

Ion Reporter™ Software 5.12.3 Release Notes

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Revision History

Revision	Date	Description
C.0	06 March 2020	Updated release notes for Ion Reporter™ Software 5.12.3. Three fixed issues and two known issues were added.
B.0	15 Nov 2019	Updated release notes for Ion Reporter™ Software 5.12.1 and 5.12.2. The following fixed issues are added to the table on page 11: IR-42413, IR-41252, IR-42622, IR-34815, IR-38705 and IR-37643. One new known issue is added: IR-42804.
A.0	12 Sept 2019	New release notes for Ion Reporter™ Software 5.12.

New Features in Ion Reporter™ Software 5.12

The Ion Reporter™ Software 5.12 release includes many new features and improvements.

OncoPrint™ BRCA analysis and results visualization improvements

OncoPrint™ BRCA analysis workflow improvements include an updated CNV baseline, multiple bug fixes, the use of a new reference transcript NM_007294.3 for BRCA1 in the exon deletion algorithm, and enhanced detection of long deletions (addition of the LongDel data type) in analysis results.

Visualization improvements include the ability to toggle between two available y-axis scales, and toggle between showing both pre- and post-VCIB corrected log2 normalized read count graphs. The color of the data shown in the graphs of OncoPrint™ BRCA analysis results for exon-level CNV has been updated to use colors that allow users to better distinguish between the exons in the BRCA1 and BRCA2 genes.

Tile size in aneuploidy CNV analyses is set by the tile size of the copy number baseline

In previous versions of Ion Reporter™ Software, you were required to ensure that the values for the aneuploidy tile size parameters in the copy number baseline creation and the analysis workflow were the same. Starting in Ion Reporter™ Software 5.12, tile size of the analysis is determined by the copy number baseline tile size.

Aneuploidy analysis results visualization improvements

- A greater Y-axis range between 2N and 3N/1N in the Whole Genome View allows for easier detection of mosaicism.
- The Whole Genome View of aneuploidy analyses include a 50% increase in pixel height, relative to width.
- A thin vertical line between chr22 and chrX was added to differentiate the data shown on these two chromosomes in the in Whole Genome View.

Immune Repertoire analysis and visualization improvements

Immune Repertoire analysis improvements include new data and visualizations including spectratyping plots by isotype; isotype usage plot including reads per isotype, lineages per isotype, clones per isotype; and somatic hypermutation (SHM) profile plots. Other improvements in searching and sorting table of data include new IGH fields in the clone summary file, a lineage summary file and new fields in the metrics file. A new compare samples feature allows for comparing clones detected across analyses, and the metrics file contains new IGH-specific fields.

Data and source updates

- The list of controlled vocabulary terms for cancer types that are shown in Ion Reporter™ Software includes additional new choices.
- You can label variants as relevant to your research in Ion Reporter™ Software. New terms are included in the list of optional user-selectable variant Classifications. You can now apply the following classification terms to variants in your analysis results: Pathogenic, Likely Pathogenic, VUS, Uncertain Significance, Likely Benign, Benign, Unknown, Suspected Benign, Suspected Deleterious, and Deleterious.
- All GRCh38 and hg19 annotation sources have been recently updated for 5.12.

Add notes to an analysis

- Include notes about analyses from the Analysis Overview screen using the “+” sign; Notes text can be used to search for the associated annotated analyses using the Search box on the Analyses Overview page.

Reporting enhancements

When you create reports in Ion Reporter™ Software 5.12, you can:

- Add data from the various views in visualizations to reports. Examples are quality control data, which were previously reported only in a separate QC report, and data from some new types of analysis results, including microsatellite instability (MSI) detection data.
- Add detailed Ion Reporter™ Software quality control data to all PFD reports.
- Add metrics and sample-specific graphical data from the CoverageAnalysis and MolecularCoverageAnalysis plugins to QC Reports generated in Ion Reporter™ Software if the data was generated by and transferred from Torrent Suite™ Software.

21 CFR part 11 electronic signatures, password policies and user activity auditing

Ion Reporter™ Software 5.12 allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing. Users can generate analysis results reports that are signed electronically, and administrators can monitor individual user activities for changes made to the software. Password policies include new options that can be set to meet institutional security policy standards.

Note: The default number of days for password expiration is 90 days in Ion Reporter™ Server 5.12. Beginning 8 days before the default 90-day password expiration date, password expiration emails are sent from the Ion Reporter™ Server to all server users in every Ion Reporter™ Software organization if password duration defaults are not changed by the administrator users of the organization. The first 90-day password expiration period begins when the server is upgraded to on Reporter™ Server 5.12.

Expanded analysis workflow capability

- Tumor Mutational Burden analysis workflows are now customizable through a variety of user adjustable parameters. Additional new features include a new simplified default germline filter-chain, the QC status of the sample that is based on a deamination score, reporting of TMB status (High, Intermediate or Low), flagging at ultra-low coverage, and a simplified visualization user interface.
- Panels whose content supports detection of microsatellite instability (MSI) will contain analysis workflows that include MSI score and status in their analysis results.
- Immune Repertoire analyses results support haplotyping for sample stratification and predictive analysis for immune-related adverse events.
- A new algorithm and data type detects 3'-5' imbalances for fusion partners. The *RNA Exon Tile Imbalance Assays* data type results can be viewed in visualizations for assays which contain the content that this algorithm requires.
- Possible Polyploid Alleles are now able to be annotated and displayed in single sample analyses run in Allele View.
- The outputs of the 16S Metagenomics analysis workflow now provides a single BIOM file showing the complete OTU table at family, genus, and species taxonomic levels.
- You can now add your own custom 16S Metagenomics databases that can be used with the 16S Metagenomics analysis workflow.

Software assessment service

An assessment service to help optimize Ion Reporter™ Software analysis workflows for new assays and established on a new version of Ion Reporter™ Software is available. Contact your Field Bioinformatics Specialist (FBS) to learn more.

Improvements to enhance software usability

- A filter chain for extended OncoPrint™ analysis results with relaxed stringency that includes OncoPrint™ annotated variants, and additional non-OncoPrint™ annotated results including non-targeted fusions, likely somatic passenger mutations, and ClinVar “Pathogenic” and “Likely Pathogenic” results.
- Filter chain logic is now displayed in the software, and the NOT logical operator is now available for use in filter chain construction.
- Filter chains can now be applied in more of the Analysis Visualization tabs.
- Filters can contain the NOT logical operator
- Filter chains can have more than one instance of a particular filter
- Gene names can be submitted as an uploaded list to the Gene Symbol filter during filter chain creation/editing
- Possible Polyploidy Alleles (PPA) enhancement for filtering and annotation these types of variants
- Ion Reporter™ Software 5.12 is compatible with and requires the Ubuntu 18.04 operating system. Please contact your local field support representative for details on having your Ion Reporter™ Server System’s operating system version and Ion Reporter™ Software version upgraded.
- Ion Reporter Server Administrators can adjust the duration of the timeout for the server.

Troubleshooting and additional export/download functionalities

- SampleID plugin display and data files are available in downloaded ZIP files
- Additional files have been added to the logs that are downloaded from the Admin tab on the System Services screen, when users click **Download All Logs**.
- IRU plugin improvements to support Deep Packet Inspection (DPI) firewalls
- Option to download validation errors when a file upload fails
- VCIB CNV Baseline creation logs now include the names of rejected BAM files
- API for downloading Current Results TSV file

Changes in terminology and data output

- Column Name harmonization: The term "Mol Freq %" is now used across all column headers in data tabs and visualizations and replaces the labels “MAF%”, “Mutant Frequency %” and “Mol Freq”, all of which represent the same data. “Mol Freq %” now occurs where those other terms previously occurred in tables and visualizations in earlier versions of Ion Reporter Software.
- For called variant allele in AmpliSeq™ HD and TagSeq analysis results, LOD value will always be given a value of “.”. This is because the actual allele frequency is available and therefore the calculated LOD is not relevant for called alleles.

End of life for Ion Reporter™ Software 5.0 analysis workflows.

Ion Reporter™ Software 5.0 analysis workflows are now retired from the Ion Reporter™ Software 5.12. Version 5.0 analysis workflows will also be unavailable after servers are upgraded Ion Reporter™ Software 5.12. Analyses that are launched through analysis workflows from Ion Reporter™ Software 5.0 and earlier will continue to be available to open and create reports for in the latest version of Ion Reporter™ Software. Each new version of Ion Reporter™ Software will continue to retire at least one of the earliest versions of the currently released Ion Reporter™ Software analysis workflows.

Issues fixed in Ion Reporter™ Software 5.12

Issue number	Description
IR-44440	Security tokens for users whose password security token is several years old were unable to access some analysis visualizations in Ion Reporter™ Software, for example, visualizations shown in the IRGV and Immune Repertoire tabs. This issue is fixed in Ion Reporter™ Software 5.12.3. For users affected by this issue, the security token generation date will be reset upon upgrade to Ion Reporter™ Software 5.12.3.
IR-44449	An issue with the use of mask files occurred on Ion Reporter™ Software 5.12.2 on Connect. When a custom mask file was uploaded to Ion Reporter™ Software 5.12.2 on Connect and added to workflows, analyses failed. The failed analyses occurred when workflows that used the uploaded custom file were launched. This issue did not affect Ion Reporter™ Server. This issue is fixed in Ion Reporter™ Software 5.12.3.
IR-42901	A rare issue was found when Ion Reporter™ Servers were upgraded to the Ubuntu™ 18.04 operating system. The issue affected variant calling. As a result, some calls were made incorrectly or discarded under some circumstances in Ion Reporter™ Server 5.12.2. This is fixed in Ion Reporter™ Software 5.12.3.
IR-38705	In Ion Reporter™ Software 5.10 Oncomine BRCA Research analysis workflow results, whole gene CNVs in the BRCA1 gene were not reported in the list of called CNV results, due to an error in coordinate mapping during CNV detection. BRCA2 gene CNVs were not impacted. BRCA1 gene CNV graph data were unaffected by the issue and still showed imbalances between the coverage of BRCA1 compared to BRCA2 in whole BRCA1 gene deletion samples in the BRCA tab of the Visualization Results screen. This issue has been fixed in Ion Reporter™ Software 5.12.
IR-42413	In Ion Reporter™ Software 5.12.0 and 5.12.1, a bug changed the order of processing of samples by the MicroSEQ ID database and the Greengenes database in the default 16S workflow. Data was first passed through the Greengenes database and any sequences not getting a hit passed to the MicroSEQ database. However, the correct order of the query of data through the two databases is to start with MicroSEQ database. This caused the algorithm to run longer when compared to Ion Reporter™ Software 5.10 analyses, and also potentially causes slight data differences due to the change in database order. This issue has been fixed in Ion Reporter™ Software 5.12.2. The order now reflects the order in Ion Reporter™ Software 5.12.0 and earlier: data is first queried against the MicroSEQ ID database, the remaining unmatched sequences are then queried against the Greengenes database. For more information, please see the algorithm white paper: https://tools.thermofisher.com/content/sfs/brochures/ion-reporter-16s-metagenomics-algorithms-whitepaper.pdf .
IR-37643	Changes made to the functionality of the MAF (Minor Allele Frequency) filter for Ion Reporter™ Software 5.12.0 and 5.12.1 did not cover all possible allele combinations and edge cases. As a result, the changes from 5.10 functionality in Ion Reporter™ Software 5.12.0 and 5.12.1 were reverted in 5.12.2, and will be postponed for a future release. The MAF filter in Ion Reporter™ Software 5.12.2 uses the functionality of the Ion Reporter™ Software 5.10 MAF filter. Therefore, filter chains that were created with Ion Reporter™ Software on Connect 5.12.0 or 5.12.1 will have a changed behavior and deliver different results from those results in 5.10.0 and 5.12.1. No other filters were impacted by the changes and reversion. RECOMMENDATION: Recreate any custom filter chains that contain the MAF filter in Ion Reporter™ Software 5.12.2, if those filter chains were first created in Ion Reporter™ Software on Connect versions 5.12.0 or 5.12.1.

Issue number	Description
IR-42622	In Ion Reporter™ Software 5.12.0 and 5.12.1, any IGH or TCR Immune Repertoire analysis consisting of multiple barcodes has an issue with the display of analysis results. Analysis results that are downloaded from the Analysis tab (in the Overview screen click Actions and select an option to download) contain a metrics file that has incorrectly placed data columns. This issue occurs because the barcodes that appear in the barcode column of the file are separated by a comma, and the file is comma-delimited. The delimiter to separate the barcodes in a multi-barcode samples is a semicolon in Ion Reporter™ Software 5.12.2, which corrects this issue.
IR-42078	In Ion Reporter™ Software on Connect 5.12.0 and 5.12.1, variants with no ClinVar annotation were not displayed as expected in the filtered out list, when a filter chain with the ClinVar filter was applied. The "Filtered Out" variant count was correct, but the display of variants in the filtered out list did not show variants which were filtered out. In Ion Reporter™ Software 5.12.2, the "Filtered Out" variant count and the variants list are both correctly displayed.
IR-34815	Results shown in the Variant Matrix tab were sometimes not visible in the Analysis Results table when the Variant Matrix Summary filter chain was used for the same analysis. This discrepancy was due to a difference in the rules that causes multi-allele variants that are present in the Variant Matrix tab to be filtered out by the Variant Matrix Summary filter chain when not all alleles pass the filter criteria used by the filter chain. This is fixed in Ion Reporter™ Software 5.12.
IR-34006	<p>The Transition Penalty value shown in the parameters reflects the true value for Low, Medium, and High Sensitivity CNV Finding parameters in Ion Reporter™ Software 5.12. The High, Medium, and Low built-in sensitivity settings for CNV Finding Sensitivity use the following transition penalty values:</p> <ul style="list-style-type: none"> • Low Sensitivity: Transition Penalty = -15 • Medium Sensitivity: Transition Penalty = -5 • High Sensitivity: Transition Penalty = -3 <p>Previously, in the CNV Finding Advanced Parameters, the Transition Penalty in all of the predefined ReproSeq analysis workflows was shown as -8 regardless of which Sensitivity setting is selected on the Main tab. The Transition Penalty value now corresponds to the selected built-in sensitivity settings. This is fixed in Ion Reporter™ Software 5.12.</p>
IR-35192	The Snapshot button in the Krona view of 16S Metagenomics results did not work in some Web browser versions. This was a known issue with Krona: https://github.com/marbl/Krona/issues/83 . This is fixed in Ion Reporter™ Software 5.12.
IR-33346	<p>Ion Reporter™ Software uses HGVS to ensure that variants annotated in the software meet HGVS compliant standards as much as possible. The Functional Annotator module in Ion Reporter™ Software 5.12 (1) follows the HGVS 3'-rule, (2) annotates full frameshift, (3) follows HGVS nonsense definition, (4) annotates MNV's in HGVS protein "Allele" format", and (5) continually incorporates other changes, bug fixes, and customer suggestion to continue to be more HGVS compliant.</p> <p>In this release, changes that can be visible in analysis results include:</p> <ol style="list-style-type: none"> 1. Ion Reporter™ Software 5.12 uses the 3' rule recommended by HGVS as written on the HGVS website. 2. Ion Reporter™ Software 5.12 calls protein variants based on the protein change, not on the DNA while still making the correct different calls also required by HGVS. <p>See the website http://varnomen.hgvs.org/recommendations/general/ for the 3' rule and the HGVS requirements. The HGVS website also contains more information and examples for the 3' rule.</p> <p>The HGVS website defines this rule as "descriptions at RNA/protein level should describe the changes observed on that level (RNA/protein) and not try to incorporate any knowledge regarding the change at DNA-level".</p>

Issue number	Description
IR-33667	In Ion Reporter™ Software, both the Allele View VCF file and the Locus View VCF file included values in the FDP and DP fields that were the same; however, each allele was displayed as a single line of record in the Allele View VCF file. This is fixed in Ion Reporter™ Software 5.12.
IR-34789	When files or reports are downloaded from the Ion Reporter™ software, there was a mismatch in time stamp data between what is shown on the screens in the software and in the downloaded file. This was due to the difference in user and server time zones. The data shown on the software screens matched the client's time zone and the data shown on the downloaded files will match the server's time zone. One exception to this difference was the visualization report where the downloaded PDF showed the client time stamp under the 'launched on' field. This is fixed in Ion Reporter™ Software 5.12.
IR-34604	An issue with sample information that was displayed in the Sample/Analyses Summary when multiple analyses that used different Research Application types were selected has been fixed. If you first selected DNA, then next selected Fusions Only and DNA, and Visualized the results, sample information was displayed correctly if, in the Visualization, you opened the results for a specific analysis from the list of multiple results in the Ion Reporter™ Genomic Viewer (IRGV) tab of the Analysis Results screen. However, if you opened an analysis result by clicking on a row in the Analyses table, the resulting Sample/Analyses Summary could show incorrect information, compared to the graphics shown on the left side of the screen.
IR-31022	Some browsers such as Chrome and Safari sometimes did not download CNV Heat map SVG files successfully analyses were visualized and exported as SVG. This is fixed in Ion Reporter™ Software 5.12.
IR-30985	TagSeq Liquid Biopsy analysis workflows, which detect CNVs, give a p-value but not a "Confidence Score" or "Confidence Interval" for each called CNV. In the Ion Reporter™ Software 5.6 IRGV, the Confidence filter setting (for example, Confidence=10.0) was displayed in the Whole Genome View, however, this information is not relevant to the data from these analyses that used TagSeq Liquid Biopsy CNV-detecting analysis workflows. This is fixed in Ion Reporter™ Software 5.12. Note: The Confidence filter setting has been replaced by a filter chain that can apply a confidence score based on a threshold that is set in the filter chain.
IR-30676	Previously you could use only Analysis name, Sample name, and Project when you searched for Immune Repertoire visualizations. You could not use barcode names for searches of Immune Repertoire visualization results. This is fixed in Ion Reporter™ Software 5.12.

Issue number	Description
IR-34478	<p>The accuracy of fusion assay annotation was improved in Ion Reporter™ Software 5.10, and included a modification of fusion reference files in AmpliSeq Lung Fusion analysis workflows.</p> <p>Details: A number of fusion annotation discrepancies were identified in the AmpliSeq Colon Lung v2 with RNA Lung Fusion single sample analysis workflow and AmpliSeq RNA Lung Fusion single sample analysis workflows. New Ion Reporter™ Software 5.10 versions of these Lung Fusion analysis workflows containing the changes listed below can be identified as they have "w1.1" in their name.</p> <p>Summary of changes: In particular, the fusion assay labeled EML4-ALK.E13A20.AB462411 was found to be inconsistent with the GenBank sequence AB462411, but is more consistent with the breakpoint reported in the literature for COSMIC fusion ID COSF408, so this has been relabeled EML4-ALK.E13A20.COSF408.1.</p> <p>Fusion target sequences for EML4-ALK.E13A20.COSF1062.1, EML4-ALK.E14A20.COSF1064, EML4-ALK.E17A20.COSF1366.1, and EML4-ALK.E17A20.COSF1367.1 have been slightly modified to more accurately reflect the breakpoints reported in the literature citations associated with their COSMIC fusion IDs. These updated sequences have been labeled EML4-ALK.E13A20.COSF1062.2, EML4-ALK.E14A20.COSF1064.1, EML4-ALK.E17A20.COSF1366.2, and EML4-ALK.E17A20.COSF1367.2, respectively.</p> <p>The following assays have been removed: (EML4-ALK.E20A20.COSF409, EML4-ALK.E6aA20.COSF411, EML4-ALK.E6bA20.COSF412, EML4-ALK.E6bA20.COSF412.1, EML4-ALK.E15A20.COSF413, EML4-ALK.E14A20.COSF477, EML4-ALK.E2A20.COSF478, EML4-ALK.E2A20.COSF479, EML4-ALK.E18A20.COSF487, EML4-ALK.E20A20.COSF730, KIF5B-RET.K15R11.COSF1255, EML4-ALK.E6A19.COSF1296, EML4-ALK.E17A20.COSF1366, EML4-ALK.E17A20.COSF1367). For the majority of these assays, a suffixed fusion assay on the panel (for example, ALK.E2A20.COSF478.1 in place of ALK.E2A20.COSF478) more closely matches the breakpoint cited in the literature associated with the COSMIC fusion identifier. The assays EML4-ALK.E6aA20.AB374361 and EML4-ALK.E6bA20.AB374362 match the COSF411 and COSF412 literature breakpoints.</p> <p>This is fixed in Ion Reporter™ Software 5.12.</p>
IR-30397	<p>The Liquid Biopsy analysis Results data columns Mol Depth, Mol Counts, Mol Freq and Detection Limit were not populated in the Liquid Biopsy tab of the Analysis Results for CNV variant rows. This is fixed in Ion Reporter™ Software 5.12.</p>

Issue number	Description
IR-27786	<p>HP-DEL was incorrectly classified incorrectly by TVC evaluator:</p> <p>The indel_as_hpindel parameter controls whether SNP filtering parameters should be applied to non-homopolymer (HP) indels (when disabled, or set to 0) rather than indel filtering parameters. In prior releases to Torrent Suite™ Software 5.4 indel classification as HP-indel, or non-HP indel, had a bug where some HP-indel deletions were incorrectly characterized as non-HP indels. This bug is fixed in Torrent Suite™ Software 5.4 but it affects filtering of affected indels when indel_as_hpindel is disabled.</p> <p>The parameter indel_as_hpindel is enabled by default in Ion 540™/Ion Proton™ germline parameter files and indel filtering is applied to all indels, so this fix will not affect the generic version of this panel. It will also not affect any other panel where this parameter is enabled (for example, AmpliseqExome).</p> <p>In all other generic panels, indel_as_hpindel is disabled by default, as indel filtering is more stringent than SNP filtering, and we expect a possible improvement in sensitivity with a possible increase in false positives. However, in testing somatic panels toggling indel_as_hpindel has no effect on indel calling.</p> <p>A change is seen in Ion 520™/Ion 530™ or Ion 318™ chips that have indel_as_hpindel disabled by default with germline calling. The indel False Positive rate increases and is offset by an increase in sensitivity. Panels that use this parameter in indel germline calling may be affected. A full return to the prior behavior of Torrent Suite™ Software 5.4 was not possible without adjusting indel-filtering parameters. Guidance would be to adjust parameters for indel calling to be more stringent.</p> <p>This is fixed in Ion Reporter™ Software 5.12.</p>
IR-23854	<p>A male or female gender previously must be specified for each sample in a trio analysis in Torrent Suite™ Software. Validation was not performed on sample genders when the trio analysis workflow was automatically launched in Ion Reporter™ Software after it is transferred from Torrent Suite™ Software. This is fixed in Ion Reporter™ Software 5.12.</p>
IR-24539	<p>When you refreshed an Ion Reporter™ Genomic Viewer (IRGV) window, a blank page appears. This is fixed in Ion Reporter™ Software 5.12.</p>
IR-23462	<p>Ion Reporter™ Software does not require you to specify the sample types for RNA/Fusions samples. However, if you did not specify RNA samples as RNA type, you do not get correct results. This is fixed in Ion Reporter™ Software 5.12.</p>

Known issues and limitations in Ion Reporter™ Software 5.12

Issue number	Description
IR-44743	The BAM file named “Demo AmpliSeq Exome CNV case” that is included with Ion Reporter™ Server is corrupted and will fail when used with workflows. Workaround: Select another Exome BAM file to demonstrate workflows until the file is updated in a later version.
IR-44642	<p>In Chrome™ browser version 80 and later, an analysis or sample becomes uneditable for other users in the organization if one user starts an edit process, then closes the browser and exits Ion Reporter™ Software before an edit action is complete. The issue occurs when these actions are done in the following screens.</p> <ul style="list-style-type: none"> • Analysis Results screen – If a user clicks a link to open an analysis, clicks Edit, or clicks Actions > Edit in the Analysis screen, then closes the Analysis Results screen before they save edits • Generate Report screen – If a user clicks Generate Report from the Analysis Results screen, then closes the Generate Report wizard before the final report generation is cancelled or published. • Define Samples screen – In the Samples tab, if a user clicks a sample link (or clicks Define Sample > Manual), to open the Define Samples page, then closes the screen before the Review step is complete and edits are saved <p>In each case, a Chrome™ browser dialog box prompts to the user to click Leave or Cancel when they click to close the screen. The same prompt might open when a user navigates to the Notification screen or Admin screen from any of the affected screens (Analysis Results screen, Generate Report screen and Define Samples screen) Ion Reporter™ Software functionality is not affected if Leave is selected. If however, Cancel is clicked, then the open screen might not close.</p> <p>To resolve this issue, the user who closed the screen must sign into Ion Reporter™ Software, using the same browser without clearing the local storage of the browser. Other users who refresh the affected screens can then make edits.</p>
IR-42804	Analysis Visualization Reports can only be created from the Generate Report button that is available in the IRGV tab in the Analysis Visualization screen. Other tabs in the Analysis Visualization screen in which that button is inactivated may have graph and table contents that may be able to be added to those reports, even though the Generate Report button in those tabs remains inactive. Click on the IRGV tab to generate the report.
IR-33625	For OncoPrint™ BRCA analysis workflows, the default canonical transcript has been changed from NM_007300.3 to NM_007294.3. If a custom transcript set is selected when the preferred BRCA1 transcript is NM_007300.3, the exon numbering for BigDup and BigDel CNV variants are still based on the numbering that is in the NM_007294.3 transcript. As a result, Exon 13 in transcript NM_007300.3 is not used in Exon Deletion/Duplication variant calling.
IR-41252	While viewing a specific chromosomal region, if you zoom in on the VCF track in Ion Reporter Genomic Viewer (IRGV), the drawing that represents the CNV can disappear from the screen. This is due to a calculation in the software of the offset for drawing representations of SNVs, CNVs, MNVs, and so on. The CNV is visible again when you zoom out to and zoom back in multiple times.
IR-41223	In Ion Reporter™ Software 5.4 and 5.6, incorrect copy numbers could be reported in targeted panels that used a VCIB copy number baseline and include chrX genes in the Pseudo-Autosomal Region (PAR,) such as AR. The copy number RAW_CN Genes could be erroneously doubled in male samples that use male baselines with REF_CN=1. This affected final copy number after any correction to tumor cellularity. The issue is fixed in Ion Reporter™ Software 5.12.
IR-41102	In Sample presets, when preset of type Controlled Vocabulary is modified, the Name, Description and Encrypted fields will be audited. The Terms field is not audited for changes. This will be fixed in a future version.

Issue number	Description
IR-37643	In rare cases, Ion Reporter™ Software filter chain filters that are based on matching genotype alleles to population variants may not work.
IR-33725	Column sorting for the "Mol Counts" and "Mol Freq" columns is not correct for ascending sort in the LOD view for liquid biopsy analyses. This is due to the way the data is stored in the internal database.
IR-40315	The OncoPrint™ TCRB-SR DNA analysis workflow w1.2 corrects an issue that affected the reporting of unproductive rearrangements in the OncoPrint™ TCRB-SR DNA assay, w1.0, released in Ion Reporter™ Software 5.10. The issue led to a failure to report unproductive rearrangements that included an in-frame variable gene and joining gene. The issue is estimated to have prevented the reporting of ~10% of unproductive rearrangements. The impact is limited to applications of the TCRB-SR DNA assay that involve the analysis of unproductive rearrangements. You are encouraged to reanalyze data use the OncoPrint™ TCRB-SR DNA analysis workflow w1.2 if you are affected by this issue.
IR-40286	Quality control for CNV calling of BAM files can fail in several ways. When CNV calling fails, analyses will complete but all CNV hotspots will be NOCALLs and no de-novo CNV calling will occur. One or more of the following can cause the CNV calling to fail: MAPD value is greater than "MAPD threshold," aligned reads less are than "min-read-count," percentage of amplicons with no reads assigned greater than "min-non-zero-amplicons-percent," percentage of reads assigned to amplicons less than "min-aligned-read-percent", or the median read count across amplicons is less than "-min-median-reads-per-amplicon."
IR-39761	In Ion Reporter™ Software 5.4, 5.6 and 5.10 chrX calls could be incorrect. In Ion Reporter™ Software 5.10, in samples with "male" gender chrX, the VCF INFO tag can be REF_CN=2, but should be REF_CN=1. Both RAW_CN and CN are affected by the incorrect REF_CN and are reported as one half of the value that it should be. In Ion Reporter™ Software 5.4 and IR 5.6 there is also incorrect handling of chrX but it is more complex and is not fully characterized.
IR-41823	In Ion Reporter™ Software, p-values in Analysis Results in the Summary view in the column named "p-value" are rounded to 5 decimal places (between 0.00001-0.99999) when displayed on the screen. Very small p-values that are less than 0.00001 are rounded to 0.00001 by default when displayed on screen. Very large p-values that are greater than 0.99999 are rounded to 0.99999 by default when displayed on screen.
IR-39002	Variant calling in Ion Reporter™ Software enables specifying minimum callable probability parameter for Limit of Detection calculation. For all DNA-detecting Ion AmpliSeq™ HD and TagSeq analysis workflows, the variant calling now enables users to specify the minimum detachable probability for the calculation of Limit Of Detection (LOD) through the "min_callable_prob" parameter. LOD is defined to be the lowest possible allele frequency in the sample such that the variant is callable with probability higher than the "min_callable_prob" value, when molecular depth is given. In Ion Reporter™ Software 5.10, the values of "min_callable_prob" in the default parameter sets for all DNA-detecting Ion AmpliSeq™ HD and TagSeq analysis workflows were set to be 0.98. In Ion Reporter™ Software 5.12, the values of "min_callable_prob" in the default parameter sets for all DNA-detecting Ion AmpliSeq™ HD and TagSeq analysis workflows are changed to be 0.95 and 0.8, respectively. As a consequence, variant calling in Ion Reporter™ Software may report lower values of LOD than does variant calling in Ion Reporter™ Software 5.10, when the same dataset that uses the default parameter settings is analyzed.
IR-39523	Differences in the predefined filter chains and similar custom filter chains can occur if both gene models (refGene and ensGene) are not taken into account. The gene model used in the analysis must match the gene model that is used by the filter chain.

Issue number	Description
IR-41847	When you visualize multiple Fusion analyses, which differ in their FASTA reference, only the FASTA reference of the first selected analysis data is loaded into the IRGV viewer. This can result in some fusion events, which have a different FASTA file than the first selected analysis, to not be correctly displayed. A workaround is to select the analysis first in which a particular fusion record of interest is present.
IR-41818	To ensure that archives and samples are successful, it is recommended that you increase the session timeout value. You can change the value in the Admin tab, under System Services , in the Configure Services against Session Timeout (Minutes) field. The recommendation is to set 1400 minutes (that is, 24 hours). The maximum limit is 3600 minutes. After the archive process is complete, set it back to the default value.
IR-37255	The on-screen preview of a visualization report can be blank if the size of the data that you add to the report exceeds the memory allocation for the browser. Click Download to download a PDF version of the report, which is not affected. The PDF report will include the visualization files that were not visible in the preview.
IR-36949	When you download Ion Reporter™ Software logs on a Microsoft Windows™ operating system, the Microsoft™ Windows 7-Zip File Manager or WinRAR software are the recommended tools to extract the downloaded ZIP files.
IR-36948	LOD reporting for TagSeq and Ion AmpliSeq™ HD assays is improved in Ion Reporter™ Software 5.10 and later. The new definition of LOD is as follows: Given the molecular depth at the locus, LOD is the lowest possible variant frequency in the sample that can be detected by the system with a true positive rate greater than 98%. Compared with Ion Reporter™ Software 5.6 and earlier, higher values of LOD are expected in Ion Reporter™ Software 5.10. By definition, given the molecular depth, a variant of a frequency that is less than LOD in the sample might still be called by the variant caller, however the variant call might not be detectable in a repeated manner with a probability greater than 98%. In general, the change on the LOD calculation and reporting does not affect the variant calling results. In future releases, the target value of true positive rate used in the LOD calculation and reflected in analysis results will be adjustable to align with requirements for specific applications. Please contact your local Field Bioinformatics Support team with any questions.
IR-34781	The non-targeted fusion detection is not fully supported for Ion AmpliSeq™ HD and TagSeq fusions analysis workflows. The molecular family counts for any non-targeted fusion candidates that are observed in the sample are not computed. The molecular family count is reported as 0, and the Detection call is made based on only the read counts, such as is the case with Ion AmpliSeq™ fusions analysis workflows.
IR-35322	The variantCaller plugin for Torrent Suite™ Software 5.10 includes the use_fd_param=1 for default Ion AmpliSeq™ HD somatic and germline settings. The parameter, when set to true, uses the new FD parameters. Ion Reporter™ Software 5.10 has partial support for FD parameters with the following limitations: <ul style="list-style-type: none"> The use_fd_param parameter is not visible in the Ion Reporter™ Software, but it can be enabled with the import of a variant calling JSON file that includes use_fd_param=1. 2. The related min_ratio_for_fd parameter (default 0.1) is not available in Ion Reporter™ Software, and it cannot be set or changed in Ion Reporter™ Software 5.10.

Issue number	Description
IR-34013	One of the output files of the CNV VCIB output is a tab-delimited text file with column headers: "gene_cn.txt." Ion Reporter™ Software 5.6 and earlier included a column labeled "chr" with entries in each CNV_ID region that use the following rule: take the corresponding contig for the hg19.fasta file and remove the prefix "chr." This would not be robust against other FASTA files, for example, assemblies that do not use "chr." Therefore, in Ion Reporter™ Software 5.10 and later this entry now uses the full contig name, for example "chr1," and so on. The order of the entries in the "Chrom" column is defined by the order in which the contig names are found in the fasta.fai index file. This might break scripts that depend on this column. For example a script that includes entries with the format used in Ion Reporter™ Software 5.10 or earlier, such as "11" will match "chr11" in the FASTA file but in Ion Reporter™ Software 5.10 or later this entry will be the same as the FASTA file. That is, "chr11."
IR-33433	In Ion Reporter™ Software, both the Allele View VCF file and the Locus View VCF file includes values in the FDP and DP fields that are the same; however, each allele is displayed as a single line of record in the Allele View VCF file.
IR-34146	VCF files that contain information for more than one sample are currently not supported using Allele (Proper) view. For example, the VCF output files for tumor-normal analysis workflows contain genotype information for both normal and tumor samples. As a result, the analysis is expected to fail, if the Allele view option is enabled in a custom Ion Reporter™ Software analysis workflow.
IR-34768	<p>If user-created Custom sample attributes share the same name as predefined Ion Reporter™ Software sample attributes, the import of samples might use either the custom or predefined attribute, depending on which attribute type is encountered first by the software. This issue is present in Ion Reporter™ Software 5.6 and later 5.10.</p> <p>Workarounds: The workaround for this issue is to, ideally, not create custom sample attributes for which an Ion predefined attribute is available. Alternatively, if using custom attributes with the same name as an Ion predefined attribute, you can confirm that the attribute values for the samples use the correct attribute after upload, or you can choose to add the values for those attributes after the samples are successfully uploaded.</p>
IR-34789	When files or reports are downloaded from the Ion Reporter™ software, there will be a mismatch in time stamp data between what is shown on the screens in the software and in the downloaded file. This is due to the difference in user and server time zones. The data shown on the software screens will match the client's time zone and the data shown on the downloaded files will match the server's time zone. One exception to this difference is the visualization report where the downloaded PDF will show client time stamp under the 'launched on' field.
IR-30952	<p>The period character (.) is not allowed at the start of the names of various elements in Ion Reporter™ Software. The following elements are not allowed to have a period at the beginning of their name: Project name, Workflow name, Sample name, any analysis Workflow Preset name, Analysis name, CNV baseline name.</p> <p>Originally, this was documented for Project name, only:</p> <p>“If the value of the Project attribute in your sample starts with a period (.), the list of analyses in the Analyses tab will be empty. The workaround is to create a Table Preference that does not include the Project column, and then apply that to your table. See the topic “Set table preferences” in the Ion Reporter™ Software Help for details.</p>
IR-31043	Custom CNV baseline creation is not supported for TagSeq-based Liquid Biopsy analysis workflows in Ion Reporter™ Software.
IR-30955	In Ion Reporter™ Software, the allele grouping (including grouping of hotspots) algorithm was improved to reduce the filtering of true variants. This can sometimes lead to some NOCALLs, due to the way alleles are grouped for evaluation. Variant detection will continue to improve in future Ion Reporter™ Software versions, as new data is gathered and analysis is performed.

Issue number	Description
IR-30957	In the results for TagSeq Liquid Biopsy analyses, CNVs contain only the start coordinate position of the variant in the 5.6 version of software. The Details section for the Immune Repertoire analysis workflow contains some components that are not applicable to the analysis workflow and resulting analyses: Annotation set, report template, parameters.
IR-31031	The Details section for the Immune Repertoire analysis workflow contains some analysis workflow components that are not applicable to the analysis workflow and resulting analyses: Annotation set, report template, parameters.
IR-31022	When visualizing analyses and exporting as SVG, some browsers such as Chrome and Safari may not successfully download the requested file. Workaround: Firefox browser may be used to download CNV Heatmap SVG files successfully.
IR-30985	TagSeq Liquid Biopsy analysis workflows, which detect CNVs, give a p-value but not a "Confidence Score" or "Confidence Interval" for each called CNV. In the Ion Reporter™ Software 5.6 IRGV, the Confidence filter setting (for example, Confidence=10.0) is displayed in the Whole Genome View, but this information not relevant to the data from these analyses using TagSeq Liquid Biopsy CNV-detecting analysis workflows. Note: The Confidence filter setting has been replaced by a filter chain that can apply a confidence score based on a threshold that is set in the filter chain.
IR-30676	You can use Analysis name, Sample name, and Project when you search for Immune Repertoire visualizations. You cannot use barcode names for searches of Immune Repertoire visualization results.
IR-30616	If a hotspot allele X is haplotyped with another allele Y, the variantCaller plugin may call the long haplotyped allele Z that consists of X and Y. As a result, Ion Reporter™ Software will report and annotate the hotspot allele X as negative, although allele X is present as part of the allele Z. For example, if X is an SNP that is haplotyped with another SNP Y, the MNP (Z) that consists of the two SNPs might be called. If you believe this has occurred, check the SUBSET file in the VCF record to determine whether an allele is a subset of a called allele. When this occurs, X will be found as a SUBSET entry in the FORMAT field in the VCF record that called Z.
IR-30650	CNV Baseline Creation for TagSeq analysis workflows is not possible if you use the existing target regions BED file in Ion Reporter™ Software 5.6 or later 5.10. The BED files for TagSeq analysis workflows in the software do not contain CNV amplicons, and therefore should not be used to create a new CNV baseline.
IR-30937	The Liquid Biopsy analysis Results data columns Mol Depth, Mol Counts, Mol Freq and Detection Limit are not populated in the Liquid Biopsy tab of the Analysis Results for CNV variant rows.
IR-29790	If you create Table Preferences to add sample attribute columns in the Samples tab with Ion Reporter™ Software 5.4 or earlier, and you want to access those preferences in Ion Reporter™ Software 5.4 or later, you must do one of the following: If you are using version 5.6, delete the entire Table Preference, then recreate it. If you are using version 5.4, recreate the Table Preference by 1) Remove the added columns. 2) Re-add the columns through the "Select Columns" dialog box.
IR-29352	In Torrent Suite™ Software 5.4, variantCaller plugin checks the tmap parameters used in the mapping step and if the initial tmap program does not have the parameters "-J 25 --end-repair 15 --do-repeat-clip --context", mapping will be re-run before variant calling is invoked. However, these parameters are not used for tmap program in Ion Reporter™ Software for non-Oncomine™ Assay and non-Hi-Q Exome analysis workflows. As a result of these differences, variants called in the Torrent Suite™ Software variantCaller plugin and variants called in Ion Reporter™ Software may not reach parity for some Ion AmpliSeq™ panels, such as CCP, CHPv2, CFTR, AML, Colon-Lung, IDP, non-Oncomine™ BRCA and non-Hi-Q Exome panels.

Issue number	Description
IR-29159	When creating a new annotation-only analysis workflow, OncoPrint™ customers with analysis workflows available to their Ion Reporter™ Software organization will see the availability of the OVAT plugin. Functionality for the OVAT plugin is highly dependent upon the panel used for the assay, so only data from supported panels should be run through analysis workflows containing the OVAT plugin.
IR-24941	When editing a filter chain, canceling during the edit should bring the user back to the filter chain, as it was before the edit with no changes. In Ion Reporter™ 5.4 Software, some edits may persist if a user cancels changes made in filter-chain dialog box. To get back to the same state before edits were made, the page needs to be reloaded to clear all edits made before the cancel.
IR-29709	In some older versions of the Firefox and Safari browsers, the choice of a filter chain will successfully filter the data and list the correct number of variants, but a statement "No Filters Selected" will be present where the filters in the filter chain and their individual filtering results should be listed. A workaround is to use the Chrome browser.
IR-24657	IGV does not load the reads & read coverage track as many times as the number of analyses you select. For example, when you select five analyses with the same input BAM file, then the reads & read coverage track loads only the first one, not all of them.
IR-24539	When you refresh an Ion Reporter™ Genomic Viewer window, a blank page appears.
IR-24080	When setting "UCSC COMMON SNPs" filter to "In", only filters in SNPs that have the "UCSC COMMON SNPs" annotation are filtered, although other variant types are contained in the full database.
IR-23854	A male or female gender must be specified for each sample in a trio analysis in Torrent Suite™ Software. Validation is not performed on sample genders when the trio analysis workflow is automatically launched in Ion Reporter™ Software after it is transferred from Torrent Suite™ Software.
IR-23462	Currently, Ion Reporter™ Software 5.2 does not require you to specify the sample types for RNA/Fusions samples. However, if you do not specify RNA samples as RNA type, you do not get correct results.
IR-23379	In DNA and Fusions analysis workflows, cellularity is not required for NTC RNA samples, but is still required for NTC DNA samples.
IR-24430	For some older analyses when the BAI file is missing from the input BAM folder, then BAM and Reads Coverage tracks do not load in IGV or Ion Reporter™ Genomic Viewer screens. The workaround is to re-run the analyses, which will generate the BAI file and cause successful loading of both the BAM and Reads Coverage tracks.
IR-25095	Due to a design change to improve MyVariants filtering in Ion Reporter™ Software 5.2, for Ion Reporter™ Software 4.0-run analyses (only), All flagged MyVariants in Ion Reporter™ Software 4.0 analyses will appear in Ion Reporter™ Software 5.2 when filtering for either IMPORTANT or IGNORE. MyVariants filtering works correctly for analyses that are run with analysis workflows from Ion Reporter™ Software 4.2 to Ion Reporter™ Software 5.2.
IR-25252	When you create an hg19 annotation set, do not use the annotation source DrugBank version 20150107. Use instead annotation source DrugBank version 1 or DrugBank version 20161212. If you use the DrugBank version 20150107 in an hg19 annotation set, you will not be able to create filter chain of DrugBank for any analysis.

Issue number	Description
IR-31487	<p>A known bug that affected the variant calling module in Ion Reporter™ Software 5.4 caused the results for variants with a very long (>1024 bytes) variant_ID to not be recorded in the VCF file. The variant_ID is a unique key for a variant that, due to improvements to ensure uniqueness for any variant in a sample, was constructed using additional variant data fields in Ion Reporter™ Software 5.2 and 5.4 compared to variant IDs from earlier software versions, which used fewer fields to construct a variant's variant_ID. Rare variants with extremely long (>1024 bytes) variant_IDs were excluded due to exceeding the byte length limit of the variant ID field. The bug was fixed in Ion Reporter™ Software 5.6. Due to this fix, if there is variant in the analysis with a variant_ID length >1024 bytes, then running version 5.2 or 5.4 analysis workflows in Ion Reporter™ Software 5.6 results in the presence of more variants than when the analysis workflows are run in Ion Reporter™ Software 5.2 or Ion Reporter™ Software 5.4. However, if there is no variant with variant_ID length >1024, the number of variants will be the same for the different analysis workflow versions.</p>

Appendix: Changes to Tumor Mutational Burden analyses

Several updates were made analysis workflows that are used for tumor mutational burden analysis in Ion Reporter™ Software 5.12. The updates apply to the OncoPrint™ Tumor Mutation Load - w3.0 - DNA - Single Sample analysis workflow, which supersedes the OncoPrint™ Tumor Mutation Load - w2.0 - DNA - Single Sample analysis workflow from Ion Reporter™ Software 5.10. Both analyses workflows are enable for tumor mutational burden by default. The updates also apply to any DNA analysis workflow that is copied, or copied and edited and has TMB enabled.

In Ion Reporter™ Software 5.12, the reported Tumor Mutational Burden (TMB) score can be calibrated and standardized for a linear curve correlation that uses customizable parameters. In Ion Reporter™ Software 5.10 and earlier, only germline calibration is available.

Algorithmic differences of the TMB processing in the two software versions are as follows:

- In Ion Reporter™ Software 5.12, TMB algorithm processing is primarily based on parameters that can be customized. **Note:** In Ion Reporter™ Software 5.10, parameters such as Variant allele frequency, Minimum base coverage and Variant type could be changed in the TMB filter chain. However, in In Ion Reporter™ Software 5.12, these parameters can be adjusted in the Tumor Mutational Burden parameter settings.
- In Ion Reporter™ Software 5.10, the default filter chain, which filters out potential germline variants, was named “Mutation Load (Somatic Mutations)”. In Ion Reporter™ Software 5.12, the default filter chain name to use with a TMB analysis workflow is “TMB (Non-germline Mutations)”.

Differences between how Quality Control (QC) metrics are reported is as follows:

Metric	Description (Ion Reporter™ Software 5.12)	Description (Ion Reporter™ Software 5.10)
Deamination	The “Deamination score” metric Reports whether QC failed or pass based upon the threshold that is defined in the parameter. Analyses do not fail if metric does not pass. The metric is reported both in Analysis Results in the software and the downloaded output file.	The metric is named “Estimated SNP proportion consistent with Deamination (mainly FFPE)”. The analysis does not fail if metric does not pass. The metric is reported both in Analysis Results in the software and the downloaded output file.
Average coverage	If average coverage is below 150, then analysis completes successfully but TMB is reported as ‘-1’. Note: This threshold cannot currently be changed by the user. It is reported in the output file only.	The Average Coverage metric does not have a defined threshold and does not impact the analysis. The metric is reported both in Analysis Results in the software and the downloaded output file.

Variant counts are calculated as follows:

Variant count type	Description	Ion Reporter™ Software 5.12 criteria	Ion Reporter™ Software 5.10 criteria
Total (germline + somatic) variants	Total number of variants prior to any filtering applied. Calculated from the annotated_variants.tsv file.	Include if variant type is 'SNV' or 'INDEL' or 'MNV'. Note: Multi-allelic positions, for example, 'MNV, SNV' or 'SNV, INDEL', are excluded.	Exclude if variant type is 'REF' or 'NOCALL'. Include rest of the variant types. Note: Multi-allelic positions containing, for example, 'SNV, INDEL' or 'SNV, INDEL, MNV', are also included.
Somatic variants	Number of filtered in variants after applying the TML filter-chain and TML parameter based filters (Ion Reporter™ Software 5.12 only).	Include the variant type(s) specified in the TML parameters. Note: Multi-allelic positions, for example, 'MNV, SNV' or 'SNV, INDEL', are excluded.	TMB algorithm version 2.0: Include if variant type contains 'SNV'. Note: Multi-allelic positions containing 'SNV, for example, 'SNV, INDEL' or 'SNV, INDEL, MNV', are included. TMB algorithm version 2.5: Include if variant type contains 'SNV' or 'INDEL'. Note: Multi-allelic positions containing 'SNV, for example, 'SNV, INDEL' or 'SNV, INDEL, MNV', are included.

The TMB score calculation and calibration are calculated as follows:

	Ion Reporter™ Software 5.12	Ion Reporter™ Software 5.10
Intermediate TMB calculation	<p>IntermediateTMB = (number of somatic variants / number of bases with sufficient base coverage) x 10⁶</p> <p>where:</p> <p><i>number of somatic variants</i>: Total number of variants in the 'somatic_variants.tsv' file that is generated using the filtering criteria defined by the TML parameters in conjunction with the TML filter-chain.</p> <p><i>number of bases with sufficient base coverage</i>: Number of bases present in the exonic regions only or entire genomic regions covered (as specified by the TML parameters). Only loci with sufficient base coverage (the threshold that is also defined in the parameters) are considered.</p>	<p>TMB algorithm version 2.0: IntermediateTMB = (number of somatic non-synonymous SNVs / number of exonic bases with sufficient base coverage) x 10⁶</p> <p>TMB algorithm version 2.5: (number of somatic non-synonymous SNVs and coding somatic indels / number of exonic bases with sufficient base coverage) x 10⁶</p> <p>where:</p> <p><i>number of somatic non-synonymous and coding somatic indels</i>: Derived from the somatic_variants.tsv file that is generated using the filtering criteria defined TML filter-chain.</p> <p><i>number of exonic bases with sufficient base coverage</i>: Number of bases present in the exonic regions covered by the assay to consider. Only loci with sufficient base coverage (>60x) are considered.</p>

TMB calibration	<p><i>If calibration min. threshold ≤ IntermediateTMB ≤ calibration max. threshold:</i></p> <p>TMB = calibration intercept + ((IntermediateTMB - calibration min. threshold) x calibration slope) + calibration min. threshold</p> <p>Otherwise:</p> <p>TMB = IntermediateTMB</p> <p>where:</p> <p><i>calibration min. threshold, calibration min. threshold, calibration intercept and calibration slope</i> are defined in the TML parameter settings.</p> <p>Note: As described in the QC metric section, IntermediateTMB is set to '-1' if average coverage is below 150.</p>	<p>TMB algorithm version 2.0:</p> <p>TMB = IntermediateTMB (i.e. no calibration is applied)</p> <p>TMB algorithm version 2.5: <i>If IntermediateTMB ≥ calibration min. threshold:</i></p> <p>TMB = calibration intercept + ((IntermediateTMB - calibration min. threshold) x calibration slope) + calibration min. threshold</p> <p>Otherwise:</p> <p>TMB = IntermediateTMB</p> <p>where:</p> <p><i>calibration min. threshold: 25 calibration intercept: 0 calibration slope: 1.4637</i></p>
Tumor Mutational Burden Filter Chain	TMB (Non-germline Mutations)	<p>TMB algorithm version 2.0: Mutation Load (Somatic SNVs)</p> <p>TMB algorithm version 2.5: Mutation Load (Somatic Mutations)</p>

The following TMB parameters can be customized. For more information, see the Ion Reporter™ Software 5.12 Help.

TMB parameters		
TMB standardization	<p>Apply the standardization of the observed TMB value to fit a linear curve. For example, fit linear curve against orthogonal TMB method. Standardization is controlled by three different parameters:</p> <p>TMB Standardization: On/Off (Default value: Off)</p> <p>TMB Standardization Factor: Slope Slope for the linear curve fitting</p> <p>TMB Standardization Factor: Intercept Intercept for the linear curve fitting</p>	Not Available
Deamination QC Threshold	Deamination value above which a sample is deemed failed for TMB reporting	Not Available

TMB-Low Threshold, TMB-High Threshold	Defined observed TMB status as 'High', 'Intermediate' and 'Low' based on these thresholds	Not Available
Other user adjustable parameters	Minimum Base Coverage, TMB Variant Minimum Allele Frequency, TMB Variant Region Type, TMB Variant Type, TMB Variant Effect Type, Variant Effect Types to be included for TMB calculation	Not Available

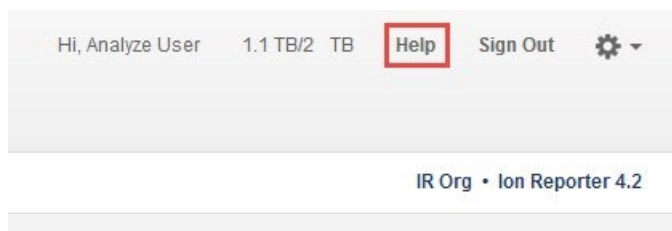
Differences in TMB value between Ion Reporter™ Software versions

The fundamentals of TMB calculation in Ion Reporter™ Software 5.12 are consistent with Ion Reporter™ Software 5.12. However for the analysis workflows that are released in both software versions (for example OncoPrint™ Tumor Mutation Load - w2.0 - DNA - Single Sample analysis workflow (5.10) and OncoPrint™ Tumor Mutation Load – w3.0 - DNA - Single Sample analysis workflow (5.12), differences in analyses results can occur in the following cases:

- The TMB parameter setting is changed from the default setting
- A significant proportion of the somatic variants is comprised of multi-allelic variants. In Ion Reporter™ Software™ 5.12, multi-allelic variants positions are excluded from counting towards number of total as well as somatic variants.

Documentation

You can access product documentation through the help link at the top right of Ion Reporter™ Software. This link opens the help in a new tab (depending on your browser settings).



Software documentation is also available at

<https://www.thermofisher.com/us/en/home/technical-resources/technical-referencelibrary/next-generation-sequencing-support-center/ngs-software-support.html>.

Compatibility with Torrent Suite™ Software

The Ion Reporter™ Uploader (IRU) plugin versions are compatible with the following Torrent Suite™ Software and Ion Reporter™ Software versions.

Version Compatibility Matrix		
Ion Reporter™ Uploader plugin	Compatibility with Torrent Suite™ Software	Compatibility with Ion Reporter™ Software
IRU plugin 5.12.0.38	Torrent Suite™ Software 5.12 and earlier	Ion Reporter™ Software 5.12 and earlier
IRU plugin 5.10.0.32	Torrent Suite™ Software 5.10 and earlier	Ion Reporter™ Software 5.10 and earlier
IRU plugin 5.6.0.41	Torrent Suite™ Software 5.6 and earlier	Ion Reporter™ Software 5.6 and earlier (including Ion Reporter™ Software 5.0 – China, Ion Reporter™ Software 5.2 – China in the future)
IRU plugin 5.4.0.44	Torrent Suite™ Software 5.4 and earlier	Ion Reporter™ Software 5.4 and earlier (including Ion Reporter™ Software 5.0 – China, Ion Reporter™ Software 5.2 – China in the future)
IRU plugin 5.2.1.2	Torrent Suite™ Software 5.2 and earlier	Ion Reporter™ Software 5.2 and earlier (including Ion Reporter™ Software 5.0 – China, Ion Reporter™ Software 5.2 – China in the future)

IonReporterUploader (IRU) plugin

A new version of the IonReporterUploader (IRU) plugin is available: Ion Reporter™ Uploader 5.12.0.38 or later. This version of the IRU plugin is already installed when Torrent Suite™ Software 5.12 is installed or upgraded.

New IRU plugin versions may become available between released of Torrent Suite™ Software or Ion Reporter™ Software. If you need to upgrade the IRU plugin on a Torrent Server of version 5.2 or higher that is connected to the Internet, you can use the off-cycle upgrades described in the Torrent Suite™ Software User Guide.

If your Torrent Server is not connected to the internet, a debian (.deb) package is available from <http://iru.ionreporter.thermofisher.com>. Use of the debian (.deb) package is the only option to update IRU in Torrent Suite™ Software 5.2 and above.

Also available on <http://iru.ionreporter.thermofisher.com> is a compressed directory (ZIP format) version of the IRU plugin for customers who want to continue using Torrent Suite™ Software 5.0 and earlier but want to upgrade IRU.

In addition, there is a ZIP file of the IRU plugin command line version (IRUCLI). This allows you to upload files to Ion Reporter™ Software from a computer other than the Torrent Server. You can also reach <http://iru.ionreporter.thermofisher.com> through the **Download Ion Reporter™ Uploader** link that is in Ion Reporter™ Software when you click Settings > Ion Reporter Uploader.

The most current IRU plugin is always available for download from <http://iru.ionreporter.thermofisher.com>.

Further information

The plugin version format is “plugin_name p.q.r.s”, where “p.q” represents the major/minor version number of the Torrent Suite™/Ion Reporter™ Software with which the plugin is used. The next number “r”, represents the patch number and “s” represents the number of builds done on this patch.

IRUCLI 5.2 and later requires a Java version of 1.7 or higher to function. Therefore, IRUCLI installed on an older Torrent Server running Java 1.6 does not work anymore unless you perform an additional setup.

Ion Reporter™ Uploader plugin configuration

Use the following Ion Reporter™ Uploader configuration setting to transfer to Ion Reporter™ Cloud:

Server: 40.dataloader.ionreporter.thermofisher.com

This is the default shown when configuring an Ion Reporter™ Cloud account in the Torrent Suite™ Software.

Note: The previous server address, 40.dataloader.ionreporter.lifetechnologies.com, will continue to work, but please use the new address going forward.

Ion Reporter™ Server: Update Operating System

To upgrade the Ubuntu™ Operating System software from 14.04 to 18.04 on your Ion Reporter™ Server, contact your Field Bioinformatics Specialist (FBS), Field Support Engineer (FSE), or Field Application Scientist (FAS) for assistance.

Ion Reporter™ Software 5.12 is designed to work with the Ubuntu™ operating system version 18.04, and is not compatible with version 14.04. Messages may periodically appear asking if you want to update your Ubuntu™ software. Do NOT update your Ubuntu™ operating system, if prompted to do so. Doing so without help from support or the Ion Reporter™ Server-specific instructions causes the Ion Reporter™ Software on the server to stop working correctly due to changes between Ubuntu™ versions.

Contact your local Ion Torrent™ Field Bioinformatics Specialist (FBS) for specific instructions and help to upgrade the Ubuntu™ operating system of your Ion Reporter™ Server. Do NOT update the Ubuntu™ operating system of your Ion Reporter™ Server without the guidance and assistance of an Ion Torrent™ bioinformatics representative. For other options and assistance with updating a Ion Reporter™ Local Server, contact your local FBS.

Update Ion Reporter™ Server Software to 5.12

To upgrade Ion Reporter™ Software to 5.12, you must have an up-to-date software license. Upgrades of the Ubuntu™ operating system is part of a separate server support contract.

The first year of software license and hardware support comes with the purchase of the Ion Reporter™ Server (4487118). In subsequent years, a combined Ion Reporter™ Software license and Hardware support contract (ZG10SCIONSERVER) is required to update the Ion Reporter™ Software through the online command line process, or as part of an on-site visit from a support representative to upgrade the software. This yearly license allows upgrading as many times as desired to as many Ion Reporter™ Software versions as are released during that year. Contact your local FBS, FSE or other service representative for online instructions to update Ion Reporter™ Software or to schedule an onsite visit.

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