

Ion Reporter™ Software 5.20 Release Notes

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Revision History

Revision	Date	Description
D	June 17, 2024	Update release notes for new Known Issue for Ion Reporter™ Server and Ion Reporter™ Software: IR-42277.
C.0	December 6, 2023	Updated release notes for Ion Reporter™ Server 5.20. Added the following Known Issues: IR-51583.
B.0	May 15, 2023	Updated release notes for Ion Reporter™ Server 5.20. Added the following Known Issues: IR-49401, IR-49355, IR-49325, IR-47983, IR-49397, IR-49407, IR-IR-49423, IR-49497, IR-49288 and added information about TVC parameters with a copied or edited workflow (IR-49379).
A.0	January 25, 2023	New release notes for Ion Reporter™ Software 5.20.

New Features in Ion Reporter™ Software 5.20

Analysis workflow improvements and feature enhancements for assays and panels

- OncoPrint™ Comprehensive Assay Plus analysis results have the following new features.
 - Reporting of a new Genomic Instability Metric (GIM).
 - Summaries for DNA mismatch repair (MMR) genes.
- Immune Repertoire assays have the following enhancements.
 - An option to select for reporting of T-cell and B-cell clonal populations with missing CDR3 region amino-acid anchors.
 - An option to select for reporting of T-cell and B-cell receptor species with singleton read evidence.
 - A choice to detect the accuracy of B-cell clonal populations with KDE rearrangements (precise alignment or fast-kmer matching).
- ReproSeq™ PGS Kits analysis results have the following new metric.
 - A new quality metric named WavinessSD (WavSD), which is calculated and displayed in tables and graphs.
- OncoPrint™ BRCA Research Assay
 - The software now uses the sample ID amplicons to determine the direction of the BRCA 1 or BRCA 2 gene CNVs for sequencing runs that use the OncoPrint™ BRCA Research Assay to distinguish between whole gene deletions and duplications.

Data and annotation source updates

- Updated hg19 and GRCh38 annotation sources.
- Updated controlled vocabulary for cancer types.

Software assessment service

- Assessment to help optimize Ion Reporter™ Software analysis workflows for new and established assays on a new version of Ion Reporter™ Software. Contact your field service engineer (FSE) or field bioinformatics specialist (FBS) to learn more.

Change to MAF filter in Ion Reporter™ Software

In Ion Reporter™ Software 5.14 and earlier, the MAF filter searches MAFs of all alleles that are associated with a locus. Alleles at the locus found by the filter are returned as filtered results if they fall within the specified MAF range (except $rma=1$). In Ion Reporter™ Software 5.16 and later, the MAF filter searches only the allele(s) that are specified by the genotype (excluding $rma=1$). The allele-based MAF filter results can be downloaded as Filtered Variants. However, the Analysis Results screen in Ion Reporter™ Software 5.16 and later continues to show the locus-based MAF values when no MAF filter is applied. As a result, users may observe some variants which have displayed MAF values within the filtered in range are not returned by the new MAF filter on the Analysis Result screen. To avoid the discrepancy, use allele view, which is not expected to have this issue because there is only one variant allele shown on each line.

End of life for Ion Reporter™ Software 5.10 analysis workflows

Ion Reporter™ Software 5.10 analysis workflows are now retired from the Ion Reporter™ Software 5.20. Version 5.10 analysis workflows will also be unavailable after servers are upgraded Ion Reporter™ Software 5.20. Analyses that are launched through analysis workflows from Ion Reporter™ Software 5.10 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.

Java version required for the Integrative Genomics Viewer (IGV)

Java 8 is required to launch IGV from Ion Reporter™ Software. Java 8 is included with the software. If you are running Java 9 or later, you must have Java 8 also for JNLP to work correctly.

Issues fixed in Ion Reporter™ Software 5.20

Issue number	Description
IR-46409	Messaging and information on RAID status on Ion Reporter™ Server sometimes included an incorrect warning symbol unless an individual drive was selected in the System Services list. This issue is fixed in Ion Reporter™ Software 5.20.
IR-47881	Occasionally on sequencing systems under a high computational load, mapping failed on some blocks of data. These blocks did not get merged into the final results and did not get picked by further processing, which led to gaps in information. This might have caused the BAM file to have missing information or otherwise not be correctly generated. When the failure to properly generate the BAM files was not correctly identified as an error, the analysis might have completed without properly failing, leading to potential missing data. This issue is fixed in Ion Reporter™ Software 5.20.
IR-47889	A RUNX1 insertion, 597dupG, results in a P200A frame shift mutation that is known to be present in a CAP sample was shown to sometimes have a false negative call if there was a signal discrepancy between the two strands. This issue is fixed in Ion Reporter™ Software 5.20.
IR-47872	In the OncoPrint™ Comprehensive Plus - w2.4 (DNA and Fusions – Single Sample, DNA – Single Sample, Fusions – Single Sample, and Annotate Variants – Single Sample) analysis workflows, a stringent SVB BSTRAND threshold caused an INDEL variant call to be suppressed in one of the system-installed controls. This issue is fixed in Ion Reporter™ Software 5.20.
IR-47743	Exons with GAIN or LOSS values were sometimes incorrectly converted to NOCALL when using the OncoPrint™ Comprehensive Plus analysis workflows. This issue is fixed in Ion Reporter™ Software 5.20.
IR-48321	The error message "30012 IOException Occurred" occurred when viewing Alpha Diversity plots in metagenomics analysis results for analyses that use only custom reference files and are launched with workflows that are copied and customized, or are newly created. This issue is fixed in Ion Reporter™ Software 5.20.
IR-48431	The hg19_clinvar_20200329.vcf annotation file contains special characters for some lines. For such cases, the Clinvar track in IGV did not show variant information. This was due to an issue in the IGV application. This issue is fixed in Ion Reporter™ Software 5.20.
IR-47724	When the hostname for an Ion Reporter™ Server was changed, additional "@IonReporterT430" queues were not present because the host cleanup script did not remove the default hostname. This issue had no impact on analyses. This issue is fixed in Ion Reporter™ Software 5.20.
IR-48456	When customizing a report template with an additional Image section, if the image section was placed below the footer of a page, that image section was not displayed on the PDF preview or final PDF. This issue is fixed in Ion Reporter™ Software 5.20.
IR-47672	Three amplicons in the OncoPrint™ BRCA Panel were identified as having lower than typical coverage on one strand. Analysis results that use the OncoPrint™ BRCA Research Somatic - 530 - w3.5 and w3.6 analysis workflows where the threshold for the minimum coverage in each strand is 100. This might have led to false negative calls due to inadequate coverage on the requirement for both strands. The threshold is updated from 100 to 20 in the analysis workflows that are included in Ion Reporter™ Software 5.20.
IR-47870	The batch archival process can sometimes stop running or fail. This issue is fixed in Ion Reporter™ Software 5.20.
IR-47869/ IR-47903	Some NAS storage devices experienced issues when using the device for archiving data. This issue is fixed in Ion Reporter™ Software 5.20.

Issue number	Description
IR-47908	Failed analysis results could occur when the Ion Reporter™ Software analysis workflow OncoPrint™ BRCA Research Somatic - 530 - w3.6 - DNA - Single Sample was used. This issue is fixed in Ion Reporter™ Software 5.20.
IR-48221	In Ion Reporter™ Software 5.18, homozygous whole gene copy number loss had duplicate entries that were incorrectly annotated as "LOH" in the Homologous Recombination Repair HRR summary table. This issue is fixed in Ion Reporter™ Software 5.20.
IR-48071	In the OncoPrint™ Comprehensive Plus - w2.3 (DNA and Fusions- Single Sample, DNA – Single Sample, Fusions – Single Sample, and Annotate Variants – Single Sample) analysis workflows in Ion Reporter™ Software 5.18.2, the stringency level for the gene-level copy loss threshold, maximum fold difference, also known as FD, caused reporting of calls that were likely false-positives. This issue is fixed in Ion Reporter™ Software 5.18.4. This threshold is updated from 0.85 to 0.70. Although 0.70 threshold is more stringent, BAP1 copy loss calls will still be called because the reported FD is 0.5.
IR-47648	The preview for final reports was not available when a custom report template from Ion Reporter™ Software 5.16 or earlier was used for an analysis launched in Ion Reporter™ Software 5.18. The issue occurred because a new report footer section that allowed users to customize a footer logo was added to Ion Reporter™ Software 5.18. The configurable footer section in the report preview was not present in earlier versions of the software. This is fixed in Ion Reporter™ Software 5.20.
IR-44980	In Ion Reporter™ Software 5.16 and 5.18, if a user signed out when an archive or restore archive was in progress, a 500 system error occurred. This is fixed in Ion Reporter™ Software 5.20.

Known issues and limitations in Ion Reporter™ Software 5.20

Issue number	Description
IR-42277	<p>Human Genome Variation Society (HGVS) sequence variant nomenclature guidelines require cDNA level duplication to be annotated as dup (that is, c.1934dup) instead of ins (that is, c.1934_1935insG). In Ion Reporter™ Software 5.20, cDNA duplications (dup) annotation implementation is incomplete. Single-base duplications are annotated as insertions (ins), not dup; multi-base dups are mostly annotated as dup with the exception of a non-left aligned minus strand dup may still be annotated as an ins. However, protein (amino acid) duplications have been functioning correctly since Ion Reporter™ Software 5.10. The dup/ins difference in annotation has no functional difference. To find a variant named as cDNA dup, you may need to look for insertions (ins) with similar positions as the dup, and check Ion Reporter™ Genomic Viewer (IRGV) or Integrative Genomics Viewer (IGV) for nearby sequences to match the dup with the ins variant. It is also recommended that users should rely on protein annotation to identify variants if available. A complete fix is planned to support single-base cDNA duplications, as well as all cases of multi-base duplications, in a future release.</p>
IR-51583	<p>Spare hard drives in RAID array produce a warning state in the RAID status for Ion Reporter™ Server and generate an email warning. Workaround: Warnings that show the Firmware state as “Hotspare,SpunUp:” can be ignored. However, all other RAID warnings and errors should be addressed.</p>
IR-49401	<p>Filtering results might differ between Ion Reporter™ Software 5.18 and Ion Reporter™ Software 5.20, due to an issue with the software. Workaround: To obtain similar results from the MAF filter as in Ion Reporter™ Software 5.18, deselect the "Include unannotated variants" checkbox when you create a filter chain that includes a MAF filter with Ion Reporter™ Software 5.20.</p>
IR-49497	<p>The sequence of the FLT3ITD insertion/duplication can in some cases deviate from the actual duplicated sequence. The position and length of the FLT3ITD insertion/duplication are reported correctly.</p>
IR-49355	<p>If Allele View is used when you view variants in the analysis results table, the Allele Ratio column refers to the ratio between one ALT allele and the REF allele, rather than the ratio between all ALT alleles to the REF allele, as is the case with Locus View. As a result, when multiple alleles are present at a location, it can be difficult to determine if multiple Present alleles are grouped together in Locus View. Workaround: Disregard the Allele Ratio column in the Analysis Results screen, and instead use the Allele Frequency% column to evaluate the prevalence of variants.</p>

Known issues and limitations in Ion Reporter™ Software 5.20

Issue number	Description
IR-49325	<p>Data that is downloaded from visualizations for which filter chains are used contain data from the filter chain that is applied in the Analysis Results screen, instead of the filter chain that is applied in the Visualization screen.</p> <p>Workaround: To download a ZIP file that contains filtered variants for the filter chain that is applied in the Visualization screen, you must first apply and save the filter chain on the Analysis Results screen for each individual analysis, then complete one of the following options.</p> <ul style="list-style-type: none"> • Select the analyses in Analyses table, then click Visualize. In the Visualization screen, click Download > Filtered Variants. The ZIP file that is downloaded will contain the expected data. • Select the analysis or analyses in the Analyses table and then click Actions>Export Filtered. <p>Alternatively, you can click Download>Current Results TSV to download a TSV file that contains data from the Visualization screen.</p>
IR-47983	<p>IonReporterUploader Command-line Utility fails for transfers of BAM files which contain read groups with different flow orders.</p> <p>Workaround: If you use the IonReporterUploader Command-line Utility, and want to transfer BAM files that include multiple flow orders, you must change the current value of cli.multiple.RG.bam.uploads.allowed from FALSE to TRUE. To change the value:</p> <ol style="list-style-type: none"> 1) Sign in to Ion Reporter™ Software, then click Settings>Download Ion Reporter Uploader. 2) Click the filename IonReporterUploader-cli.zip, then download the file to the target computer. 3) Extract the downloaded IonReporterUploader-cli.zip file, and then copy the IonReporterUploader-cli directory to a convenient location on the target computer. 4) In a command-line utility, enter: <pre>cd IonReporterUploader-cli/etc vi IonReporterUploader.properties</pre> 5) Change the value for cli.multiple.RG.bam.uploads.allowed to TRUE.
IR-49423	<p>When you download the results of multiple completed analysis results that use Microbiome, Metagenomics, or Immune Repertoire analysis workflows.</p> <p>Workaround: Download the analysis results individually instead of as a batch.</p>
IR-49288	<p>When the bulk archival/restore process is aborted, all of the entities except for the last one that is in the queue will be aborted. When the archival/restore completes for the last entity, you can resume using the software.</p>

Known issues and limitations in Ion Reporter™ Software 5.20

Issue number	Description
IR-49407	<p>When you copy, or edit, any analysis workflow and upload one of the following target regions BED files, the plan.json file that is associated with the panel does not update the TVC parameter settings if the panel was downloaded to a local computer from AmpliSeq.com.</p> <ul style="list-style-type: none"> · Ion AmpliSeq™ TP53 Panel · CFTR NxGenMDX Panel · Ion AmpliSeq™ Pharmacogenomics Panel · WG00527 <p>Workaround: When you use these BED files from AmpliSeq.com, ensure that you import the BED file directly (with the  AmpliSeq Import option) to Ion Reporter™ Software, instead of uploading the panel from a local computer. Note: You must have an AmpliSeq.com account and access code to complete this procedure.</p> <p>If the files were not downloaded directly to Ion Reporter™ Software and an analysis workflow was previously created for use with the panel, you must do the following to ensure the panel is updated correctly with the TVC parameters from the plan.json file:</p> <ol style="list-style-type: none"> 1) Delete the panel and any analysis workflows that contain the panel. 2) Delete any analysis workflow that is used with the copied or edited analysis workflow. 3) Create a new analysis workflow and in the Reference step, click  AmpliSeq Import to upload the panel files directly from AmpliSeq.com. The analysis workflow is now ready to use with your panel.
IR-49397	<p>If an analysis that uses an OCA Plus analysis workflow contains samples with extreme ploidy, for example, ploidy that is greater than or equal to 11, the analysis might fail.</p> <p>Workaround: Copy an analysis workflow from the OCAplus analysis workflow. In the Copy Number step, select "No Baseline, Don't call CNVs" from the Baseline dropdown menu. This will prevent the analysis from failing, and will also disable CNV analysis results. SNV/InDels and Fusion analysis results will not be affected.</p>
IR-49244	<p>A rare issue can affect analysis results that contain COSMIC variants. The issue can occur for analysis results that use the COSMIC annotation source that is included in Ion Reporter™ Software and contain a reference sequence with multiple base pairs such as complex variants and other types of variants. During the Annotation step of the software analysis, when such called variants are annotated by the COSMIC annotation source that is present in the software, the software is unable to fully match and annotate these called variants with COSMIC variant if the option to include locus, allele or genotype hit level matches is used.</p> <p>Note: The default hit level for COSMIC annotation source is "locus" hit level in all Ion Reporter™ Software pre-installed analysis workflows.</p> <p>Workaround: Use a customized analysis workflow that has the hit level parameter set to "Overlap" for the COSMIC annotation source. The Overlap hit level ensures that the called variants will get a COSMIC annotation based only on genomic positions of the variants, regardless of which reference sequence is used.</p> <p>Important: When you use a custom analysis workflow that has the hit level set to "Overlap," the variant can get annotated with numerous COSMIC variants if there are many COSMIC variants around the genomic position of your variant.</p>

Known issues and limitations in Ion Reporter™ Software 5.20

Issue number	Description
IR-48754	If multiple Immune Repertoire analysis workflows are launched at the same time, one or more analyses may stall or make the server inaccessible temporarily. To avoid this issue, do not launch multiple Immune Repertoire analysis workflows. The Ion Reporter™ Server will become unavailable for up to 24 hours and then become available again automatically. Contact your Field Support Engineer (FSE) if the server does not come back up automatically after 24 hours.
IR-48306	Exon Tile baseline is failing with error "Segmentation fault (core dumped)" in rare cases. In tests, this failure occurs once in 150 baseline creation attempts. Workaround: a relaunch of the baseline creation will likely resolve the failure since this issue rarely occurs.
IR-48869	When using Google Chrome™ browser version 105.0.5195.102, if you are not able to select all samples or analysis from table, upgrade Chrome to version 105.0.5195.125 to resolve the issue.
IR-49228	dbSNP annotations include links to the external home page for the dbSNP database. Due to recent URL changes made by the dbSNP website, when you click on a specific annotation in the dbSNP column in Ion Reporter™ Software, a dbSNP website opens to a 404 page error. Workaround: To open the direct link for the annotation, edit the URL of the main dbSNP web page that is opened for a variant, as shown in this example. If the URL is for example: https://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=rs974924 . Change the URL as shown in this example: https://www.ncbi.nlm.nih.gov/snp/rs974924
IR-49191	If you use some custom scripts to process the VCF file that is downloaded from Ion Reporter™ Software, the VCF file generated by DNA and Fusions analysis workflows for use with the OncoPrint™ Myeloid Assay can contain the two following headers: ##INFO=<ID=SVTYPE,Number=1,Type=String,Description="Type of structural variant"> ##INFO=<ID=SVTYPE,Number=A,Type=String,Description="Type of structural variant"> This is an incorrect VCF file type and will result in an error message. Workaround: Remove the following line from the header and re-run the script that generated the error message to correct the VCF format of the file: ##INFO=<ID=SVTYPE,Number=A,Type=String,Description="Type of structural variant">
IR-44509	You can use the visualizations in the software to confirm deletions and duplications. Whole gene deletion or duplication results can be confirmed in the pre-corrected view. Workaround: Whole gene deletion or duplication results can be confirmed only in the pre-corrected view. That is, if a BRCA1 deletion or duplication (BRCA1DEL or BRCA1DUP) or a BRCA2 deletion or duplication (BRCA2DEL or BRCA2DUP) for a GeneCNV subtype is shown in the Call Details of the CNV variants table, review the visualization to verify the direction of the GENE CNV subtype. Click Pre-corrected and Compare the SampleID (sid) amplicons to the BRCA1 or BRCA2 genes.
IR-47896/ IR-47610	If large numbers of VCR or BAM files are uploaded with the IonReporterUploader command-line utility on a Microsoft™ Windows computer, uploads may stop. Workaround: Add a maximum of five files each time that files are uploaded.

Known issues and limitations in Ion Reporter™ Software 5.20

Issue number	Description
IR-48185	<p>In rare cases when barcoded and non-barcoded samples are combined in the same sequencing run, a NullPointerException error is received when downloading variant files in Ion Reporter™ Software.</p> <p>Workarounds: 1) Delete coverageAnalysis plugin output in Torrent Suite™ Software, then re-upload samples using the IRU plugin and complete the analysis on Ion Reporter™ Software. 2) Upload the BAM file using IRUCLI command-line utility by downloading it from Torrent Suite™ Software, then complete the analysis in Ion Reporter™ Software.</p>
IR-47734	<p>In Ion Reporter™ Software 5.14, changes were made to genotype parsing that impacted some valid VCF files with a genotype that includes a single allele such as those on the Y chromosome, male nonpseudoautosomal regions of X, or mitochondrion. The issue impacts only VCF files that are not generated by Torrent Suite™ Software, Ion Reporter™ Software, or Genexus™ Software. This issue does not impact VCFs generated by these Thermo Fisher Scientific software products.</p>
IR-47689	<p>DRA (Disease Research Area) annotations include links to the external main MESH database main web page. When a user clicks on a specific annotation on the DRA column in Ion Reporter™ Software, a MESH main page opens, instead of the direct link to the page with details for an annotation. Workaround: Use these steps to open the direct link for the annotation: From the main web page that is opened, for example: https://meshb.nlm.nih.gov/search#%2Frecord%2Fui%3Fui=D008577, copy and paste the ID "D008577" into the search box on the page, then select the MESH Unique ID option and click "exact match" to start a search. This will open the direct link for the annotation: https://meshb.nlm.nih.gov/record/ui?ui=D008577.</p>
IR-47612	<p>The preview for visualization reports and final reports preview are not available if you use version 14 of the Safari® browser on macOS® Big Sur. Workaround: Use Google Chrome™ browser or Mozilla Firefox™ browser to view the report previews.</p>
IR-49290	<p>In Ion Reporter™ Software 5.20, some analysis workflows such as those for OncoPrint™ Comprehensive Assay Plus, may take 5 minutes or more to import into Ion Reporter™ Software. Most analysis workflows take approximately 1 minute to import.</p>
IR-49275	<p>PFAM annotations include links to the website for the PFAM database. Due to recent URL changes made by the PFAM website, when you click on an annotation in the PFAM column in Ion Reporter™ Software, the current PFAM website opens and redirects it to the new PFAM website at https://www.ebi.ac.uk/interpro/entry/pfam. The links might not redirect to the new website after March 2023.</p> <p>Workaround: If the redirect action to the new PFAM website does not work, go to the new PFAM website at: https://www.ebi.ac.uk/interpro/entry/pfam to search for the PFAM annotations listed in analysis results in Ion Reporter™ Software.</p>
IR-47706	<p>For Fusion isoforms such as MTAP-CDKN2B_AS1_004.M7C5, some fusion panels use a "Do Not Report" flag internally to suppress false calls caused by background coverage. The final VCF file correctly excludes the output for the call. However, the "Fusion Overall Call" in the top of the Analysis Results screen reports the call.</p> <p>Workaround: We recommend that the call in the "Fusion Overall Call" and intermediate files be ignored unless the call is present in both the Analysis Results fusion data and the final VCF export.</p>

Known issues and limitations in Ion Reporter™ Software 5.20

Issue number	Description
IR-48096	<p>Although you can download results files for metagenomics analyses from the Analyses screen with the Actions> Download menu, the downloaded results will include only the following single plots: Chao1.txt, Shanon.txt, Simpon.txt and observed Species.txt. The content of these files is calculated by from alpha diversity results by using all of the genus, species, and family data combined, and therefore does not match the results shown in the Visualization/Downloads section of the metagenomics analysis results.</p> <p>To download all results files for metagenomics analyses, use the Download results files for all samples link, shown in the Visualization/Downloads section of the metagenomics analysis results.</p>
IR-47647	<p>When using Microsoft™ Internet Explorer browser version 20H2, the status of all accounts on the IonReporterUploader plugin configuration screen are red or 'waiting', even though some of the accounts are available to select in the screen.</p> <p>Workaround: Use Google Chrome™ browser or Mozilla Firefox™ browser to view the correct statuses for accounts when configuring the IonReporterUploader plugin.</p>
IR-47510	<p>Upon launching the AmpliSeq™ Microbiome Health analysis workflows for the same sample, the abundances calculated and reported on the 16S rRNA Gene tab in Ion Reporter™ Software 5.18 and later (Family Relative Abundance% and Gene Relative Abundance% columns in the visualization) might show some minor variation from one run to another. This occurs when there are reads that have multiple best hits upon mapping to the reference; the mapping method used by the software picks any one target at random, which leads to different estimations. 16S regions are very similar to each other, so it is very likely that a given read is equidistant from several reference sequences. As a result, such results are expected. These variations in analysis results do not impact the overall quality of the results.</p>
IR-44638	<p>In Ion Reporter™ Software 5.14 and later, a software update to the Minor Allele Frequency filter which allows users to select the Include Unannotated Variants option causes a discrepancy between the total number of variant rows shown in the table and the counts given in the software for the total number of Filtered In and Filtered Out variants.</p> <ul style="list-style-type: none"> - In Ion Reporter™ Software 5.12 and earlier, the Include Unannotated Variants checkbox for the filter is disabled, so you cannot not select this option, and the Total Variants count does not add up to 100 percent of the variants because unannotated variants are not included in the count of Total Variants. Instead, the Filtered Out and Filtered In variants includes only variants that meet the criteria entered for Filtered In variants, such as a Range of 0.0 to 0.5 plus the Filtered Out variants, but does not include the unannotated variants. <p>In Ion Reporter™ Software 5.14 and later, if the Include Unannotated Variants checkbox is selected for the filter, the count of Total Variants (both Filtered Out and Filtered In) adds up to 100 percent of the variants, as expected because the unannotated variants are included in the Total Variants count. If the Include Unannotated Variants checkbox is not selected, the count of total variants might not add up to 100 percent.</p>
IR-47610	<p>While uploading a large number of multiple VCF files with the IonReporterUploader command-line utility on a windows computer, uploads may stop. Workaround: Add a maximum of 5 VCF files for each file upload.</p>

Known issues and limitations in Ion Reporter™ Software 5.20

Issue number	Description
IR-45389	<p>If exon-levels CNVs are detected in a panel, not all of the CNVs may be represented for that gene in CNV heatmaps. This occurs due to how the software identifies CNVs.</p> <p>In the CNV heatmap for a set of CNVs, for a specific genomic segment, ploidy for variants is analyzed in the ascending order of the variant positions based on the following rules:</p> <ul style="list-style-type: none"> • If a single variant with the highest ploidy is identified, the software analysis will 1) begin with one variant before the segment, and 2) include all of the variants after the segment that have the highest ploidy, and 3) include the single variant that was identified as having the highest ploidy variant. • If there are multiple variants identified as having the highest ploidy, the following variants are included in the analysis: 1) the last identified variant that has the highest ploidy, and 2) one variant before the multiple variants and, 3) all variants after the multiple variants. <p>NOCALL variants are not counted.</p>
IR-31124	<p>When visualizing REFERENCE calls for hotspot alleles in the Liquid Biopsy tab of Analysis Results, some records might have empty values in Mol Counts, Mol Freq, Detection Limits. This happens when consecutive REF calls occur. The empty values are equivalent to values reported in the first record of the group of consecutive REF calls that are found in the genome, and shown in the BED file, or as sorted by the software in consecutive rows of the Analysis Results table.</p>
IR-44334	<p>In audit records, all actions performed by the user are categorized as Add or Modification. Audit Records that include records of a deletion are captured as a Modification. Workaround: Users can view the status of the deleted record as part of the audit record details. To find the record of a deletion, look at the Status field in the audit details. If a record has been deleted, the status will be Deleted.</p>
IR-41058	<p>When using the option to download variants as a current results TSV file from the Analysis Results screen, the column order and variants that are exported in the TSV file are the same as the variants shown in the table of analysis results in Ion Reporter™ Software. However, due to the way data is stored and then sorted in the software, the sort order between the screen and the TSV file might be different. The variants in the TSV file are always sorted by the Locus column.</p>
IR-49232	<p>For completed analyses that use the OncoPrint™ Comprehensive Plus - w3.0 analysis workflows, the value of MSI QC is blank on the Analysis Visualization screen when the MSI QC passes for the sample. However, if the QC for MSI calculation fails, a correct error message is displayed. This issue occurs with the OncoPrint™ Comprehensive Plus - w3.0 analysis workflows that are included in Ion Reporter Software 5.20 and for the OncoPrint™ Comprehensive Plus analysis workflows that are included with previous versions of the software.</p>
IR-42886	<p>When a CNV record from MyVariants is exported, the export might contain comma-separated ploidy values. When importing the record back into Ion Reporter™ Software, after making edits to the file, the Copy Number field is not imported if the CNV record includes multiple copy number values. Workaround: For a successful import, edit the file to either 1) remove all ploidy values or 2) keep a single ploidy value in the column.</p>

Known issues and limitations in Ion Reporter™ Software 5.20

Issue number	Description
IR-45381	The analysis name is incorporated into Immune Repertoire output files. Long sample names, and/or analyses names which are based on long sample names, can prevent some PDF output files from being generated by the Immune Repertoire analysis workflows. The issue is due to limitations on the number of characters that can be used in file names. To resolve this issue, reduce the length of the analysis name, then re-launch the analysis, or reduce the length of the sample name in Ion Reporter™ Software, then reanalyze the sample.
IR-41021	The list of filters for some filter chains that appear in the Filter Options section of the Analysis Results screen are not ordered consistently. However, the results of the filtering for the variants will appear the same each time that a filter chain is applied. This does not affect how filter chains function.
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-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-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	In the IRGV & Generate Report tab, if you zoom in on the VCF track while viewing a specific chromosomal region 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 in to a point where the retrieved data block of data from the index file is different from the previous block of data.
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-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 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-41823	In Ion Reporter™ Software, p-values in Analysis Results in the Summary view in the column named "p-value" are rounded to five 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-39523	Differences in the predefined filter chains and similar custom filter chains can occur if both gene models (refGene and ensGene) are not considered. The gene model used in the analysis must match the gene model that is used by the filter chain.

Known issues and limitations in Ion Reporter™ Software 5.20

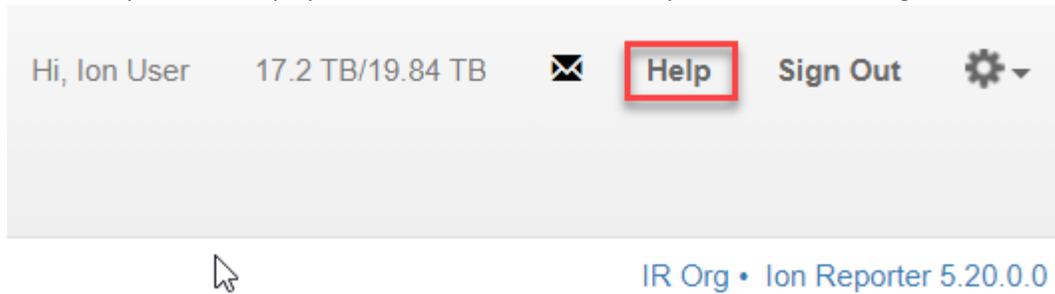
Issue number	Description
IR-34781	<p>The non-targeted fusion detection is not fully supported for Ion AmpliSeq™ HD and TagSeq fusions analysis workflows. The molecular family counts for any non-targeted fusion candidates that are observed in the sample are not computed. The molecular family count is reported as zero, and the Detection call is made based on only the read counts, such as is the case with Ion AmpliSeq™ fusions analysis workflows.</p>
IR-35322	<p>The variantCaller plugin for Torrent Suite™ Software 5.10 and later includes the use_fd_param=1 for default AmpliSeq™ HD somatic and germline settings. The parameter, when set to true, uses the new FD parameters. Ion Reporter™ Software 5.12 and later has partial support for FD parameters with the following limitations:</p> <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. • 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 5.12 and later analysis workflows. <p>Contact your Field Bioinformatics Scientist (FBS) for further assistance if you would like to change these parameters in Ion Reporter™ Software.</p>
IR-33433	<p>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.</p>
IR-34146	<p>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.</p>
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 5.8 and later.</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	<p>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 computer time zone and the data shown on the downloaded files will match the server 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.</p>
IR-29790	<p>If you use Table Preferences to add sample attribute columns in the Samples tab that were created in Ion Reporter™ Software 5.4 or earlier, in order to access those preferences in Ion Reporter™ Software 5.6 or later, you must delete the entire Table Preference, then recreate it.</p>

Known issues and limitations in Ion Reporter™ Software 5.20

Issue number	Description
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™ Software 5.4 and later, some edits might 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 Google Chrome™ browser.
IR-23462	Currently 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. Workaround: Re-run the analyses, which will generate the BAI file and cause successful loading of both the BAM and Reads Coverage tracks. Ion Reporter™ Software 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.

Documentation

You can access product documentation through the help link at the top right of Ion Reporter™ Software. This link opens the help system in a new tab, based 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

In Ion Reporter™ Software on Connect requires an access code is required to configure user accounts for the IonReporterUploader plugin and to use Ion Reporter™ Software command-line utility (IRUCLI).

To create an access code for use with the IonReporterUploader plugin setup and to use Ion Reporter™ Software command-line utility (IRUCLI):

1. Sign into Ion Reporter™ Software.
2. Click  **(Settings) > Manage Tokens**.
3. Click **Set New Access Code**, then enter an access code in the New Access code field.

The access code must contain at least six characters. The maximum length of the access code is 50 characters.

4. Select an expiration time in the Access code Age dropdown menu, then click Save and Generate.

Note: The IRU token is for use with the IonReporterUploader command-line utility and is not required for this procedure.

The access code that you must use to set up the IonReporterUploader is shown in the **Manage Tokens** dialog box and is available on the clipboard. Save this access code for use in future account setups. Alternatively, you can reset the access code as needed.

The IonReporterUploader 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
Ion Reporter™ Uploader plugin 5.20.0.6 or later	Torrent Suite™ Software 5.18 and earlier	Ion Reporter™ Software versions 5.12, 5.14, 5.16, 5.18, and 5.20
Ion Reporter™ Uploader plugin 5.18.0.22 or later	Torrent Suite™ Software 5.18 and earlier	Ion Reporter™ Software versions 5.10, 5.12, 5.14, 5.16, 5.18

IonReporterUploader plugin

A new version of the IonReporterUploader plugin is available: Ion Reporter™ Uploader plugin 5.20 or later.

New plugin versions might become available between released of Torrent Suite™ Software or Ion Reporter™ Software. If you need to upgrade IonReporterUploader plugin on an Ion™ Torrent Server of version 5.8 or later that is connected to the Internet, you can use the off-cycle upgrade procedures 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 IonReporterUploader plugin in Torrent Suite™ Software 5.8 and later.

Ion Reporter™ Software command-line utility (IRUCLI)

A ZIP file of the Ion Reporter™ Software command-line utility (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 IonReporterUploader plugin and Ion Reporter™ Software command-line utility (IRUCLI) are 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 “r” represents the patch number and “s” represents the number of builds done on this patch.

IRUCLI 5.6 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.

IonReporterUploader plugin configuration

Use the following IonReporterUploader plugin configuration setting for Ion Reporter™ Software accounts to transfer data to Ion Reporter™ Software on Connect:

Server: 40.dataloader.ionreporter.thermofisher.com

This is the default shown when configuring an Ion Reporter™ Software on Connect 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.

Impact to TVC parameters with a copied or edited analysis workflow (IR-49379)

When you copy an analysis workflow from any workflow, the Torrent Variant Caller (TVC) parameters will be the same as the workflow which was copied in most cases. In a small number of cases, if a new target regions BED file that was imported from AmpliSeq.com is selected, the TVC parameters are set based on the values that are contained in the imported files. Panels that can include TVC parameters are Community Panels, Ready-to-Use Panels, and custom panels from AmpliSeq.com.

When you copy an analysis workflow from any customized workflow, and then change the target regions BED file, if the selected target regions BED file was from a system-installed analysis workflow or imported from AmpliSeq.com, the TVC parameters and the values associated with the BED file (if any) are used to update the TVC parameters of the edited workflow. Otherwise, the parameters associated with the analysis workflow that is copied will be used to update the parameters of the analysis workflow that is edited (with the selection of a target regions BED file).

If a new target regions BED file will be selected, use **Actions -> Copy** instead of **Actions -> Edit** to ensure that the correct TVC parameters are used.

Ion Reporter™ Server: Update Operating System

Ion Reporter™ Software 5.20 is designed to work with the Ubuntu™ operating system version 18.04 and is not compatible with earlier Ubuntu™ operating system versions. Messages can 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.

To upgrade the Ubuntu™ Operating System software 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.

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 an Ion Reporter™ Local Server, contact your local FBS.

Update Ion Reporter™ Server Software to 5.20

To upgrade Ion Reporter™ Software to 5.20, 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.

The information in this guide is subject to change without notice.

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