

NextSeq 1000/2000 Software Release Notes

NextSeq 1000/2000 Control Software 1.7.0
RTA 3.10.30 & 4.12.2
IAG 1.14.5
DRAGEN 4.2.7
Installation Instructions

April 2024

INTRODUCTION

These Release Notes detail new features, improvements, and known issues for the NextSeq 1000/2000 Sequencing System Control Software for compatibility with XLEAP-SBS kits and DRAGEN 4.2. The software can be downloaded from the Illumina software download page.

For more information on previous releases, visit the Illumina Support Site:

- NextSeq 1000/2000 v1.5.0 Software Release Notes (document #200030868)
- NextSeq 1000/2000 v1.4.1 Software Release Notes (document #200012109)
- NextSeq 1000/2000 v1.4.0 Software Release Notes (document #200005228)
- NextSeq 1000/2000 v1.3.1 Software Release Notes (document #200004033)
- NextSeq 1000/2000 v1.3.0 Software Release Notes (document #1000000160800)

For more information on the NextSeq 1000/2000, see the NextSeq 1000/2000 Product Documentation (document #200027171) available on the NextSeq 1000/2000 Sequencing System support pages.

The software package includes:

- NextSeq 1000/2000 Control Software 1.7.0

NEXTSEQ 1000/2000 CONTROL SOFTWARE 1.7.0

NEW FEATURES

- Support for P4 compatibility for all kits
- Support for XLEAP-SBS reagent kits for:
 - P1 100 cycles, 300 cycles, 600 cycles
 - P2 100 cycles, 200 cycles, 300 cycles, 600 cycles
 - P3 100 cycles, 200 cycles, 300 cycles (note, P3 50 cycles not available with XLEAP-SBS Chemistry in lieu of P4 50 cycles)
 - **Note:** Software will continue to support all Standard SBS chemistry kits
- Support for DRAGEN 4.2 workflows
 - Requires updated v9 reference genomes which are necessary for DRAGEN 4.2 compatibility with Control Software 1.7
 - **Note:** Control Software v1.7 no longer supports onboard DRAGEN workflows below version 4.2, and requires download of the compatible reference genomes. Customers should also use DRAGEN workflows version 4.2 or higher if running DRAGEN in the Cloud on BaseSpace or ICA.
- Added Secondary Analysis field at review screen
- Updated user interface "Average %Q30" to "≥ Q30" at sequencing screen. This update does not change the content available
- Enabled error message for when user tries to use mismatched Standard SBS and XLEAP-SBS consumables
- Updated software libraries for both NextSeq1000/2000 Suite Installer and Orchestrator

Note: SAV version 2.5.12 is recommended for runs completed with NextSeq 1000/2000 using v1.7.0 Control Software, SAV version 3.0 is also compatible. The installer for SAV version 3.0 can be found on the NextSeq 1000/2000 support page.

BUG FIXES

- N/A

RTA 4.12.2

NEW FEATURES

- New q-table trained on XLEAP-SBS consumables with Q40 bin. The third Q-score bin has been altered to contain all bases greater than or equal to Q30.
- More accurate representation in early cycle Q-scores

BUG FIXES

- N/A

RTA 3.10.30

NEW FEATURES

- N/A
- **Note:** RTA 3.10.30 will continue to enable support for Standard SBS consumables

BUG FIXES

- N/A

IAG 1.14.5

NEW FEATURES

- Support for two versions of RTA
 - RTA 4.12.2 enables support for XLEAP-SBS chemistry consumables
 - RTA 3.10.30 supports standard SBS chemistry consumables
- RTAExited.txt written whenever RTA exits for any reason (previously only written when RTA finished successfully)
- RTA.cfg file is hidden
- Reduced extraneous warning messages during normal operation to save disk space
- Maintenance of cybersecurity compliance

BUG FIXES

- N/A

DRAGEN 4.2.7

Please follow all steps below for a successful software installation.

Note: If you are installing new DRAGEN 4.2.7 workflows, reference the following documents for a summary of new features:

- DRAGEN Amplicon v4.2.7 (document #200052657)
- DRAGEN BCL Convert v4.2.7 (document #200052659)
- DRAGEN Germline v4.2.7 (document #200052828)
- DRAGEN Enrichment v4.2.7 (document #200052831)
- DRAGEN RNA v4.2.7 (document #200052832)
- DRAGEN Single Cell RNA v4.2.7 (document #200052833)

NEXTSEQ 1000/2000 CONTROL SOFTWARE INSTALLATION INSTRUCTIONS

Please follow all steps below for a successful software installation

NOTE: If v1.5 or higher version software is currently installed, the user can upgrade to v1.7 Control Software directly.

If v1.1 software is currently installed, the user must first upgrade to v1.2 software following the instructions found in the v1.2 software release notes and then upgrade to v1.5 software following the instructions found in the v1.5 software release notes.

Key Recommendations for Software Installation Success:

- Log in as an admin user to enable the software update. Attempting to install on any other account will result in installation failure.
- Within the Process Management tab, ensure there is nothing in progress. If processes are running, the software installation may fail.

PREPARATION

These instructions are intended for the v1.7.0 Control Software upgrade only.

Note: The total installation time (not including file download times) is approximately 25-30 minutes. **After these software updates are installed, they cannot be reverted.** If you are running onboard DRAGEN workflows below version 4.2, please note you will not be able to do so after updating to Control Software v1.7. Control Software v1.7 requires a minimum of DRAGEN version 4.2 workflows for onboard analysis (including DRAGEN BCL Convert).

1. Back up any sequencing runs that are currently on the instrument.
2. Confirm that in **Process Management**, Run, Analysis, Network and Cloud all show Completed for all runs.
3. Make sure that you have the password to the ilmadmin account. The login credentials will be required repeatedly throughout the installation process.
4. To log in to the ilmadmin account:
 - a. If you are already logged in as ilmuser and in Control Software, select the Control Software menu, then select **Exit Application** to access the desktop.
 - b. Select the power button icon in the upper right corner and log out of ilmuser.
 - c. After you are on the login screen, select ilmadmin, and then enter the password to log in.
 - d. The control software automatically launches once you are logged in.

ONLINE INSTALLATION

Follow the "Preparation" steps outlined above before continuing.

1. Install Software Suite

- a. Select the software update alert in the Control Software notification panel and follow the prompts to upgrade the software suite.

- b. The Control Software will prompt you to restart application.
- c. Upon restart, a popup menu prompt will appear for the upgrading of kernel and driver versions. Follow the prompts to proceed with the kernel and driver update.
 - o **Note:** During this process, the screen will go blank and enter boot. Do not intervene, wait for the image to return to user login menu.
- d. Log in as ilmnadmin whenever prompted and follow the prompts to continue the upgrade process for the software suite.
- e. When the installation process completes, you will see a message that confirms installation completion. Select **OK** to restart NextSeq 1000/2000 Control Software.
- f. 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. If the image does not return after 5 minutes, proceed to the next step to power cycle the instrument by manually toggling the power switch.
- g. Follow the prompt to power cycle the instrument.
 - o **Note:** Once the instrument light turns off, toggle the power switch at the back of the instrument one time to complete the power cycle. Wait for at least 30 seconds, then turn the instrument back on via the back toggle switch. Then press the pulsing power button on the front of the instrument. System initialization takes about 5 minutes to complete.
- h. Power on the instrument and log in as ilmnadmin. The Control Software automatically launches.
 - o **Note:** An alert appears indicating that DRAGEN workflows need to be updated. Proceed to the DRAGEN workflow installation instructions below to resolve the alert.
- i. Confirm that the version is updated to v1.7.0 or above in the **About** screen in the Control Software menu.

OFFLINE INSTALLATION

Follow the "Preparation" steps outlined above before continuing.

1. The upgrade installation files require up to 3 GB of free space on your desktop. See file sizes below.
 - a. To access your desktop, select the Control Software menu in the Control Software, and then select **Minimize Application**.
 - b. Delete or transfer files off the desktop that are not needed.
 - c. Empty trash to free space from the drive.
 - i. Select **Applications**, **Favorites**, and then **Files** from the upper left corner of the desktop to launch the file explorer.
 - ii. On the left panel of the file explorer, right click the Trash icon and select **Empty Trash**.
2. Download the software suite installer from the support site.
 - a. **Do not extract these files; leave them as .tar.gz.**
 - i. v1.7.0 SW Suite Installer (1 GB)
 - b. For instruments not connected to the internet, using a separate device, download the software suite installer from the NextSeq 1000 and 2000 Software Downloads page on the Illumina Support Site and transfer the files to your instrument using a portable drive.
 - c. For instruments connected to the internet, launch Chromium to download the software suite installer from the Illumina Support Site.
 - i. While in desktop view, open the Applications menu on the upper left corner. Select **Internet**, and then select **Chromium**.
 - ii. **Note:** After launching Chromium, you may receive a prompt to unlock your login keyring. You can safely ignore and cancel out of the prompt. Use Chromium to browse to the NextSeq 1000 and 2000 Software Downloads page on the Illumina Support Site, and download the software suite installer.
3. After the files have finished downloading or have been transferred to the instrument using a portable drive, ensure the files are located on the desktop, then maximize the Control Software by selecting it from the toolbar.
4. Install software suite
 - a. Launch Control Software
 - i. Open Control Software menu
 - ii. Select **Software Update**
 - iii. Select **Install From File**
 - iv. Navigate to the desktop and select the System Suite Installer
 - v. Select **Update**
 - b. Enter the ilmnadmin password whenever you are prompted throughout the software install process.
 - c. The Control Software will prompt you to restart the application.
 - d. Upon restart, a popup menu prompt will appear for the upgrading of kernel and driver versions. Follow the prompts to proceed with the kernel and driver update.
 - o **Note:** During this process, the screen will go blank and enter boot. Wait for the image to return to user login menu.
 - e. When the installation process completes, you will see a message that confirms installation completion. Select **OK** to restart NextSeq 1000/2000 Control Software. 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. If the image does not return after 5 minutes, proceed to the next step to manually toggle the power switch.
 - f. Follow the prompt to power cycle the instrument.

- **Note:** Once the instrument light turns off, toggle the power switch at the back of the instrument one time to complete the power cycle. Ensure there is no portable drive connected to the instrument before powering back on. Wait for at least 30 seconds, then turn the the instrument back on via the back toggle switch. Then press the pulsing power button on the front of the instrument. System initialization takes about 5 minutes to complete.
 - g. Confirm that the version is updated to v1.7.0 or above in the **About** screen in the control software menu.
 - **Note:** An alert appears indicating that DRAGEN workflows need to be updated. Proceed to the DRAGEN workflow installation instructions below to resolve the alert.
5. Final steps
- a. After installation completes, delete all the downloaded installation files from the desktop and empty the trash to save space.

DRAGEN WORKFLOW INSTALLATION INSTRUCTIONS


Please ensure Control Software v1.7.0 is installed before proceeding with DRAGEN Workflow 4.2 installation.

Control Software v1.7.0 does not support DRAGEN workflows below version 4.2.

The upgrade to Control Software v1.7.0 does not wipe older versions of DRAGEN workflows. After performing the Control Software upgrade, you must uninstall all DRAGEN workflow versions 3.10 and below, uninstalling BCL Convert last, before installing DRAGEN BCL Convert 4.2.

UNINSTALL DRAGEN WORKFLOWS

Uninstall DRAGEN Workflows

- Navigate to the DRAGEN screen
- See the **Version** section
- Select the delete icon  to uninstall the workflow.
 - **NOTE:** If you are uninstalling several workflows, BCL Convert must be deleted last.

ONLINE INSTALLATION

These instructions are intended for online installation of DRAGEN pipelines on the NextSeq 1000/2000.

1. Make sure that you have the password to the ilmnadmin account.
2. Log in to the ilmnadmin account:
 - a. 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.

- b. Select the power button icon in the upper right corner and log out of ilmnuser.
 - c. After you are on the login screen, select ilmnadmin, and then enter the password to log in.
 - d. The Control Software automatically launches once you are logged in.
3. The DRAGEN installation requires up to 13 GB of free space on your desktop.
4. Select the **DRAGEN** screen in the Control Software.
5. Click on **check online** button in the DRAGEN workflows section.
6. A popup will show which workflows have an update available for installation.
7. Select which pipelines you would like to update.
 - o **NOTE:** For online updates, DRAGEN BCL Convert must be installed before other workflows of the same DRAGEN version
8. Select download and install.
9. You will be prompted to enter the ilmnadmin password once the download completes and proceeds to install.
10. After installation is complete, you will be navigated back to the DRAGEN screen and can view the updated list of installed DRAGEN workflows.

OFFLINE INSTALLATION

These instructions are intended for offline installation of DRAGEN pipelines on the NextSeq 1000/2000.

1. Make sure that you have the password to the ilmnadmin account.
2. Log in to the ilmnadmin account:
 - a. 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.
 - b. Select the power button icon in the upper right corner and log out of ilmnuser.
 - c. After you are on the login screen, select ilmnadmin, and then enter the password to log in.
 - d. The control software automatically launches once you are logged in.
3. The DRAGEN installation files require up to 4.3 GB of free space on your desktop. See file size below:
 - o DRAGEN BCL Convert workflow – 4.3 GB
 - o Other DRAGEN workflows – around 10 KB
4. The DRAGEN installation requires up to 13 GB of additional free space on your desktop.
 - a. To access your desktop, select the Control Software menu in the Control Software, and then select **Minimize Application**.
 - b. Delete or transfer files off the desktop that are not needed.
 - c. Empty trash to free space from the drive.
 - i. Select **Applications**, and then **Files** from the upper left corner of the desktop to launch the file explorer.
 - ii. On the left panel of the file explorer, right click the Trash icon and select **Empty Trash**.
5. Download the DRAGEN workflows from the Illumina Support Site. **Do not extract these files; leave them as .tar.gz**
 - a. For instruments not connected to the internet, using a separate device, download the DRAGEN workflows from the NextSeq 1000 and 2000 Software Downloads page on the Illumina Support Site and transfer the files to your instrument using a portable drive.
 - b. For instruments connected to the internet, while in desktop view, open the Applications menu on the upper left corner. Select **Internet**, and then select

- Chromium.** Browse to the NextSeq 1000 and 2000 Software Downloads page on the Illumina Support Site, and download the DRAGEN workflows.
- **Note:** After launching Chromium, you may receive a prompt to unlock your login keyring. You can safely ignore and cancel out of the prompt.
6. After the files have finished downloading, or have been transferred to the instrument using a portable drive, ensure the files are located on the desktop, then maximize the Control Software by selecting it from the toolbar.
 7. 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 workflow *.tar.gz package.
 - **Note:** DRAGEN BCL Convert must be installed before other workflows
 - d. In the Control Software DRAGEN window, select **Install**.
 - e. At the login prompt, enter the ilmnadmin password.
 - f. View the updated list of installed DRAGEN workflows.

REFERENCE GENOMES

DRAGEN 4.2 requires v9 reference genomes. Reference genomes v8 and below can be uninstalled.

1. Uninstall Reference Genomes.
 - a. From the control software menu, select **Disk Management**.
 - b. For the genome you want to delete, select **Delete Genome**.
 - c. In the dialogue box, select **Yes, Delete Genome**.
 - d. Repeat steps **b** and **c** for each genome you want to delete.
2. Import new Reference Genomes
 - a. Compatible Reference Genomes can be found at the NextSeq 1000/2000 support site. See file size below:
 - Reference Genomes:
 - A. thaliana - 204 MB
 - Bacillus cereus ATCC 10987 - 8.9 MB
 - Chm13 - 8.8 GB
 - DRAGEN Compression Reference Genome - 2.3 GB
 - E. coli K12 - 7.4 MB
 - Hg19 - 7.8 GB
 - Hg38 - 8.2 GB
 - Hs37d - 7.7 GB
 - Mm10 - 7.2 GB
 - PhiX - 28 KB
 - Rhodobacter sphaeroides - 7.1 MB
 - Rn6 - 7.3 GB
 - WBcel235 - 175 MB
 - Multigenome Graph References:
 - Chm13 - 9.9 GB
 - Hg19 - 9.4 GB
 - Hg38 - 9.7 GB
 - Hs37d5 - 9.2 GB
 - b. Download Reference Genomes that you wish to install to the Desktop.
 - c. Navigate to the DRAGEN section.
 - d. See the **Genomes** section.
 - e. Select **Choose** to import new Reference Genomes.
 - f. Navigate to the Reference Genome file (*.tar.gz) on the Desktop, and then select **Open**.

- **Note:** if using a portable drive, ensure you copy the Reference Genome files from a portable drive to the Desktop, and open from the Desktop.
- g. Once uploading the Reference Genome file is complete, click **Import** to import the genome.
 - **Note:** During run review, the Control Software will check if the correct Reference Genomes are installed on the instrument and produce an error if they are not installed.

RESOLVED ISSUES

N/A

KNOWN ISSUES

Error messaging

- When error occurs in Pre Run Check, a blank error is shown. Retry Pre Run Check upon blank error message.
- If accessing reports from the system check, operating system locks system check data. When attempting to delete locked system check data, the application displays an "unknown error occurred" message to the user.
- The following message may appear and prevent software suite installation from starting: "Update cannot continue because the following actions are currently in progress: data file copy." This indicates that a file transfer is currently in progress. If this issue persists after file copy is complete, please contact Illumina Technical Support.
- Upon successful completion of the software update, an error message stating: "An unexpected error has occurred with system integration, power cycle the instrument per system guide instructions and retry" may appear. Power cycle the instrument and the error will no longer appear.

Notifications

- History notifications have mismatched dates with the current notifications tab in the notification panel. This does not impact output or function.
- When Local Run Setup and Proactive Support Only modes are selected, there is no notification when output folder is not set.

Other

- Upon navigating to the DRAGEN management screen, if a copy is in progress, the user is unable to perform license update, genome install, or DRAGEN install.
- During the software suite installation, the screen may remain blank after the firmware upgrade process. If the image does not return after 5 minutes, proceed to toggle the rear power switch and continue.

RTA

- There is an intensity increase after cycle 25 which is an expected artifact of the RTA version 3.10.30.
- The RTA version in RunParameters.xml will populate RTA 4.12.2, even if RTA 3.10.30 is used. There is no impact to run configuration or performance.