HiSeq Instrument Software Release Notes

HiSeq Control Software (HCS) v2.2.38

Real Time Analysis (RTA) v1.18.61

Recipe Fragments (RF) v1.5.14

.net 4.5.1

Sequencing Analysis Viewer (SAV) v.1.8.37

BaseSpace Broker v2.1.0.1

For HiSeq® 1000 HiSeq® 1500 HiSeq® 2000
and HiSeq® 2500 Systems

July 2014

Introduction

These Release Notes detail the key changes to instrument software components for the HiSeq 1000, 1500, 2000 and 2500 since the package containing HCS v2.0.12, RTA v1.17.21.3, Recipe Fragments v1.3.61, SAV 1.8.20 and BaseSpace Broker 2.0.13022.1628.

If you are upgrading from a version prior to HCS v2.0.12, please review the release notes for HCS v2.0.12 for a list of features and bug fixes introduced in that version.

FPGA upgrade to v3.10.3 or later is mandatory prior to the installation of this software package. If you need to update your FPGA version, please contact Illumina Technical Support. FPGA updates must be completed by a trained Illumina staff person. Note: if your HiSeq is already using HCS 2.0.5 or higher, your FPGA has already been updated to 3.10.3 or higher. You can confirm the FPGA version by going to the Menu, About screen in HCS.

HiSeq configuration file settings, with the exceptions of changes made to HiSeqControlSoftware.Options.cfg, will not be preserved through this software update.

Any custom recipe files will be over written by the Recipe Fragments installation: customers should recreate their custom recipes after receiving this update.

The BaseSpace Send Instrument Health data option setting will be reset by this software install to be “on”. Customers should update their BaseSpace Send Instrument Health settings after receiving this update.

Note that for HiSeq v4 runs, there may be a noticeable increase in the reported intensity by cycle displayed in SAV after template generation is completed after cycle 5. This is because after this point, the reported intensities include only clusters included in the final template.

The software package includes:
- HiSeq Control Software (HCS) v2.2.38
- Real Time Analysis (RTA) v1.18.61
- Recipe Fragments (RF) v1.5.14
- .net 4.5.1
- Sequencing Analysis Viewer (SAV) v.1.8.37
- BaseSpaceBroker2.1.0.1

I. HCS v2.2.38

FEATURES:

Control lanes are no longer required

- The control lane option has been removed from all run modes: internal testing by Illumina has shown that a control lane is unnecessary when using RTA 1.18.61, which includes new features to support low diversity sequencing. See the RTA 1.18.61 section (below) for more details. When sequencing challenging samples such as a single amplicon, a 10% PhiX spike-in is recommended in place of a control lane.

Enhanced integration with TruSeq SBS kits

- HiSeq run mode names that appear in the HCS interface have been updated to match the corresponding TruSeq SBS kit product. The new run modes are TruSeq v3 (formerly High Output), TruSeq Rapid (formerly Rapid Run) and HiSeq v4 (if applicable).
- For HiSeqs enabled with the optional HiSeq v4 upgrade, a new HiSeq v4 run mode is also automatically available. This mode will not appear on systems that are not enabled to use it. This new run mode enables output of up to 1 Tb of data in 6 days, when running 2 flow cells.
- For HiSeq v4 runs, users are prompted to load PE reagents prior to starting the run.
- Read lengths, drop-down menus for indexing schemes, run setup options such as save cifs, etc., will all display default values and available options based on the selected run mode. Some options are not available with certain modes, due to the high data output:
    - When running in HiSeq v4 mode, the option to save cif files is not available.
    - When using v4, zipped bcl files are required.

Enhanced flow cell rehyb capabilities

- In HiSeq v4 mode, flow cell rehybs can now be performed on-instrument during read 1, read 2, and index reads. With other run modes, this is unchanged and rehybs must be performed on the cBot.
- For all run modes, when a rehyb is selected the Rehyb panel will now prompt for loading of Paired End reagents, if applicable.

Other changes

- Sample sheet validation has been updated to allow different indexing schemes in different lanes. Both Casava format and HAS format sample sheets are supported.
- HCS and the HCS installer have been updated to run with .NET 4.5.
- Q-scoring binning is now selected by default. This setting can be unselected during run setup.
- Improved run setup workflow: BaseSpace integration option is now presented at the beginning of run configuration.
- The option to save all thumbnails or a subset of swath tile thumbnails has been added. Thumbnail images are important for troubleshooting run issues, but unselecting this option can reduce the total data footprint of run folders. Default is save all thumbnails, for all run modes.
- The embedded help documentation (available under Help) has been deprecated. Refer to the System User Guide for information on instrument operation.
- Improvements have been made to camera functionality to reduce the risk of image corruption errors. These changes consist of millisecond slow-downs in focus mode switching. This change has no impact on overall run time.
- HCS will now create an additional, temporary run folder during template generation data processing. All data (including template cycle data) is transferred to the standard run folders in drives D and E after template generation is completed, and

the standard run folder is used thereafter in the same manner as previous versions of HCS. This change was made to improve disk I/O performance during template generation.

  - The standard run folder for a run from flow cell position A will be created at the start of the run in the D drive (HiSeq Temp). The standard run folder for a run from flow cell position B will be created at the start of the run in the E drive (HiSeq Temp). This is unchanged from prior versions of HCS.
  - The temporary, template cycles run folder for a run from flow cell position A will be created at the start of the run in the E drive (HiSeq Temp), while the template cycles run folder for a run from position B will be created at the start of the run in the D drive (HiSeq Temp). Data is written to these folders during the template generation cycles (1-5 for v4 runs; 1-4 for all other run modes). After template cycles are completed, all data is moved to the standard run folder which is used exclusively for data output thereafter.
  - These temporary, template cycle folders have a negligible impact on overall data footprint, and can be deleted at the end of the run.

DEFECT REPAIRS:

- A fix was made so that custom kit selections on the reagent panel are now preserved.
- An issue leading to the accumulation of cif files in BaseSpace Temp has been fixed: previously, if a user selected to save cif files and used BaseSpace for run monitoring, run data, or the “send instrument health” option, cif files would accumulate in BaseSpace Temp. This issue will now no longer occur.

KNOWN ISSUES:

- A “Not enough quota is available” error appears intermittently upon install. There is no negative impact on HCS functionality. Select “close” to clear this error.
- “IO exception” error is thrown intermittently upon initialization of HCS. There is no negative impact on HCS functionality. Select “close” to clear the error.
- The HCS software now initializes in the background. This means that when the HCS software package is launched, no status of progress will be presented to the user until initialization is complete. Initialization of HCS will take approximately 5-6 minutes.
- When specifying a sample sheet during run setup, the use of Custom Recipes is not currently possible. The following workaround is available:
  - Start the run using the desired Custom Recipe <u>without</u> specifying a sample sheet at run setup.
  - Once the run starts, manually copy the sample sheet into the Temp directory copy of the Run Folder, and restart RTA using the RTA.bat file. The sample sheet must be named “SampleSheet.csv”.
- If no individual lanes are selected for alignment to PhiX, all lanes will be aligned to PhiX.
- HCS hangs onto indexed sample sheet if user goes back and sets up non-indexed run. The workaround is to cancel run setup and reset the run.

II. RTA v1.18.61

FEATURES:

- Optimized RTA performance:
    - RTA v1.18.61 uses 8 processing threads instead of the 4 used in previous versions. This has no overall impact on processing speed, but was necessary to support new features added to enhance performance when sequencing low diversity samples (see below).
    - New image sharping methods applied to HiSeq v4 mode only.
    - Five cycle template generation is used for v4 mode only: other run modes continue to use 4 cycle template generation.
- Added features to improve low diversity sequencing: these features were originally released on the MiSeq platform as part of RTA version 1.17.28 for MiSeq, released with the MiSeq 2.2 software update in March 2013. These features have been added for all run modes.
    - RTA 1.18.61 for HiSeq includes optimizations to the estimation of the color normalization matrix and phasing and prephasing rates. These optimizations improve the ability of RTA to handle low diversity samples. These include samples with unbalanced genome compositions (such as AT- or GC-rich genomes) or samples with low sequence diversity (such as amplicon sequencing).
    - Because of these improvements, it is no longer necessary to designate a control lane in HCS to estimate matrix and phasing. Internal testing during development showed excellent performance with samples including genomes with a range of GC content, and several different single amplicons with 10% PhiX spike-ins. The option to designate a control lane has been removed from HCS 2.2.38. When sequencing low diversity samples, such as a single amplicon, a 10% PhiX spike-in is recommended in place of a control lane.
    - Color matrix estimation now uses the first 11 cycles of sequencing data, and the same matrix is used to correct all reads. Template generation continues to use the first 4 cycles of sequencing (or 5 in the case of v4 runs), and this process uses a matrix calculated from these first 4 or 5 cycles. Once the template is established, this initial matrix is discarded and the first 11 cycles of intensity data are used for the final matrix estimation.
    - A new empirical phasing correction is now applied. For each cycle of sequencing, unique phasing corrections are calculated to maximize data quality. This is repeated for each cycle and applied on a per-cycle basis. The reported phasing shown in SAV is the tile median slope of the observed phasing corrections for cycles 1-25.
    - Phasing/prephasing is reported as “0” in SAV for an index read.
- Added new v4-specific Qtables for HiSeq v4 mode chemistry only.

- Calculation of on-the-fly indexing QC information, reported in the Indexing tab in SAV, will automatically be disabled when more than 300 indices are included in a run. This is to ensure that RTA performance does not decrease with very high indexing levels.

DEFECT REPAIRS:

- RTA will now properly calculate on-the-fly indexing QC information, reported in the Indexing tab in SAV, for HAS format sample sheets. With the previous version of RTA 1.17.21.3 for HiSeqs, this information was not calculated when and HAS format sample sheet was used, but it was properly calculated when using a Casava format sample sheet.

KNOWN ISSUES:

- When Qscore binning is selected, Qscores will reflect binning in the copy of the run folder saved to the Output folder (as expected). However, Qscores will not be binned in the copy of the run folder saved to the HiSeq Temp folder.

III. Recipe Fragments v1.5.14

FEATURES:

- Includes HiSeq v4 chemistry recipes and HiSeq v4 Mode rehyb recipes.

DEFECT REPAIRS:

- None.

KNOWN ISSUES:

- None.

IV. .net 4.5.1

- This is a software utility developed by Microsoft which is used by these latest versions of HCS and RTA and which must be installed on the HiSeq PC. This is performed automatically in the software suite installation process. Please see these links for complete information.
    - Offline installer: http://www.microsoft.com/en-us/download/details.aspx?id=40779

V. Sequence Analysis Viewer 1.8.37

NEW FEATURES:

- SAV Software has been updated to support all Illumina sequencers. The flow cell chart will update automatically to display the correct tile layout for any of these instruments.
    - Please note: On NextSeq 500 and HiSeq X systems, the Index tab will not be populated unless bcl2fastq conversion and demultiplexing is completed. For more information on performing demultiplexing and bcl to fastq conversion of NextSeq data, please refer to the NextSeq 500 System User Guide and the bcl2fastq 2.0 User Guide.

DEFECT REPAIRS:

- The copy to clipboard function on the Imaging tab of SAV now properly copies the table information in the order in which it is displayed in SAV. With previous versions of SAV, the copied data would display data columns in a different order than is displayed in the Imaging tab in SAV.
- Data for lane 1 of a multi-lane flow cell is now properly displayed in the Summary tab.
- An issue impacting the Qscore Heatmap display of binned Qscore data has been fixed; the Qscore heatmap is now able to properly display cases where the maximum and minimum Qscores are equal.
- The Qscore distribution slider function will now properly handle binned Qscore data. With previous versions of SAV, the slider would attempt to display a resolution finer than the width of the bin.

KNOWN ISSUES:

- None.

VI. BaseSpace Broker 2.1.0.1

NEW FEATURES:

- BaseSpace Broker now utilizes an archiving system for the brokerstate.xml and transferred.dat files. These files are now archived when a run successfully completes uploaded to BaseSpace or is stopped. Files that have been inactive for a week will also be automatically archived. These files can be found in a new Archive folder located at D:/Illumina/basespace broker/Archive.
- Improved the efficiency of the process by which the broker checks run upload status; inactive or completed runs listed in the Archived files are now ignored at startup.
- A run status in BaseSpace is now set to “Running” when the broker starts sending the run, as opposed to the previous implementation where it was set to “Running” when the run is updated.
- Enhanced logging: Added intermittent logging and logging of the last known exception error (if applicable). This information is written to D:\Illumina\BaseSpace Broker_logs.
- BaseSpace Broker now uploads data directly and securely to Amazon.com’s cloud storage service. This improves overall efficiency of the upload process. BaseSpace account authentication communications through the broker are still sent to Illumina’s BaseSpace API servers. This new feature requires the instrument computer clock to be synched with internet time servers, and automatic adjustment for daylight savings time must be enabled. Contact Illumina Technical Support if assistance is needed. Direct upload sends data to the following urls: *.basespace.illumina.com and *.s3.amazonaws.com.

KNOWN ISSUES:

- Delay in upload of data to BaseSpace.
  - In rare instances, users may observe a delay in uploading the data to BaseSpace. If this occurs, the Broker may be idle for a few hours. Eventually the data is uploaded successfully.