

HiSeq Instrument Software Release Notes

HiSeq Control Software (HCS) v2.2.58

Real Time Analysis (RTA) v1.18.64

Recipe Fragments v1.5.21

.Net 4.5.1

Sequencing Analysis Viewer (SAV) v1.8.46

Batch Installer v1.3

BaseSpace Broker v2.4.1.4

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

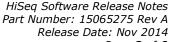
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Introduction

These release notes detail the key changes to instrument software components for the HiSeq 1000, 1500, 2000 and 2500 since the package containing HCS v2.2.38, RTA v1.18.61, Recipe Fragments v1.5.14, SAV 1.8.37 and BaseSpace Broker 2.1.0.1.

If you are upgrading from a version prior to HCS v2.2.38, please review the release notes for HCS v2.2.38 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.

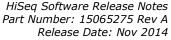
The software package includes:

- HiSeq Control Software (HCS) v2.2.58
- Real Time Analysis (RTA) v1.18.64
- Recipe Fragments v1.5.21
- .Net 4.5.1
- Sequencing Analysis Viewer (SAV) v1.8.46
- BaseSpaceBroker v2.4.1.4
- Batch Installer v1.3

I. HCS v2.2.58

NEW FEATURES:

- Enabled support for Rapid Run mode using HiSeq Rapid v2 chemistry. This workflow only proceeds with a HiSeq Rapid v2 reagent kit and components. HiSeq Rapid v2 workflow includes the option for a new 500 cycles reagent option (with 525 max cycles remaining) and maximum 251 cycles per read.
- Low diversity samples supported for all HiSeq systems.
- Added improved user controls over wash options:







- Maintenance wash workflow updated to support only maintenance wash solution (Tween 20 and ProClin 300) protocol. NaOH based wash workflow no longer supported.
- The user can now choose between maintenance wash or water wash after every high output mode sequencing run, whether the run was successful, aborted, or errored out. A maintenance wash will no longer be enforced after each sequencing run.
- User can resume or rehyb a run on the instrument in Rapid Run mode and Hiseq v4
 High Output mode during PE turnaround. User can only resume a run on the
 instrument in TruSeq v3 High Output mode, and rehyb for resumed runs must be
 done on cBot.

IMPROVEMENTS:

- Previous run attempt reagent ID fields are reported in the RunParameters.xml file.
- Software start up screen added back to UI.
- Improved wording surrounding gasket change prompts during wash.

DEFECT REPAIRS:

- Software will warn user that wash is unavailable with an option to "wait for availability" when attempting to perform a wash on a FC while the other side FC is in paired end turn chemistry step.
- Reduced dither group size to prevent run failures and swath dropouts caused by resonant frequencing in Z stage and out of focus TDI scans.
- When resuming a run in Rapid Run mode, the SBS reagent kit ID is pre-populated along with the cluster reagent kit ID (kit includes PE, clustering, and indexing reagents)..
- When resuming a run in TruSeq v3 or HiSeq v4 high output mode, the SBS reagent kit ID is pre-populated along with the PE and indexing reagent kit IDs.

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 appears intermittently upon initialization of HCS. There is no negative impact on HCS functionality. Select **Close** to clear the error.
- 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 without specifying a sample sheet at run setup.
 - When 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 keeps 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 v1.18.64

NEW FEATURES:

• Updated Q tables for HiSeq Rapid v2 workflow.

Q-score Bins	Mapped Q-
	scores
10-19	13
20-24	22
25-29	27
30-34	32
35-39	38
40+	40

DEFECT REPAIRS:

- Increased CIF copy buffer size to 64mB to prevent CIF copy errors.
- Fixed When Qscore binning is selected, Qscores will reflect binning in the copy of the run folder saved to the Output folder and the HiSeq Temp folder.

KNOWN ISSUES:

None.

III. Batch Installer v1.3

NEW FEATURES:

• None.

IMPROVEMENTS:

- Updated system to recognize HiSeq v4 mode for all HiSeq systems.
- Added logging and diagnostics for installation procedure.

IV. Recipe Fragments v1.5.21

New Features:

- Added new recipe to support Rapid Run mode using HiSeq Rapid v2 chemistry.
 - Maintenance wash recipe updated to support only maintenance wash solution (Tween 20 and ProClin 300) protocol. NaOH based wash workflow no longer supported.

DEFECT REPAIRS:

None.





KNOWN ISSUES:

None.

V. .Net 4.5.1

- No changes in this release.
- This is a software utility developed by Microsoft that is used by HiSeq X Control Software and RTA and must be installed on the HiSeq X PC. Installation of .Net 4.5.1 is automatically included in the software suite installation process. Please see this link for more information.
 - Offline installer: http://www.microsoft.com/en-us/download/details.aspx?id=40779

VI. Sequence Analysis Viewer 1.8.46

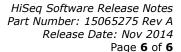
This version no longer supports Genome Analyzer run files. GA run files should not use any SAV version later than 1.8.37

NEW FEATURES:

• Added the ability to export Imaging table to .txt file.

IMPROVEMENTS:

- The Tile Status Tab and TruSeq Controls tab have been deprecated.
- Addition of a dropdown option for displaying 10 most recent runs loaded.
- Flow cell chart in Analysis screen updated to display outliers.
- Added dashed lines in to data by cycle chart to indicate start of each read.
- Added a key to the Data by Lane chart.
- The run level summary values within the summary tab for the following fields have been removed:
 - Yield Perfect (G)
 - o Yield <= 3 errors (G)</p>
 - % Perfect [Num Cycles]
 - o % <= 3 errors [Num Cycles]</p>
 - % Intensity Cycle 20
- Updated the phasing/prephasing calculations from median to mean in the run level summary table.
- The functionality to export IVC plots has been removed from the summary tab.
 Each plot can still be copied to the clip board by right clicking on the plot in the Analysis tab.
- A number of improvements have been made to the the Imaging table:
 - Improved column heading formatting
 - Added Tile Number and read drop downs
 - Removed Index Column
 - Removed Contrast Min (per channel)





- Removed Contrast Max (per channel)
- Improved performance in the speed at which data is loaded.
- Improved error handling of missing InterOp files.
- The display will now automatically refresh after the run data is loaded.
- The order and color of bases on Run Screen → Intensity plots have been updated:
 - Base A is now Red (changed from Black).
 - Base C is now Green (changed from Red).
 - Base G remains Blue.
 - Base T is now Black (changed from Green).

DEFECT REPAIRS:

None.

KNOWN ISSUES:

- Thumbnails are displayed in SAV after template build. Thumbnails exist on the disk in the runfolder\thumbnails directory.
- Windows Control Panel, Programs and Features can show entries for previously installed version after updating.

VII. BaseSpace Broker 2.4.1.4

IMPROVEMENTS:

Increased reliability and recovery abilities.

DEFECT REPAIR:

 Addressed a memory leak that could cause increasing memory consumption during a sequencing run.

KNOWN ISSUES:

- An aborted or canceled sequencing run may not be fully uploaded to BaseSpace due to the current polling interval of Broker service.
- 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.