***Launching Illumina NextSeq 2000***

*Walker Orr, 11/01/2023*

## Thawing the Reagent Cartridge

|  |
| --- |
| Option 1: Thawing in a water bath.   1. Take the reagent cartridge out of the box but ***leave it in the foil bag***. 2. Prepare a 25C water bath to a depth of 9.5-10 cm. 3. Place the reagent cartridge in the water bath (it will float) for 6-8 hours (do ***not*** exceed 8 hours). 4. Remove the cartridge from the water bath and pat dry with paper towels. |
| Option 2: Thawing in the refrigerator.   1. One day prior to your sequencing run, take the reagent cartridge out of the box but ***leave it in the foil bag.*** 2. Place the cartridge on the bench with the label face up so air can circulate on all sides. 3. Leave the reagent cartridge on the bench for 6 hours. 4. Place the cartridge in a 2-8C refrigerator with the label face up so air can circulate on all sides. 5. Leave the reagent cartridge in the refrigerator for 12-72 hours (do ***not*** exceed 72 hours). |
| Option 3: Thawing on the bench   1. One day prior to your sequencing run, take the reagent cartridge out of the box but ***leave it in the foil bag.*** 2. Place the cartridge on the bench with the label face up so air can circulate on all sides. 3. Leave the cartridge on the bench for 9-16 hours (do ***not*** exceed 16 hours). |

## Preparing a Samplesheet

|  |
| --- |
| 1. Go to Basespace (basespace.illumina.com). |
| 1. Go to runs. On the runs page, click new run > run planning. |
| 1. On the first screen (“run settings”, select NextSeq 1000/2000 as the instrument. For secondary analysis, choose **local**. |
| 1. Under “configuration,” choose “Illumina DRAGEN BCL Convert – 3.8.4.” For “library prep kit” and “index adapter kit,” choose “not specified” (at the very bottom of the dropdowns). |
| 1. You will now be asked for more information (“Configuration: Illumina DRAGEN BCL Convert - 3.8.4”). |
| 1. Enter the index reads length (10 for Twist UDI plate) and the read length (150 for 300 cycles, 250 for 500 cycles, etc). |
| 1. Enter your sample name and the adapter sequences. You can also bulk import these using a template spreadsheet. |
| 1. Under the “Analysis Setting” heading, enter the sequences for the Twist Universal Adapters. These are:  |  | | --- | | AGATCGGAAGAGCACACGTCTGAACTCCAGTCA | | AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT |   For read1 and read2, respectively. Allow one barcode mismatch for each read. Under “FASTQ Compression Format” choose **gzip**. |
| 1. You will be prompted to a preview screen. Choose “export” to generate your sample sheet. **DO NOT CHANGE YOUR SAMPLESHEET NAME**, as this can create problems for the sequencer. |

## Final Library Preparation and Run Initiation

|  |
| --- |
| **Quantitation and Preliminary Pooling**   1. Quantitate each library using Qubit and obtain the size using TapeStation D1000. 2. Convert to nanomolar concentration using the illumina website, or this formula: 3. Thaw Illumina’s RSB/Tween for use in the pool. 4. Dilute libraries to 10 nM in nuclease-free water. 5. Dilute libraries to 2 nM each in RSB/Tween in a DNA LoBind tube. This will be 2 ul of 10 nM library in 8 ul of RSB/Tween. You will need a total of 25 ul library. 6. Double-check concentration with Qubit HS if desired. |
| **Final Dilution and PhiX Spike-In**   1. Allow flow cell and reagent cartridge to come to room temperature (15 minutes to 1 hour). You will also need the 2 nM pool, 1 nM PhiX (-20C) and more RSB/Tween. 2. Prepare a final dilution (650 pm) of pooled libraries by mixing 7.8 ul 2 nM library pool with 16.2 ul of RSB/Tween. 3. Pool 1 ul 1 nM PhiX with 24 ul 2 nM library for a total of 25 ul (~2% PhiX spike-in). Vortex final pool briefly, spin down, and set aside on ice.    1. If using stock 10 nM PhiX: Prepare 20 ul 1 nM PhiX by combining 1 ul 10 nM PhiX with 9 ul RSB/Tween (always prepare fresh). |
| **Loading Cartridge and Starting the Run**   1. Open the cartridge bag and remove carefully. 2. Invert cartridge 10 times to mix reagents (side-to-side). 3. Open flow cell package carefully. Mount the flow cell in the cartridge. Hold the flow cell by the grey tab to expose the flow cell. 4. Using a new P1000 pipette tip, pierce the library reservoir and push the goil to the edges to enlarge the hole. Discard the tip. 5. Add 20 ul diluted of final pool to the bottom of the reservoir by slowly lowering the pipette tip to the bottom of the reservoir before dispensing.    1. Avoid touching the foil.    2. You will not be able to see the bottom of the well, so you have to feel it. 6. Bring the loaded cartridge to the sequencer. 7. Press “Start” and follow the prompts.    1. Make sure to choose “external folder” (i.e., the hard drive) for the output folder location. 8. You will load the machine with the flow cell pointing internal to the machine. The 600 cycles kits cartridge will stick out a bit from the sequencer, as this cartridge holds more reagents. 9. Wait for the sequencer to do its warm-up routine. This takes around 20 minutes for all the checks. |