NEXTSEQ 550 Siyu Sun

References

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

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 \bullet 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), \bullet reagent wash cartridge, and the empty waste collecting cartridge to place
 - click start, it will take ~20min.

wash buffer load here



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



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- ulletsoftware; or
- you could manually type in the information ullet
- once you make sure every info is there, you click "save run" then minimize the software window ullet

you could select "Import Sample Sheet" to directly upload your pre-generated sample sheet.csv to the



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"





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- created run to the NextSeq RUO software.
- make sure everything is looking correct

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• after log-in to "local run manager", you are able to extract the previously

• you can see all the info about your experiment here, and noble check to



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"

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

2.4 load the buffer cartridge

- remove the used ones \bullet
 - *used ones should be rinsed with water and solution can be disposed into the sink; the container itself should be disposed into regular trash can
- 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

- Protective rubber cover
- 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"

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- you will see the machine are starting to process your library...it will give you an expected time of run completion
 - still need to take 3~4 hours to generate the fasta files for you
- time and your contact, so that if anything happened, you get notified by others

• Notice that this time is the time of the sequencing completion; the machine will

I also recommend placing a sticky note on the machine, write the expected running

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final step: clean-up

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

make sure you dispose the solutions, the reservoir, the cartridges to the