



HiSeq[®] 3000 and HiSeq[®] 4000 Instrument Software Release Notes

HiSeq Control Software (HCS) v3.3.76

Real-Time Analysis (RTA) v2.7.6

Recipe Fragments v3.3.9

Sequencing Analysis Viewer (SAV) v1.10.2

BaseSpace Broker v2.9.0.2

Run Copy Service v1.0.19.0

For HiSeq[®] 3000 and HiSeq[®] 400 Systems

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Introduction

These Release Notes detail the key changes to instrument software components for the HiSeq 3000 and HiSeq 4000 since the release of the previous HiSeq Control Software (HCS) package. The previous release contained HCS v3.3.52, RTA v2.7.3, Run Copy Service 1.0.19.0 and Recipe Fragments v3.2.7.

If you are upgrading from an earlier version than HiSeq 3000 and HiSeq 4000 Control Software v3.3.52, review the release notes for HCS v3.3.52. These release notes contain a list of features and bug fixes introduced in that version.

The software included in this release is customer-installable and does not require additional support from trained Illumina service personnel.

The Recipe Fragments installation overwrites any custom recipe files. Therefore, recreate any custom recipes after receiving this update.

The BaseSpace Send Instrument Health data option is activated during this software installation. After receiving this update, update your BaseSpace Send Instrument Health settings.

The software package includes:

- HCS v3.3.76
- RTA v2.7.6
- Recipe Fragments v3.3.9
- Microsoft .Net Framework 4.5.1 – no change
- SAV v1.10.2 – no change
- BaseSpace Broker v2.9.0.2
- Run Copy Service v1.0.19.0– no change

I. HiSeq Control Software v3.3.76

NEW FEATURES:

- Addition of BaseSpace domain configurability.
- Simplified system health log files generated in run folders.
- Sample sheet validation now allows single and dual indexing across lanes in a flow cell.
 - Exception: single and dual indexes cannot be pooled within a lane.

IMPROVEMENTS:

- Improvement to auto-center retry attempts.
 - When auto-center is unsuccessful for a particular lane, HCS retries alignment in the next lane until all 8 lanes have been attempted (total of 16 retries per flow cell, 8 retries per flow cell surface).
- Updated the disk space check to occur before reagent priming.

DEFECT REPAIRS:

- The Pause feature is enabled.



- HCS users can now pause a run to check run components or replenish reagents. After a paused run is resumed, RTA automatically restarts.
- The recommended procedure for staggering runs on Flow Cell A and Flow Cell B have been updated. For instructions, see the *HiSeq 4000 System Guide* (document # 15066496).

KNOWN ISSUES:

- Selecting the Abort button to stop a run during imaging puts the camera in an unknown state and stops the adjacent run.
 - To recover from this issue, restart HCS.
 - The sequencing run for either flow cell cannot be resumed.

II. RTA v2.7.6

DEFECT REPAIRS:

- Improved phasing and prephasing estimation.
- Improved the run time of intensity correction algorithms.
- When RTA cannot write files on the S (SSD) drive, instead of crashing, the software logs an error and subsequent sequencing data for that tile is corrupt.

KNOWN ISSUES:

- PhiX error rate quantized at 0.2% at lowest allowed spike-in of 0.1%. Illumina recommends using a minimum spike-in of 0.5% to recover.

III. Recipe Fragments v3.3.9

IMPROVEMENTS:

- Updated the following recipe fragments:
 - Reagent and flow cell barcode masks
 - Paired-end (PE) reagent names

DEFECT REPAIR:

- None

KNOWN ISSUES:

- None

IV. Sequencing Analysis Viewer 1.10.2

- No changes in this release.

V. BaseSpace Broker 2.9.0.2

NEW FEATURES:

- Addition of BaseSpace domain configurability

IMPROVEMENTS:



- Updated how frequently system health logs are uploaded to BaseSpace Sequence Hub:
 - During a sequencing run: after every cycle.
 - Between sequencing runs: once every 24 hours or after HCS closes.

DEFECT REPAIR:

- None

KNOWN ISSUES:

- None

EXTERNAL CONSIDERATIONS:

- For customers with network firewall limitations, the addition of basespace-data-east.s3-external-1.amazonaws.com to whitelist addresses is required to enable BaseSpace Sequence Hub functionality.

VI. Run Copy Service v1.0.19.0

- No changes in this release