

# **HiSeq Instrument Software Release Notes**

**HCS v2.0.12**

**RTA v1.17.21.3**

**Recipe Fragments v1.3.61**

**Illumina BaseSpace Broker v2.0.13022.1628**

**SAV v1.8.20**

***For HiSeq® 2000 and HiSeq® 1000 Systems***

**June 2013**

## Introduction

These Release Notes detail the key changes to instrument software components for the HiSeq 2000 and 1000 since the package containing HCS v1.5.15.1, RTA v1.13.48, Recipe Fragments v1.3.26, and SAV v1.8.4.

If you are upgrading from a version prior to HCS v1.5, please review the release notes for HCS v1.5 for a list of features and bug fixes introduced in that version.

FPGA upgrade to v3.10.0 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.

The software package includes:

- HiSeq Control Software (HCS) v2.0.12
- Real Time Analysis (RTA) v1.17.21.3
- Recipe Fragments (RF) v1.3.61
- BaseSpace Broker v2.0.13022.1628
- SAV v1.8.20

## I. HCS v2.0.12

### FEATURES:

#### **Storage features**

- HCS provides 2 new data compression options. When used in combination, these methods reduce the data footprint by more than 50%, without affecting data quality. This allows storage of twice the amount of run data with the same disk storage space. Both of these options are available during run setup. Please refer to the Illumina white paper "Reducing Whole-Genome Data Storage Footprint" for more details on these methods. This document is available on Illumina.com at ([http://support.illumina.com/downloads/reducing\\_whole-genome\\_data\\_storage\\_footprint.ilmn](http://support.illumina.com/downloads/reducing_whole-genome_data_storage_footprint.ilmn)).
  - BCL zipping: BCL files produced during base calling will be zipped. This option reduces the run folder by approximately 36%.
    - When using BaseSpace for data storage and analysis, bcl file zipping is required.
    - The Illumina software package bcl2fastq is required for bcl to fastq conversion of zipped bcl files. Please see Illumina.com to obtain installers and more information on bcl2fastq.  
([http://support.illumina.com/downloads/bcl2fastq\\_conversion\\_software\\_184.ilmn](http://support.illumina.com/downloads/bcl2fastq_conversion_software_184.ilmn))
  - Q score binning: Q scores are assigned to one of 7 empirically optimized bins. This option provides an additional 15% reduction in data size.
    - The use of Q score binning is an optional setting.
- HCS continues to include the functionality to optionally save CIF files (intensity files) in the run output folder. Enabling this option will significantly increase the disk space needed for storage of the run folder.

#### **Efficiency improvements**

- Optimized scanning parameters and memory usage.
  - Improved method in thumbnail images saving.
  - Improved method in saving cif files.

- Please Note: Status.htm file, Status\_files and reports folders, and ImageSize.dat file are no longer generated. This information continues to be available in the InterOp files.
- HCS now enables the functionality to de-select lanes for sequencing use. This will skip image processing of the deselected lanes. De-selection of a lane must be specified prior to the start of a sequencing run, including the first base. Note: All flow cells can only be used once. Even if a lane is de-selected in a sequencing run, it will no longer be usable in future runs.

### **Instrument wash protocols**

- To maintain instrument run performance, the following mandatory wash workflows have been implemented. Maintenance wash steps take approximately 3 hours and involve a 3 step wash of water and sodium hydroxide. Water washes take approximately 20 to 60 minutes, depending on whether the Paired End module positions are included. Please see the HiSeq system user guide for more details. ([http://support.illumina.com/sequencing/sequencing\\_instruments/hiseq\\_2000.ilmn](http://support.illumina.com/sequencing/sequencing_instruments/hiseq_2000.ilmn))
  - A maintenance wash (including a gasket change) is now required at the end of a sequencing run. This maintenance wash will not be required when choosing to abort a run from the First Base Report check.
  - A maintenance wash is required if the instrument is idle for more than 10 days.
  - A water wash is required if the instrument is idle for more than 1 day.

### **Improved error handling**

- HCS now presents clearer messages for the following errors, including suggested customer action prior to contacting customer support. Please note the error message text recorded in log files has not changed from HCS version 1.5.
  - Time out errors: HCS software will display "Time out error. Stop the run and shut down the software, power cycle the sequencing system, re-start the software, and resume the run."
  - Focus errors: HCS software will display "Focus error. Attempt to restart the run. If unable to restart, re-hybridize the flowcell. If the problem persists, contact Illumina technical support."
  - Chemistry fluidics valve errors: HCS software will display "Valve error. Stop the run and shut down the software, power cycle the sequencing system, re-start the software, and resume the run. If the problem persists, contact Illumina technical support."
  - Scan errors: HCS software will display "Scan error" and inform the user that a swath was skipped.
  - Laser errors: HCS software will display "Laser error. Wait 5 minutes for laser power to stabilize and re-start the run."

### **Sample Sheet validation**

- If a sample sheet is specified during run setup, HCS validates the sample sheet. This validation is performed to ensure successful downstream data analysis.
  - Sample Sheets are only required for Storage and Analysis BaseSpace runs; they are optional for "Run Monitoring Only" BaseSpace runs or for runs which do not use BaseSpace. (See below for further information on BaseSpace features.)
  - HCS validates the sample sheet formatting, which can be in either CASAVA format (generated by Illumina Experiment Manager v1.4.75 and earlier) or HiSeq Analysis Software format (generated by Illumina Experiment Manager v1.5 or higher).

- HCS validates the sample sheet to ensure that all indices within any one lane are unique.
- HCS will warn the user of any inconsistencies between the number and length (in bp) of the index reads specified during run setup, and the number and length of the index sequences listed in the sample sheet. The length of the index read specified in the run setup can be equal to or greater than the length of the index sequences listed in the sample sheet.
- A mix of indexed and non-indexed samples, even in different lanes, will not pass the HCS sample sheet validation process, and not compatible with BaseSpace. A mix of indexed and non-indexed samples can be processed by Illumina's bcl2fastq software.

### **Other changes**

- An AreaPerPixelmm2 value was added to the runParameters.xml file.
- The Instrument State file is now in binary format and is no longer an xml type file.
- The order of information entered during run setup has been changed. The user now enters run setup information in the following order: storage, flow cell setup, advanced options, recipe, and reagents, followed by final review of run setup.

### **BaseSpace features**

- HCS enables optional upload of run data to BaseSpace. BaseSpace can be used for storage and analysis or run monitoring only. If BaseSpace is used, customers are able to share data with other BaseSpace users.
  - Run monitoring only mode: Only data on run performance is transferred to the specified BaseSpace account. This includes the InterOp files, RunInfo.xml file, RunParameters.xml file and the sample sheet file (optional). This enables use of Sequence Analysis Viewer functionality in BaseSpace, allowing users to monitor runs from any location by logging into BaseSpace.
  - Storage and Analysis mode: Both run performance and actual run data are transferred to BaseSpace, allowing use of BaseSpace for run monitoring, data storage and data analysis.
  - Use of BaseSpace requires a BaseSpace account: See <http://basespace.illumina.com> to set up a free account.
  - When using BaseSpace in Storage and Analysis mode, the sample sheet can be in either CASAVA format (generated by Illumina Experiment Manager v1.4.75 and earlier) or HiSeq Analysis Software format (generated by Illumina Experiment Manager v1.5 or higher).
  - If a run is rehybridized, this rehybridized data will be smoothly transferred to BaseSpace.
  - The data connection to BaseSpace will smoothly resume if the run is stopped temporarily and then resumed, or if the internet connection is temporarily lost.
- When electing to use BaseSpace for Storage and Analysis, a message appears on the Storage screen linking to details regarding BaseSpace-supported workflows.
  - Message states: "Note that BaseSpace currently supports a select number of analysis workflows. Click [here](#) to check if the analysis you wish to perform is available in BaseSpace."
- When electing to use BaseSpace as the only storage method (i.e., no local copy of the data is being saved), a warning is displayed on the Storage screen.

- Warning states: "You have chosen to send data to BaseSpace without keeping a copy on premises. We recommend that you also keep a copy of the data on premises."
- HCS enables customers to choose to send instrument health data to Illumina. If this option is elected, generic run metrics will be sent to Illumina and used by Illumina to monitor and improve product quality. The "send instrument health data" option is found under the HCS Menu, under Tools and Options. Please refer to the document "Customer Information on Instrument Health Data Options with HCS 2.0" for more information.

#### KNOWN ISSUES:

- On HiSeq 2000 systems, during a sequencing run, if a wash is started on the other flow cell position, the sequencing run in progress will be automatically paused in a safe state. When the wash is complete, the sequencing run will not be automatically resumed: the sequencing run must be manually resumed by the user.
- During sequencing run setup, if a Sample Sheet is loaded, the progress points displayed on the Run Configuration tab are absent after the Sample Sheet is loaded.
- 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.
  - 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.
- Cif files improperly accumulate in the BaseSpace Temp folder when Save Cif files and instrument health data are selected, or if BaseSpace is used for either Run Monitoring Only or Data Transfer and Analysis. As a workaround, when saving Cif files, disable the instrument health data option, and do not use BaseSpace for either Run Monitoring Only or Data Transfer and analysis.

## II. RTA v1.17.21.3

#### FEATURES:

- Supports BCL zipping and Q score binning. Please refer to the Illumina whitepaper "Reducing Whole-Genome Data Storage Footprint" for more details on these methods. This document is available on Illumina.com.
- Safe stop and safe pause functions are now implemented. RTA will stop at a safe point prior to exiting the application.
- Optimized I/O in order to reduce RTA processing time.
- Improved file copying to network output folder.
- Improved file saving and loading.

## KNOWN ISSUES

- If a sample sheet created by IEM v1.5 or greater is used, RTA is unable to properly read the index sequences listed in the sample sheet. The only impact of this is that in SAV, the indexing tab information will not be populated (noted below under SAV known issues). If a Casava-format sample sheet or one generated with IEM v1.4.75 or earlier is used, the indexing tab information will be properly handled by RTA and the indexing tab will be properly populated in SAV. Basecalling of the index read cycles and downstream fastq generation and demultiplexing are not impacted in any way by this issue. As a workaround, if the use of the index tabs in SAV is needed, use a Casava-format sample sheet or one generated with IEM v1.4.75 or earlier.

## III. Recipe Fragments v1.3.61

### FEATURES:

- Includes additional wash recipes, providing post-run wash, water wash and maintenance wash functionality.

## IV. Illumina BaseSpace Broker v2.0.13022.1628

### FEATURES:

- Supports integration of HiSeq instruments with BaseSpace.
- Supports BaseSpace username validation from HCS, including Terms and Condition approval.
- Supports data copy from instrument to BaseSpace.

## V. Sequencing Analysis Viewer v1.8.20

### FEATURES

- Automatically adjust display for a 2 lane run configuration (only applicable on HiSeq2500) or an 8 lane run configuration (High Output runs).
- Automatically adjusts Q score distribution and heat map graphics to support binned or non-binned Q scores.
- SAV now remembers the file path from previous analysis.
- SAV supports four digit tile naming convention, allowing use on MiSeq runs using MCS 1.2.3 and above, as well as HiSeq, HiScanSQ, and Genome Analyzer runs.

### KNOWN ISSUES

- When displaying binned Q score data, the slider will attempt to display a resolution that is finer than the width of the bin.
- If a sample sheet created by IEM v1.5 or greater is used, the indexing tab information will not be populated, due to a known issue in RTA (noted above). If a

Casava-format sample sheet or one generated with IEM v1.4.75 or earlier is used, the indexing tab information will be populated.

- Data for lane 1 is not displayed.