



DRAGEN Single Cell RNA v3.7.4 for NextSeq 1000/2000

Release Notes

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INTRODUCTION

These Release Notes detail the initial release of the DRAGEN Single Cell RNA Pipeline on NextSeq 1000/2000.

INSTALLATION INSTRUCTIONS

In order to install DRAGEN Single Cell RNA v3.7.4, the Control Software must first be updated. Please follow all the steps below for a successful software installation. Note that if you are installing multiple DRAGEN workflows, the only steps that need to be repeated for each DRAGEN workflow are those detailed under "Install DRAGEN workflows".

PREPARATION

* **These instructions are intended for the v1.2 Control Software upgrade only.**

1. The total installation time (not including file download times) may take up to 2 hours.
2. **For offline customers**, make sure you receive an updated DRAGEN license file:
 - o Contact customerservice@illumina.com and request offline DRAGEN license for reinstallation. Save this **license.zip** file to the desktop after logging into ilmnadmin.
3. Make sure that you have the password to the ilmnadmin account.
4. To log in to the ilmnadmin account:
 - o If you are already logged in as ilmnuser and in control software, select the control software menu, and then select **Exit Application** to access the desktop.
 - o Select the power button icon in the upper right corner and log out of ilmnuser.
 - o After you are on the login screen, select ilmnadmin, and then enter the password to log in.
 - o The control software automatically launches once you are logged in.
5. The upgrade installation files require up to 16 GB of free space on your desktop. See file sizes below.
 - o To access your desktop, select the control software menu in the control software, and then select **Minimize Application**.
 - o Delete or transfer files off the desktop that are not needed.
 - o Empty trash to free space from the drive.
 - Select **Applications**, and then **Files** from the upper left corner of the desktop to launch the file explorer.
 - On the left panel of the file explorer, right click the Trash icon and select **Empty Trash**.
6. Launch Chromium to download the software upgrade installers from the Illumina Support Site.
 - o While in desktop view, open the Applications menu on the upper left corner. Select **Internet**, and then select **Chromium**.
 - **Note: After launching Chromium, you may receive a prompt to unlock your login keyring. You can safely ignore and cancel out of the prompt.**
 - o Use Chromium to browse to the NextSeq 1000 and 2000 Software Downloads page on the Illumina Support Site.
7. Download the following software upgrade installers from the Illumina Support Site and save them to the desktop. **Do not extract these files; leave them as .tar.gz.**



- For online customers:
 - DRAGEN Workflows (13 GB)
 - DRAGEN compression reference file (1.5 GB)
 - For offline customers:
 - v1.2 SW Suite Installer (1 GB)
 - DRAGEN Workflows (13 GB)
 - DRAGEN compression reference file (1.5 GB)
 - Save the **license.zip** file to desktop. **Do not extract this file; leave it as .zip.**
8. After the files have finished downloading to the desktop, maximize the control software by selecting it from the toolbar.
9. To begin installation:
- Follow the "Online Installation" steps below if your system is connected to the internet.
 - Skip to the "Offline Installation" steps if your system is not connected to the internet.

ONLINE INSTALLATION

* Follow the "Preparation" steps outlined above before continuing.

1. Install SW suite

- a. Select the software update alert in the control software notification panel and follow the prompts to upgrade the software suite.
- b. When the installation process completes, you will see a message that confirms installation. After closing the prompt, the control software automatically relaunches.
- c. Follow the prompt for the firmware update.
 - **Note: During this process, the screen will go blank. Wait for the image to return and the process to complete.**
- d. Follow the prompt to power cycle the instrument.
 - **Note: Make sure to toggle the power switch at the bottom of the instrument to complete the power cycle after the instrument shuts down.**
- e. Power on the instrument and log in as ilmnadmin. The control software automatically launches.
 - **Note: An alert appears indicating that DRAGEN workflows need to be updated. Proceed through the next steps to resolve the alert.**
- f. Confirm that the version is updated to 1.2 in the **About** screen in the control software menu.
- g. Proceed to the next step to install DRAGEN workflows.

2. Install DRAGEN workflows

- a. Select the control software menu, then select **DRAGEN**.
- b. In the Version section, select **Browse for New Version**.
- c. In the browse window, navigate to the desktop and double-click the DRAGEN Single Cell RNA workflow *.tar.gz package.
- d. In the control software DRAGEN window, select **Install**.
- e. At the login prompt, enter the ilmnadmin password.
- f. Confirm that the DRAGEN workflow that is installed is updated to 3.7.4.
- g. Proceed to the next step to install the DRAGEN compression reference file.



3. **Install DRAGEN compression reference file**
 - a. Select the control software menu, and then select **DRAGEN**.
 - b. In the Genome section, select **Choose** under Import New Reference Genomes, navigate to the **dragen_compression_ref.tar.gz** file, and then select **Open**.
 - c. Select **Import**.
 - d. Proceed to the next step to update the DRAGEN license.
4. **Update DRAGEN License**
 - a. Open control software menu.
 - b. Under License Renewal select on **Renew Online**.
 - c. Verify the license is valid and not expired in the control software **About** tab.
5. **Final steps**
 - a. After installation completes, delete all the downloaded installation files from the desktop and empty the trash to save space.
 - b. Log back into ilmnuser to begin sequencing.

OFFLINE INSTALLATION

* Follow the "Preparation" steps outlined above before continuing.

1. **Install SW suite**
 - a. Launch control software
 - o Open control software menu.
 - o Select **Software Update**.
 - o Select **Choose...** under **Browse for Software Update**.
 - o Navigate to the desktop and select the installer
 - o Select **Update Now**.
 - b. Follow the password and other prompts.
 - c. When the installation process completes, you will see a message that confirms installation. After closing the prompt, the control software automatically relaunches.
 - d. Follow the prompt for the firmware update.
 - o **Note: During this process, the screen will go blank. Wait for the image to return and the process to complete.**
 - e. Follow the prompt to power cycle the instrument.
 - o **Note: Make sure to toggle the power switch at the bottom of the instrument to complete the power cycle after the instrument shuts down.**
 - f. Confirm that version is updated in the **About** screen in the control software menu.
2. **Install DRAGEN workflows and DRAGEN compression reference file**
 - a. Follow the "Install DRAGEN workflows" and "Install DRAGEN compression reference file" steps above. Then proceed to the DRAGEN license update procedure below.
3. **Update DRAGEN License**
 - a. In control software
 - o Open control software menu.
 - o Select **DRAGEN**.



- Under License Renewal select **Choose Offline**.
 - Navigate to the desktop and select the **license.zip** file.
 - Select **Update**.
- b. Verify the license is valid and not expired in the control software **About** tab.

4. Final steps

- a. After installation completes, delete all downloaded files from the desktop to save space unless multiple DRAGEN workflows will be installed, in which case the files should be deleted after the final DRAGEN workflow is installed.
- b. Log back into ilmnuser to begin sequencing.

FEATURES

- The DRAGEN Single Cell RNA pipeline is a high-performance secondary analysis pipeline for single-cell gene expression data
- The pipeline supports input from multiple common library prep systems, and the output is compatible with many downstream analysis tools.
- The pipeline supports the following operations:
 - Aggregate and Per-sample Fast QC metric plots
 - Optional ability for the user to save FASTQ files at the end of the run
 - Optional ability for the user to enable DRAGEN FASTQ compression, which offers up to a 5x reduction in FASTQ file sizes
 - Demultiplexing of separate sequencer base calls into individual samples
 - Use of cell barcodes to group reads to recover single-cell data
 - Sequence alignment of reads: Reads are mapped and aligned to a reference genome
 - Quantification of gene expression: UMIs for each cell and gene are counted and duplicates are removed after error correction
 - Dataset filtering and QC: Noncellular barcodes and low-quality cells are filtered, and QC metrics are calculated
 - Output of cell x gene expression matrix
- The output is compatible with open source single-cell analysis tools such as Scanpy / AnnDta, Seurat, etc.
- Downstream analysis such as UMAP projections, SAM / Leiden clustering, Marger gene discovery, can be run on the output.
- The output is highly consistent with established tools, yet 3x faster
 - Correlation of per-cell gene expression results with STARsolo: $r > 0.985$
 - 1.4 billion reads, 8018 cells: 38 min, compared to 125 min for STARsolo
- DRAGEN will generate a graphical interactive HTML report with QC metrics, cell clustering and marker genes.

RESOLVED ISSUES

N/A



KNOWN ISSUES

None