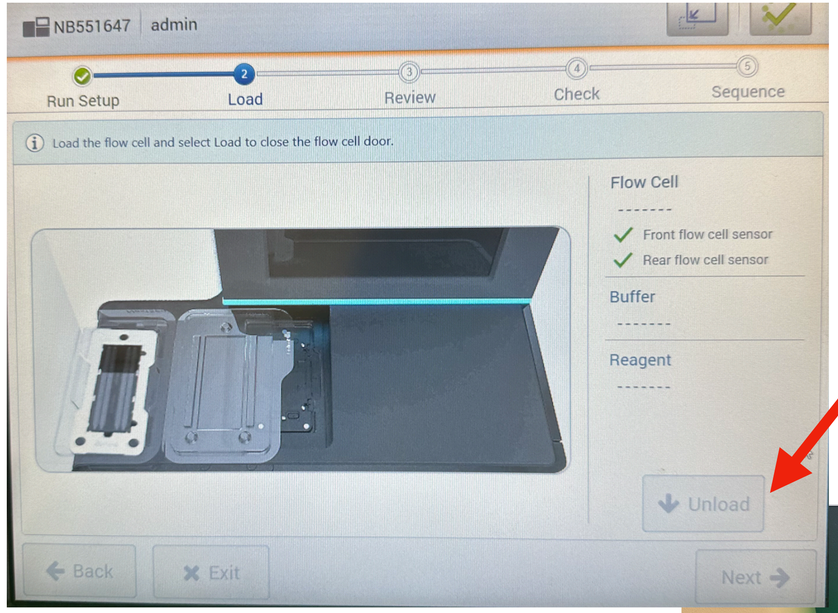
- you can see all the info about your experiment here, and noble check to make sure everything is looking correct



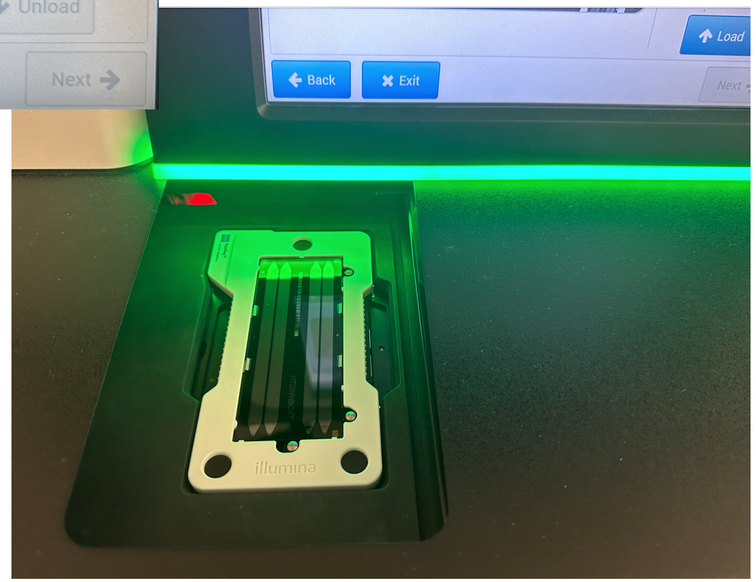
- select “Next”

2.2 load flow cell

- make sure the flow cell is placing at RT for at least 30min — so that no condensation generated
- ** it will take a while for the machine to automatically open the flow cell placing stage
- remove the used flow cell out
- put the new flow cell on the stage as prompt by machine
- select “Load”
- select “Next"



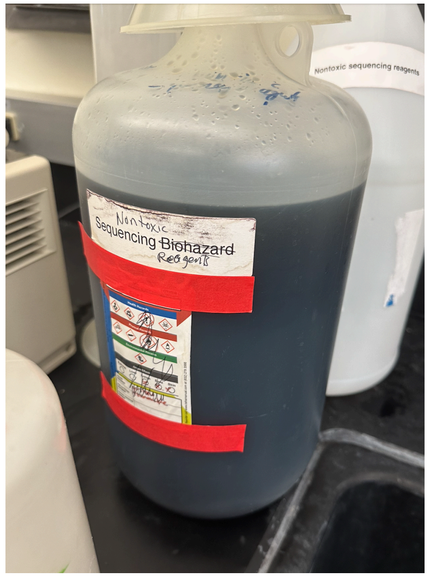
“Load”
“Next”

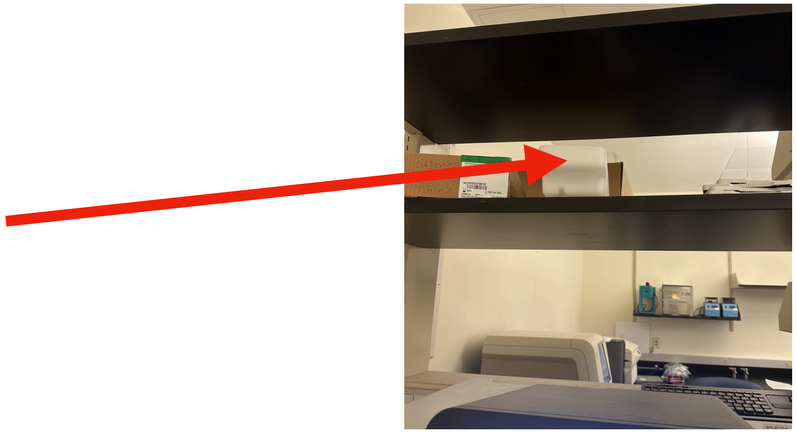


2.3 Empty/replace the Spent Reagent Container

- open the buffer compartment door
- took the container out, empty/clean the container, the waste should be disposed into the <u>sequencing reagent bottle</u> next to the sink
- *there is a spare one on the shelf, if need it, use it

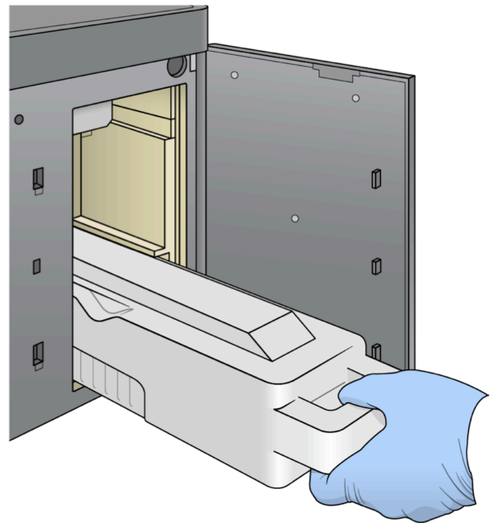
Figure 13 Remove the Spent Reagents Container





- slide the empty Spent Reagent Container into the buffer compartment (bottom) until it stops

Figure 14 Load the Empty Spent Reagents Container



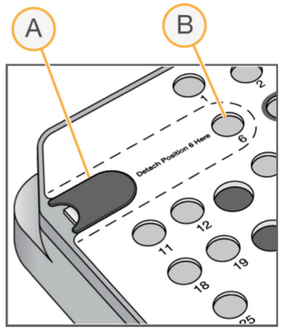
2.4 load the buffer cartridge

- remove the used ones
    - *used ones should be rinsed with water and solution can be disposed into the sink; the container itself should be disposed into <u>regular trash can</u>
- slide the new buffer cartridge into the top stage until it stops
- close the buffer compartment door, and select “Next”

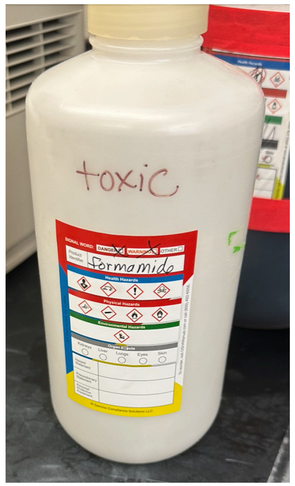
2.5 load the reagent cartridge

- reagent cartridge need to pre-thaw, and place in RT for at least 30min before loading
- open the reagent compartment door
- remove the used reagent cartridge
  - used reagent cartridge need to be properly clean-up: step1. remove the rubber cover, press down to eject the reservoir; step2. dispose the solution containing formamide to the bottle next to the sink labeled “Toxic” and “formamide”, the reservoir itself goes to the red-biohazard trash can; step3. the remaining cartridge need to be rinsed, and dispose into the regular trash can

Figure 17 Removable Position #6

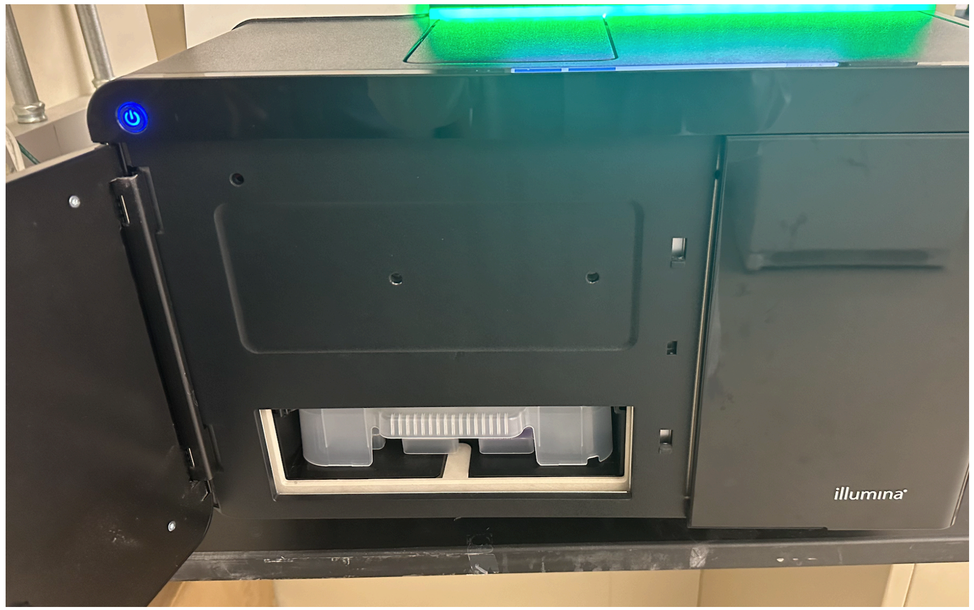


A Protective rubber cover
B Position #6



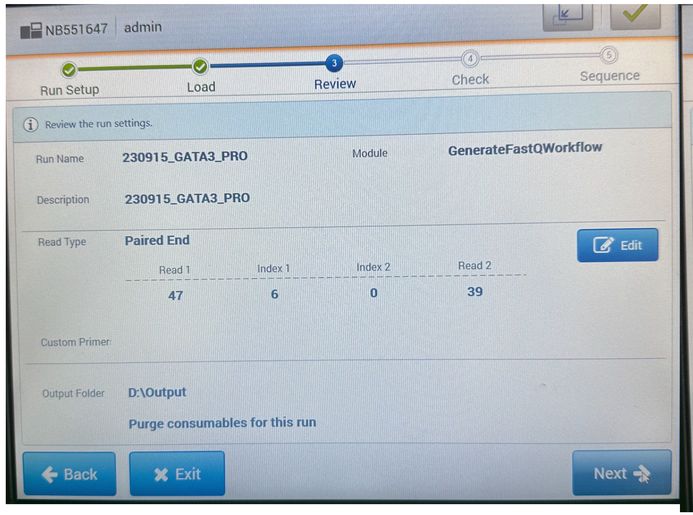
Reservoir that are ejectable and contains formamide

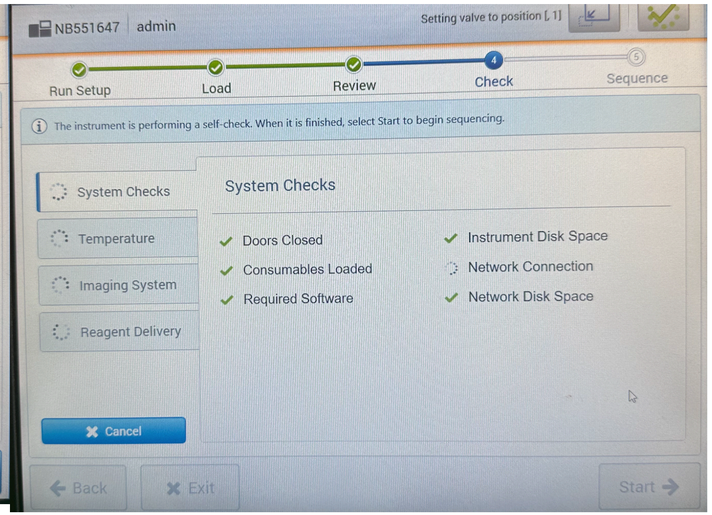
- load your library into the reagent cartridge
  - poke the foil with a clean tip, then load with pipette at the designated area
- slide the reagent cartridge into the stage until it stops
- close the door
- select “Load”, then “Next”



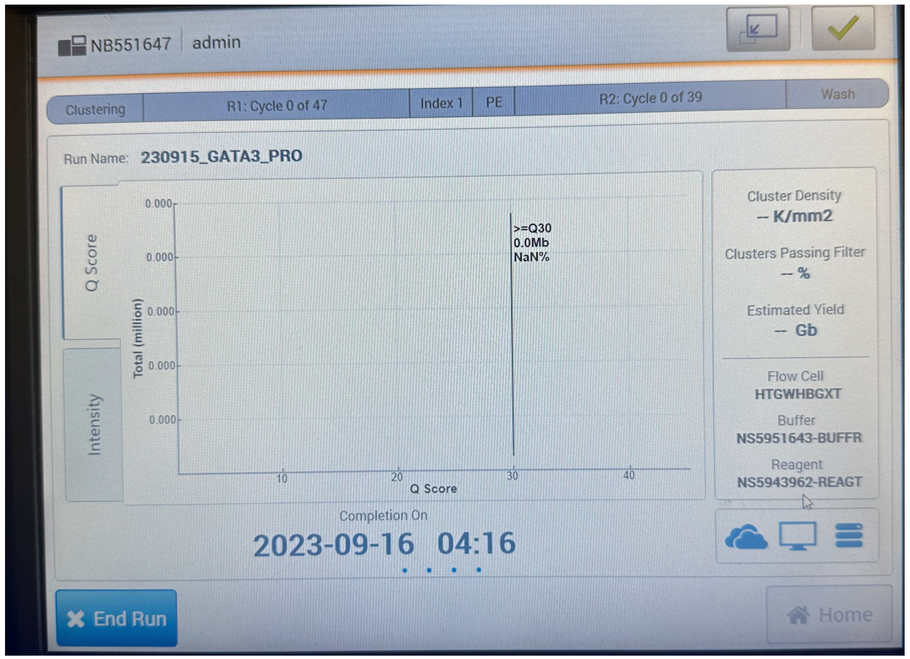
step3. monitor run progress

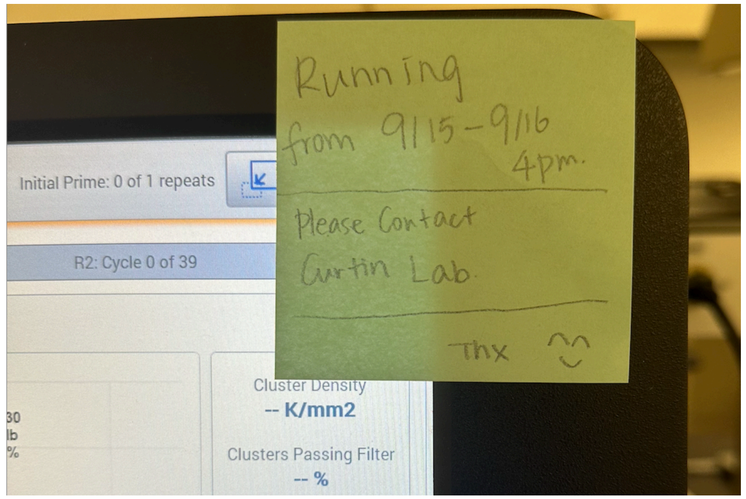
- review—check—start
- at this point, you will once again review the details of your run, if everything looks good, click “Next”
- machine will then take several minutes to automatically check the temperature, systems …, once done, click “Start”





- you will see the machine are starting to process your library…it will give you an expected time of run completion
    - Notice that this time is the time of the sequencing completion; the machine will still need to take 3~4 hours to generate the fasta files for you
- I also recommend placing a sticky note on the machine, write the expected running time and your contact, so that if anything happened, you get notified by others





final step: clean-up

- after you done with your run, make sure to clean up everything.
- make sure you dispose the solutions, the reservoir, the cartridges to the right place.
- finally, wish your run successful!