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NovaSeq Software Release Notes

NovaSeq Control Software 1.8.1

NovaSeq Service Software 1.8.0

Real Time Analysis 3.4.4

Universal Copy Service 2.7.3

Firmware 1.26.10

Recipes 1.8.0

Install Notes

For NovaSeq 6000 RUO

October 20, 2023

Introduction

These Software Release Notes detail new features, improvements, and known issues for the NovaSeq™ 6000 Sequencing System for improved sequencing robustness. Contact your field representative to schedule an upgrade to NovaSeq Control Software (NVCS) v1.8.1.

For more information on the NovaSeq 6000, see the NovaSeq 6000 Sequencing System Guide (document # 1000000019358) available on the [NovaSeq 6000 Sequencing System support pages](#).

The software package includes:

- NovaSeq Control Software 1.8.1
- NovaSeq Service Software 1.8.0
- Real Time Analysis 3.4.4
- Universal Copy Service (UCS) 2.7.3
- Firmware 1.26.10
- Recipes 1.8.0

I.   NovaSeq Control Software 1.8.1

NEW FEATURES:

- Added option to check flow cell vacuum pressure prior to run start.
- Added option to perform flow cell vacuum check manually.
- Added logging for pressure sensor logs during post-run wash.
- Added additional Proactive notifications for when instrument is in ""Down"" state or instrument is shut down.
- Updated laser power curve to reflect read length rather than total cycle count.
- A new Standalone Recipe installer for high altitude and potential reduction of Side A to Side B variability is available for use. This may only be used with NVCS 1.8. Contact field service for installation.
- BaseSpace Credentials are no longer saved. The user must type in username and password during each Run Setup.

IMPROVEMENTS:

- LGM warm up check removed from Initialization, lowering occurrence of LGM failures during initialization & reducing initialization time.
- Updated messaging in the following workflows for clarity:
  - Reverse complement workflow
  - Forward strand workflow

- o Buffer drawer attempted opening

- Reduced default configuration value "FcFlatnessIndex" mitigating focus/image quality reduction in sequencing runs.

- Added logging of the FC Flatness Pre-Run Check results to Proactive.

- Removed free-text fields from Instrument Performance data logs.

FIXED ISSUES:

- Report the correct System Suite Installer and Universal Copy Service (UCS) software versions in the Instrument Performance Data logs.

  - Fixed a bug where UI was blank during post run wash.

  - Changed message associated with Reagent Chiller door open during sequencing from an error to a warning.

  - Suppressed maintenance wash warning during sequencing run.

  - "Options.cfg", "Simulation.cfg", and "Simulation.out" backup when Control Software launches.

  - Fixed an issue preventing complete deletion of runs from the Process Management window.

  - Fixed a bug where sample sheets of arbitrary names were not automatically converted to the name "SampleSheet.csv" for upload to BaseSpace. BaseSpace expects the sample sheet to be named "SampleSheet.csv". The original sample sheet is also copied to the output run folder.

  - Updated NovaSeq control software to pass "*Out.bin" to UCS removing the possibility of TileMetrics.bin from being added to the upload contract.

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KNOWN ISSUES:

- UCS Version may be missing from the Proactive Dashboard.

- In rare occurrences, the reagent chiller door sensor warning during Pre-Run Checks may prevent continuation for both flow cell sides (instead of only the failed side) during a dual flow cell run. User is required to exit the workflow and setup runs for both sides again.

- 10X Genomics users: Demultiplexing using mkfastq (cellranger, cellranger-arc, cellranger-atac, or spaceranger) can cause reads to be assigned to the Undermined FASTQ files.

  - o 10X Genomics has released a workaround available on their [Knowledge Base](#)

II.   NovaSeq Service Software 1.8.0

NEW FEATURES:

- Added option to send tests performed in Service Software to Proactive upon control software launch.
- Added Leak Sensors tab under fluidics.

IMPROVEMENTS:

- Updated specifications based on analysis of manufacturing data.

BUG FIXES:

- Fixed a bug where SP flow cells were not read at load flow cell screen.
- "Options.cfg", "Simulation.cfg", and "Simulation.out" backup when Service Software launches.
- Run counter no longer includes Mock runs in calculation.

III.   Real Time Analysis 3.4.4

- **Same version as prior released software – no changes made**

IV.   Universal Copy Service 2.7.3

NEW FEATURES:

- Enable UCS on Linux based systems.
- Added a check to ensure the output folder is not in the Run folder, causing an infinite copy loop.
- Improved the startup time of UCS when a large number of files were queued to upload.
- Added the ability for Arrays to upload to ICA.
- Enhanced upload efficiency for Arrays.
- Switched to Rclone for file upload. Note: Due to this change, the modified date of the copied file now corresponds to the date/time the file was transferred to the Output folder. In previous versions the modified date/time was not updated when the file was moved to the Output folder. Rclone also includes an MD5 hash data integrity check for file transfer.
- Enabled ICA integration for NovaSeq Control Software.
- Added a manifest file that lists all files being copied.

BUG FIXES:

- Fixed an issue where the BaseSpace auth token was refreshed too frequently.
- Fixed an issue when UCS would deadlock when deleting a run.

- Fixed an issue where poor internet connectivity may cause the same file to get uploaded to BaseSpace multiple times.

- Fixed an issue where the run may not get marked as complete even though all files were sent.

- Increased UCS start up time by reducing the number of retry on missing files.

- Fixed an error when the API Service would hang with consecutive Delete/Create calls.

- Fixed an issue where Rclone processes weren't getting cleaned up properly when shutdown.

- Fixed an issue where certain temporary files were not getting deleted.

- Fixed an issue where certain valid characters in a file path caused errors.

- Fixed an issue where UCS would not recover when the same Windows user would log into different Windows sessions.

- Fixed an issue where Rclone logs were not getting deleted.

- Fixed an issue where multiple instances of UCS were being created.

KNOWN ISSUES:

- If the software is upgraded to NovaSeq Software Suite Installer v1.8, you may experience that large file (estimated to be roughly 10GBs or larger) uploads that take longer than 10 minutes to upload will fail data integrity check and will be requeued for upload.

- If using mounted filesystems which compress data size, the data integrity check may fail due to the file size being different than expected. If issue is encountered, please call technical support for next steps.

V.   Firmware 1.26.10

BUG FIXES:

- Decreased frequency of run failures caused by imaging timeout.

VI.   Recipes 1.8.0

IMPROVEMENTS:

- Updated the minimum sequencing temperature from 20°C to 25°C in order to support sequencing robustness in high humidity. This temperature change only occurs during imaging and at the end of these steps to return to baseline temperature: Deblock step for the last buffer wash, i7 index read preparation and OBCG Part 2. This causes intensity for S2 and S4 runs to be approximately 10-15% lower than prior SW versions, however there is no impact to Quality Metrics (there is no impact to overall Q30 and Q30 of the last 10 cycles).

- Dispense flow rate at specific steps reduced (S4 Only) in order to mitigate risk of flow cell leaks on instrument.

- Added in step to Backflush BB3 to Waste in order to decrease risk of clogs in the waste lines.

- Added in step to send bleach through the Waste lines during post run wash in order to decrease risk of clogs in the waste lines.

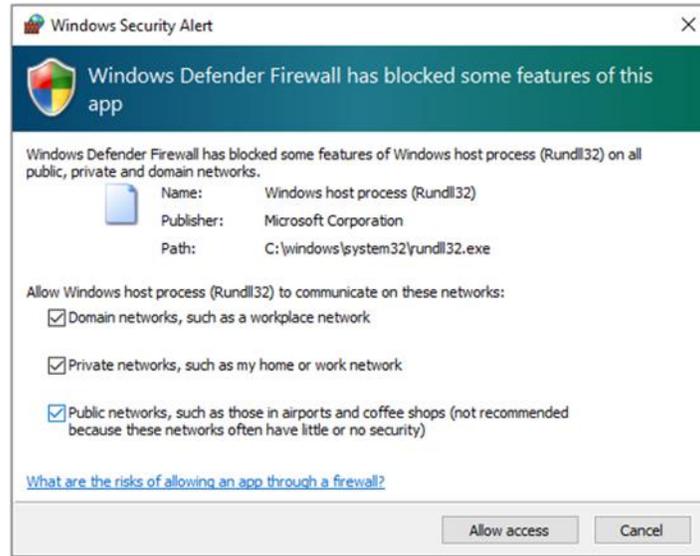- Renamed reagent names to reflect v1.5 consumables.

VII. Manual Install Notes

- Before upgrading, check the Recipe Fragment Version currently installed within the NovaSeq Control Software About screen (screenshot below).
    - The 1.8.1 System Suite Installer will install the 1.8.0 Recipe Fragment version.
    - Please contact Illumina tech support techsupport@illumina.com to schedule an FSE visit to restore Recipe Fragment Version 1.8.1 if previously installed.
    - No action needed if previously using Recipe Fragment Version 1.8.0.
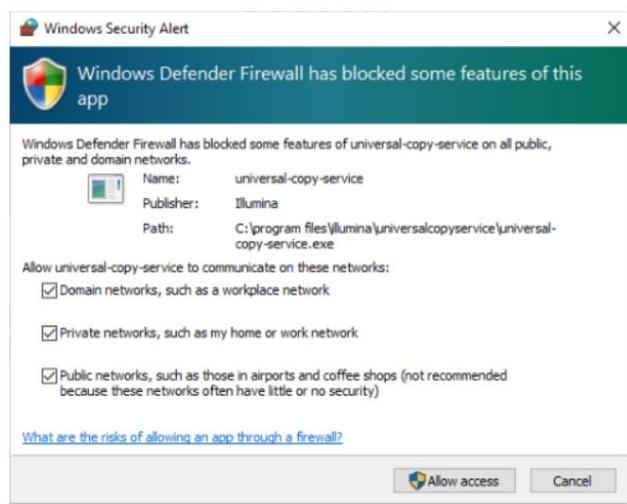

- Prior to performing a manual software upgrade, <u>power cycle</u> the instrument and ensure the following:
    - The system is powered on.
    - The system is not currently performing a sequencing run or system wash.
    - The system is not currently uploading sequencing run data.

- Log in using a Windows administrator account on the NovaSeq, then download the installer from the Illumina Support Site and unzip to C:\Program Files\Illumina.

- Run the NovaSeq Software Suite Installer.

    o Confirm time on system matches local time.

    o If prompted, ensure firewall settings allow network communication for NVCS (see below).

- Reboot system as prompted by NovaSeq Software Suite Installer.

- On first-time launch of NovaSeq Control Software 1.8, navigate to Settings, update any options as necessary, and click Save.

    o If prompted, ensure firewall settings allow network communication for UCS (see below).

KNOWN ISSUES:

- A manual power cycle is **required** to complete the installation if "Restart" button on the "Setup Successful" screen does not function.

- Login screen appears blank or continually loads on first-time launch of NovaSeq Control Software 1.8 when Settings have not been saved once after update.
  - o If seen, please follow these steps:
    - Navigate to Settings.
    - Switch to a different Hosting Region (e.g. switch to CAC1, then back to USE1).
    - Deselect BaseSpace Sequence Hub, hit save, then reselect BaseSpace Sequence Hub, hit save.
    - Attempt to set up run with BSSH Login.
  - o If the issue is not resolved, please call technical support for next steps.

- On rare occasions, the installer can fail to communicate with one of the devices on the system in the required time and the software update process will not complete.
  - o If seen, please power cycle and run the installer package again.
  - o If the issue persists, please call technical support for next steps.
  - o If encountering this issue via BSSH download, please call technical support for next steps.

- On rare occasions, UCS or Rclone processes may persist in the background and cause install to fail.
  - o If seen, please follow these steps:
    - Power cycle.
    - Navigate to C:\ProgramData\Illumina\Universal Copy Service and move the Runs and Logs folder to another location to ensure that the Universal Copy Service folder is empty.
    - Run the installer package again.
  - o If the issue persists, please call technical support for next steps.

- On some occasions, the NovaSeq Control Software 1.8.1 will crash when moving to the BaseSpace login screen after the first-time installation of 1.8.1.
  - o If seen, please follow these steps:
    - Power cycle.
    - Run the 1.8.1 system suite installer again and select Repair.
    - Power cycle again.
  - o If the issue persists, please call technical support for next steps.

Release History

Revision	Release Reference	Originator	Description of Change
00	1082437	J. Nguyen	Universal Copy Service update
01	1091812	J. Nguyen	Added instructions to re-run System Installer to overcome crash observed on some customer instruments