MiSeq Software Release Notes

MCS 2.4

RTA 1.18.54

MiSeq Reporter 2.4

BaseSpace Broker v2.1

MiSeq Recipes 3.0

For MiSeq Systems

April 30, 2014

Introduction

These Release Notes detail the key changes to software components for the MiSeq since the package containing MCS v2.3, MiSeq Reporter v2.3.32, RTA v1.18.42, BaseSpace Broker v2.0.12195.16151, and MiSeq Recipes v3.0.0.

This release is optional, but highly recommended due to the features and bug fixes described in these Release Notes. Please review these changes before updating your software.

If you have installed MCS v2.4.0, please update to MCS 2.4.1 to resolve an issue found with sample sheet validation.

If you are upgrading from a version earlier than MCS v2.3, please review the release notes for MCS 2.3, etc. for a list of features and bug fixes introduced in that version.

For more information about the MiSeq system and how to use the software included with it, please see the product and support pages at illumina.com

http://www.illumina.com/systems/miseq.ilmn

http://support.illumina.com/sequencing/sequencing_instruments/miseq.ilmn

To update this software suite, your MiSeq must already be running MiSeq versions 2.1, 2.2, 2.3, or 2.4.0. If you have an earlier MiSeq software version, you can update your software using the MiSeq Updater 2.1, which can be found on the Illumina website at the following location:

http://support.illumina.com/downloads/miseq_software_updater_and_reference_genomes_updater.ilmn

The software package includes:

- MiSeq Control Software (MCS) v2.4*
- RTA v1.18.54
- MiSeq Reporter v2.4
- BaseSpace Broker v2.1
- MiSeq Recipes 3.0

* MiSeq Updater v2.4.0 contained MCS v2.4.0. An issue with sample sheet validation was found in this version. MiSeq Updater v2.4.1 has since been updated with MCS v.2.4.1 to address this defect. MiSeq Updater v2.4.1 includes the four other software applications listed above to allow customers to upgrade from MiSeq v2.3 directly to MiSeq v2.4.1. The only software application changed in this package was MCS.

I. MiSeq Control Software (MCS) v2.4

NEW FEATURES:

- Run time reduction when using MiSeq v3 Reagents. This reduction is due to an optimization of stage movements when using v3 kits. No other changes have been made to instrument operation.

- A new technical support file bundling feature was added. This feature can be found under the Manage Files menu in MCS, under a new tab named Bundle Logs. This feature provides an easy way to collect run and log files frequently requested by Illumina Support for use in troubleshooting. See the MiSeq System User Guide for more details on this new feature.
- A new cumulative wash history file, WashLog.xml, is now generated under D:\Illumina Maintenance Logs. This file is intended to provide a history of instrument wash practices, for use in laboratory record-keeping.
- Added hardware initialization retry logic in order to improve functional robustness. This was added so that in the event of an unexpected component initialization failure, MCS will automatically retry the initialization.

DEFECT REPAIRS INCLUDED IN MCS V2.4.0:

- A random access memory (RAM) leak issue has been fixed. In previous versions of MCS, this issue led to the MCS application consuming an increasing portion of the available RAM.

DEFECT REPAIRS INCLUDED IN MCS V2.4.1:

- The pre-run sample sheet validation has been fixed. In MCS v2.4.0, an issue was introduced that impacted the use of sample sheets that listed Custom Amplicon or Small RNA as the analysis workflow.

KNOWN ISSUES:

- None.

II. RTA v1.18.54

NEW FEATURES:

- None.

DEFECT REPAIRS:

- A bug leading to a discrepancy in the percentage of reads passing filter reported in the filter files as compared to the stats files has been fixed. With previous versions of RTA, this discrepancy could lead to differences in the percent reads passing filter reported in Sequence Analysis Viewer compared with downstream results after BCL to FASTQ conversion.

KNOWN ISSUES:

- None.

III. MiSeq Reporter v2.4

NEW FEATURES:

- The 16S Metagenomics workflow now includes an additional HTML report in the analysis subfolder of the run output folder. This report uses the same data as the standard 16S reports, but includes additional visualizations and summaries. The MiSeq Reporter Metagenomics Workflow Reference Guide has been updated with details on how to use this report. Note for Internet Explorer users: IE9.0 or later is required to view all of the plots.

- The default value for the file copy timeout has been increased. This enhancement was made to improve the management of the final copy step, which transfers results to the MiSeq Output directory. The default time limit is now 30 minutes, an increase from the prior limit of 15 minutes in previous versions of MiSeq Reporter.
- The reporting of algorithm versions and parameter settings applied during analysis has been improved for all workflows, with this information now being included in the BAM and vcf/gVCF results files. The BAM header portion of BAM files now includes the aligner name and version. VCF files now include the dbSNP database version if available
- An enhancement has been added to the MiSeq Reporter annotator so that it now uses the most recent dbSNP file present, provided dbSNP files are named following the convention dbsnp[version].txt, e.g., dbsnp137.txt. Please note, MiSeq Reporter only reads dbsnp files obtained from Illumina. More specifically, MiSeq Reporter uses the following logic:
    1. Look for dbsnp<ver>.txt
    2. If there are multiple versions, use the one with the highest number (latest available release) and return that number
    3. If no file matches, search for snp<ver>.txt (this logic provides backward compatibility)
    4. Follow same rule as above (#2)
    5. If no file matches, search for dbsnp.txt
- Enabled variant annotation for the Amplicon - DS Workflow. Variant results from this workflow are now annotated in the same way as all other workflow variant results, and results are provided in the merged VCF files only.

DEFECT REPAIRS:

- Amplicon - DS Workflow:
    - Previously, when BAM file results for regions with zero or low coverage were passed to the Amplicon - DS workflow, a "null reference" exception error would result. This issue has now been fixed so that this workflow can properly process low coverage BAM results.
    - An issue leading to low confidence variants failing to be filtered out has been fixed: Now, low confidence variants adjacent to homopolymer regions (defined as 8 or more single bp repeats) are correctly filtered out.
- TruSeqAmplicon Workflow:
    - There was a visualization bug where the 'Details' tab in the MiSeq Reporter user interface contained no information on Sample and Variants. The bug is now fixed.
- Somatic Variant Caller option:

- Memory usage has now been adjusted to handle gVCF output files properly. This improvement was made to resolve an issue where using the Somatic Variant Caller with gVCF output could result in out-of-memory errors.
- An issue leading to improper "low variant frequency" filtering of reference calls when using the Somatic Variant Caller with gVCF outputs has been corrected.
- An issue leading to a 1bp shift in the intervals in which variant calling was applied has been corrected. This issue only occurred with use of the Somatic Variant caller. With previous versions, variants at the start or end of the targeted region could be missed: This is now fixed.
- Previously, when using the Enrichment workflow with the Somatic Variant Caller, variant calling would be performed over the entire genome: a fix has been made so that now, when using the Enrichment workflow with the Somatic Variant Caller, variants are now called only inside the targeted region as intended.

- Metagenomics 16S Workflow:
  - The percent abundance table displayed in the detailed view in MiSeq Reporter properly handles species with very low abundance. With this change, all low abundance species (defined as species with less than 0.25% abundance each) are combined into one entry in table, named Other.
- Targeted RNA Expression Workflow:
  - A fix was made to the stringency applied during the alignment step so that very short amplicons are properly handled. This fix reduces the possibility of false positive alignments and increases the accuracy of gene counts.
- StitchReads setting:
  - A fix was made to the StitchReads function so that now reads are stitched even if the read lengths differ after adapter trimming, or differ simply due to the run configuration. Previously an "index array out of bounds" error would occur in this situation. With this fix, reads with different lengths can now be stitched.

KNOWN ISSUES:

  - The 16S Metagenomics workflow generally matches the top 3 hits to classify each read. In rare cases, species level identification can lead to misclassification, rather than stepping back to a higher taxonomic level to identify the reads. This particular error can occur when the top hit is not fully classified to a species level match in GreenGenes, but the second best hit is classified.
- When using the AmpliconDS workflow with samples containing an exceptionally high frequency of indels, the somatic variant caller may run out of memory. It will not be possible to complete analysis of such a sample. If this situation occurs, a "Vcf needs to be ordered" exception error will appear in the error log file.

IV. BaseSpace Broker v2.1

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 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. This change was made to improve instrument startup times.
- 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.

DEFECT REPAIRS:

- None.

KNOWN ISSUES:

- Installing this update will overwrite the broker configuration settings in the C:\Illumina\BaseSpace Broker\Illumina.BaseSpace.Broker.exe.config. If you have customized the broker configufation (e.g. proxy settings), please create and retain a copy of the Illumina.BaseSpace.Broker.exe.config file before installation.

V. MiSeq Recipes 3.0

- Note: No changes were made to this application. The MiSeq Recipes 3.0.0.1 will be uninstalled and then reinstalled as part of the MiSeq 2.4 software update. Any modifications made to the default recipes will be over-written and will need to be repeated after the software update is complete.