

HiSeq 3000 and HiSeq 4000 Instrument Software Release Notes

HiSeq Control Software (HCS) 3.3.52

Real-Time Analysis (RTA) v2.7.3

Recipe Fragments v3.3.7

Sequencing Analysis Viewer (SAV) v1.10.2

BaseSpace Broker v2.5.2.28

Run Copy Service v1.0.19.0

For HiSeq 3000 and HiSeq 4000 Systems

June 2016

Introduction

These Release Notes detail new features, improvements, and known issues for the HiSeq 3000 and HiSeq 4000 instrument software components since the release of the previous HiSeq Control Software (HCS) package. The previous release contained HCS v3.3.41, RTA v2.5.2, Recipe Fragments v3.3.4, SAV v1.8.46, BaseSpace Broker v2.4.0, and Run Copy Service v1.10.14.

The software included in this release must be installed by Illumina service personnel. FPGA upgrade to v10.37.13 or later is recommended prior to the installation of this software package. Additionally it is also recommended that the ARM9 firmware on the chemistry module board be upgraded to v2.042 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.

For more information on the HiSeq system, including HCS 3.3.52 and RTA 2.7.3, please refer to the HiSeq[®] 3000 System Guide or the HiSeq[®] 4000 System Guide, both available on illumina.com.

For more information and detailed instructions on bcl2fastq2 conversion software, refer to the bcl2fastq2 User Guide, available on illumina.com.

The software package includes:

- HiSeq Control Software (HCS) 3.3.52
- Real-Time Analysis (RTA) v2.7.3
- Recipe Fragments v3.3.7
- Sequencing Analysis Viewer (SAV) v1.10.2
- BaseSpace Broker 2.5.2.28
- Run Copy Service v1.0.19.0

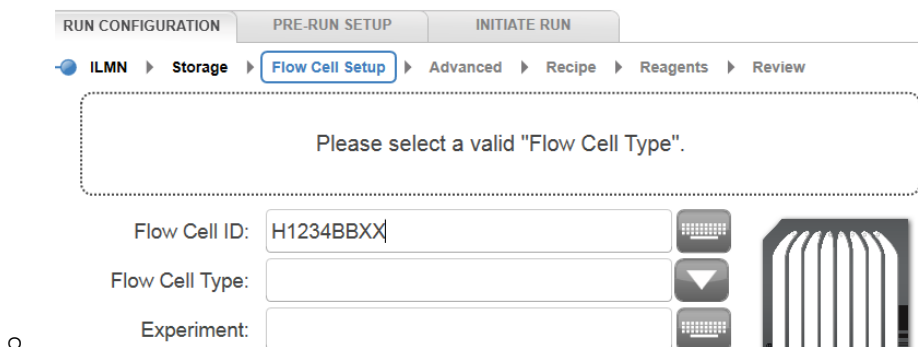
I. HiSeq Control Software (HCS) 3.3.52

NEW FEATURES:

- Added support for single read flow cells. HiSeq 3000 4000 SR flow cell is selectable through the Flow Cell Setup screen on the Run Configuration tab.
- Added BaseSpace Onsite support.
- Improved % passing filter for lanes 1 and 8.
- The following status icons have been added to the interface:
 - BaseSpace Broker
 - Run Copy Service
- Added Performance Monitor to HCS Menu | Tools to record computer hardware performances.
 - This feature defaults to "off" on install.
 - Feature is intended for Illumina Field Staff to troubleshoot hardware issues on instrument control PC (feature not intended for customer use).

IMPROVEMENTS:

- Vacuum sensor and chiller door logic changed to ignore false positive sensor warnings.
- Added BaseSpace Broker version to the runParameters.xml file.
- Updated disk space usage check to more accurately assess data output by cycle. There must be 6.5 GB per cycle of space on the O:/ drive. For details, see the HiSeq® 3000 System Guide or the HiSeq® 4000 System Guide.
- Gasket change required with 10-day maintenance wash.
- The following checkboxes were removed from the Storage Panel because HCS performs the associated actions by default:
 - Checkbox for Q-score binning
 - Checkbox for zipping BCL files
 - Checkbox for selecting an output folder
- When starting a run, the flowcell type box doesn't automatically populate in the Flow cell set up screen, unlike the previous HCS previous version. The user is required to select the appropriate flow cell type (Paired read or Single read) so that the correct recipe for indexing is called designated in HCS

**DEFECT REPAIRS:**

- Run set up can now be configured for First Base Report to pause for user confirmation prior to continuing the sequencing run. Illumina recommends that the run is not paused at this point, and that SAV is used to assess run metrics.

KNOWN ISSUES:

- Pausing an ongoing run to start a second flow cell might stop the flow cell that is running, and the run cannot be resumed.
 - To avoid a run-stopping event, follow the pause procedure described in the HiSeq 3000 System Guide or the HiSeq 4000 System Guide.
- Selecting the Abort button to stop the run for a flow cell on one side during imaging puts the camera in an unknown state and stops the run for the flow cell on the adjacent side.
 - To recover from this issue, restart HCS.
 - The sequencing runs for either flow cell cannot be resumed.

- Upon closing HCS a warning may display: - "Scanner Warning Camera2 was not initialized before calling Scanner Uninitialized ()". The warning is incorrect, and cameras are still being properly uninitialized at shutdown.

II. Real-Time Analysis (RTA) v2.7.3

NEW FEATURES:

In a change from the workflow on the 2500, where individual lanes could be selected via a checkbox in the run set up to align to PhiX, RTA v2 will attempt Phi X alignment to clusters in tiles from all lanes. It is not configurable.

IMPROVEMENTS:

- Added new Q-table for HiSeq 3000 and HiSeq 4000 trained on a broad range of genomic samples.
- Updated matrix correction algorithm to improve performance on MethylSeq and low diversity samples.

III. Recipe Fragments v3.3.7

NEW FEATURES:

- Support added for Single Read flow cells. See "New Features" section in HiSeq Control Software for more information.

DEFECT REPAIRS:

- None.

KNOWN ISSUES:

- None.

IV. Sequencing Analysis Viewer (SAV) v1.10.2

NEW FEATURES:

- Added Zip My Run button to Summary tab.
- Add non-indexed row to Summary tab table.
- Added Minimum and Maximum contrast to imaging tab table.
- Added folder drag and drop into RunFolder text box.
- Loading tab calculations on demand instead of all at once.

IMPROVEMENTS:

- Change %Q30+ to exclude last cycle of read.
- Change total yield to include only non-N PF basecalls, rather than taking number of PF reads and multiplying by number of cycles in read minus one.
- Change projected total yield to extrapolate from existing cycles.
- Removed %NC from Corrected % and made separate column in imaging tab table.

- Renamed Corrected % to % Base.
- % Base and % No Call are calculated only from PF reads.
- Analysis tab status shows a range of values for in progress runs.
- All drop-down menus have the same look and feel.
- Dates in the Imaging tab are iso-8601 compliant.

DEFECT REPAIRS:

- Clear data when current load has errors.
- Removed outlier coloring in flowcell chart (outlier-tolerant scaling is still present, though).
- Q-score distribution chart flips from GB to MB at 100 MB instead of 10 MB.
- Fixed scatter plot z-scaling.

KNOWN ISSUES:

- Corrected intensity is 0 for most recent RTA versions.
- Q-score histogram includes the last cycle of reads when calculating yields, while summary tab does not.
- Zip My Run button uses excessive memory to run. This may impact an ongoing run if "zip my run" is selected while HCS is running, particularly on systems that have existing memory constraints.

V. BaseSpace Broker 2.5.2.28

IMPROVEMENTS:

- BaseSpace Broker updated to ensure correct copy policy association with upload mode (Instrument Health, Run Monitoring, Storage and Analysis).
- Limited Broker thread count and resource utilization.

DEFECT REPAIR:

- None

KNOWN ISSUES:

- None

VI. Run Copy Service v1.0.19.0

NEW FEATURES:

- New file copy user interface display indicating the following:
 - File copy in progress but network connection slow.
 - RCS not running.
 - RCS running but not copying.
- Configurable value that defines the limit of for the number of files that have not been copied.