Sequencing libraries on the NextSeq 1000

As of 2022-03-09

Contents

Buffers / reagents

Qubit 1x dsDNA HS kit

Fisher Q33230

Qubit assay tubes

Fisher Q32856

PhiX control library

Illumina FC-110-3001

NextSeq 1000 reagent kit

See options here

Steps

Day One

Thaw the cartridge

1. Remove box containing the cartridge from -20°C.
2. Remove the cartridge from the box. **Do not remove cartridge from silver foil bag**
3. Position cartridge at room temp so that label faces up and air can circulate on sides and top.
4. Thaw at room temperature for 6 hours.
5. Transfer to 4°C and thaw for at least 12 hours. **Do not exceed 72 hours**

Set up run on Base Space

6. Navigate to this page
7. Click on **RUNS** within the blue section at the top of the page.
8. Click “New run” and select “INSTRUMENT RUN SETUP”.
9. Fill out the information related to your run.

   For paired reads, the number of cycles per read should be $\leq$ (total cycles / 2) + 1.
   For example, a P2-300 flow cell can accomodate 150 + 1 cycles per read.
   The NEBNext libraries for Illumina resemble TruSeq libraries and can be trimmed like TruSeq:
   Adaptor Read1 AGATCGGAAGAGCACACGTCTGAACTCCAGTCA
   Adaptor Read2 AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT

Day Two

Qubit

Only use the tubes specific to the Qubit instrument

10. Dilute the high and low standards with 10 μl of standard : 190 μl of working buffer.
11. Dilute each of your libraries with 1 μl of library : 199 μl of working buffer.
12. Vortex standards and libraries and then read on the Qubit.

Table 1: Library concentrations as measured by the Qubit

library	ng/μl

Convert concentrations to molarity

13. Use the following equation to go from ng/μl to nM.:

$$[ng/\mu l] * \ 1/660 \ g/mol * 1/average \ library \ size \ (bp) \ * 1E6 \ = [nM]$$

Table 2: Library concentrations in nM

library	ng/μl	nM

Dilute libraries to 2 nM

14. Dilute your libraries to 2 nM using nuclease-free H_2O

Table 3: Diluting libraries to [Working]

library	Stock (nM)	Working (nM)

Determine the target concentration for the pool of libraries to be loaded onto the flow cell.

Table 4: Illumina's suggested loading concentrations for pools containing variously prepared libraries.

Library Type	Loading concentration (pM)
Ampliseq for Illumina Library PLUS	750
Illumina DNA Prep	750
Illumina DNA Prep with Enrichment	1000
Illumina Stranded Total RNA with Ribo-Zero Plus	750
Illumina Stranded mRNA Prep	750
Illumina DNA PCR-Free	1000
TruSeq DNA Nano 350a	1200
TruSeq DNA Nano 550	1500
TruSeq Stranded mRNA	1000
100% PhiX	650

[a] The NEBNext libraries for Illumina are analagous to TruSeq libraries.

15. For this sequencing experiment, we will aim for the following loading concentration **of the pool**: _____
    Pooling concentration is defined by the sum of the molarity of each library within the pool

Dilute libraries and then pool

The minimum volume that you will need for the pool is 24 μl
Always aim to pipette at least 2 μl

16. Use Illumina's online pooling calculator to combine libraries into one pool for loading onto the flow cell.
17. Create your multiplexed pool.

Table 5: Pooling libraries

library	Vol (μl)
RSB with Tween 20	

Prepare PhiX control

For a ~1% PhiX control…

18. In a Lo-bind tube, add 2 μl of 10 nM PhiX library to 38 μl of RSB with Tween 20.
19. Vortex briefly and then centrifuge at 280 g x 1'.
20. Keep on ice until ready to combine with pool

Create loading-ready pool

21. In a fresh Lo-bind tube labeled ready, combine 24 μl of pooled libraries from above with 1 μl of PhiX at 0.5 nM.
22. Keep on ice until ready to load the flow cell.

Loading the flow cell

23. Remove cartridge *and* flow cell from 4°C and allow to sit at room temperature for at least 15' and no more than 60'.
24. Right before loading, remove both cartridge and flow cell from their respective foil bags.
25. Invert the *cartridge* ten times.
26. Remove the flow cell from its foil package (use the gray tab with the label on the tab facing up) and push to insert into the front of the cartridge. *You will hear a click*
27. Once inserted, pull back and remove the gray tab.
28. Using a new P1000 tip, pierce the Library reservoir and push the foil to the edges to enlarge the hole.
29. Discard the pipette tip to prevent contamination.
30. Add 20 μl of pool to the *bottom* of the reservoir by slowly lowering the pipette tip to the bottom of the reservoir before dispensing. *Avoid touching the foil*

Cartridge into the machine

31. Follow instructions given by the machine.