



Release notes

Agilent Alissa Reporter v1.3

Agilent Alissa Reporter is an intuitive and streamlined next-generation sequencing (NGS) data analysis solution. This cloud-based software-as-a-service (SaaS) platform delivers high-performance detection of germline and somatic variants through integrated genome browsing, and a built-in quality control (QC) dashboard.

Integration of the NVIDIA Clara Parabricks platform with the secondary analysis pipelines enables users to benefit from fast, panel-specific data analysis solutions. Alissa Reporter completely automates data upload, analysis, and export, including direct export from your Amazon Web Services (AWS) account.

Key features and functionality

New Applications

The following new RUO applications are made available to customers with the release of Alissa Reporter v1.3:

- Alissa Reporter for SureSelect Cancer CGP DNA
- Alissa Reporter for SureSelect Cancer CGP RNA
- Supplementary ITD (internal tandem repeat) analysis enabled in somatic Human All Exon v7/v8 and custom DNA applications

In this release, Alissa Reporter will support the following Research Use Only (RUO) data analysis applications:

Analysis Type	SureSelect Panel	Description	Part Number
Germline	Human All Exon V7	- SNV/indel	G5395AA
	Human All Exon V8	- Exon-level CNV calling	G5396AA
	Custom panels	- ITD	G5398AA
Somatic	Human All Exon V7	- SNV/indel	G9591AA
	Human All Exon V8	- Gene-level CNV calling	G9592AA
	Custom panels	- ITD	G9593AA
	Cancer CGP DNA	- SNV/indel, CNV, TL,	G5393AA
	Cancer CGP RNA	RNA gene fusion,	G5394AA
	Cancer CGP DNA & RNA	TMB, MSI	M5771AA

Optimized algorithms for variant analysis

SureSelect Cancer CGP DNA

- The SSEL Cancer CGP DNA application supports SNV/indel calling (up to 0.1% allele frequency, verified up to 5%). Parameters such as allele frequency and variant calling quality are calculated for each SNV/indel variant call.
- In case of the presence of a matched/unmatched reference sample, a 'tumor/normal' mode can be used for analysis, which will remove germline variants (SNV/indel) identified in the reference sample.
- The application supports somatic CNV calling down to gene-level resolution (using a reference sample). Parameters such as copy number, mean log2 ratio, and calling quality are calculated for each CNV variant call.
- The application supports both inter- and intra-chromosomal somatic translocation calling (without using a reference sample). Parameters such as variant frequency and variant call quality are calculated for each translocation call.
- The application generates a single VCF file containing SNV/indel, CNV and translocation variant calls and all relevant calling parameters. Also multi-sample VCF at run level can be produced if required.
- The application supports post-analysis filtering to classify certain variants as 'retained' or 'discarded' based on provided cut-off values (e.g. minimum allele frequency)
- The application supports Micro Satellite Instability (MSI) calculation for both 'tumor-only; and 'tumor/normal' scenarios.
- The application supports Tumor Mutation Burden (TMB) calculation for both 'tumor-only' and 'tumor/normal' scenarios
- The application calculates and reports relevant Quality Control (QC) metrics (e.g fraction of unique reads) at run level and sample level (both for the target and reference sample).
- The application calculates and reports "inaccessible regions" – regions below a certain coverage threshold for SNV/Indel calling or regions that cannot be used for CNV calling.
- The application supports all relevant Cancer CGP SureSelect chemistries (i.e. XTHSV2). The application supports applicable deduplication methods (positional and MBC single/hybrid/duplex consensus).
- The application can handle large samples well above the recommended sequencing depth. Samples with more than 500M reads are down-sampled to 500M reads prior to initiating the actual sample analysis. Down-sampling has successfully been tested with samples up to 2x100GB zipped FASTQ input files.

- The application can take a dedicated type ("targeted gene panel") of in silico filters ("virtual panels") into account.
- The application is compatible with existing general functionality such as:
 - Downloadable files including VCF file, PDF report, etc.
 - QC dashboard.
 - SNV/Indel pileup.
 - CNV viewer.
 - Translocation viewer.
 - Automated or on-demand transfer of all relevant analysis results to Alissa Interpret (including TMS and MSI results).
 - Automated import of sequencing files from and export of results to Amazon Web Services (AWS).

SureSelect Cancer CGP RNA

- The application supports somatic RNA fusion calling. Parameters such as variant frequency and variant call quality are calculated for each fusion variant call.
- The application generates a VCF file containing RNA fusion calls represented as translocations. The application supports the generation of a multi-sample VCF at run level.
- The application supports post-analysis filtering to classify certain variants as "retained" or "discarded" based on provided cut-off values (e.g., minimum variant frequency).
- The application supports exon skipping determination for MET exon 14 and EGFR vIII.
- The application calculates and reports relevant Quality Control (QC) metrics (e.g., fraction of unique reads).
- The application calculates and reports "inaccessible regions" – regions that cannot be used for RNA fusion calling.
- The application supports all relevant Cancer CGP SureSelect chemistries (i.e. XTHSV2). The application supports both positional as "no dedupe" deduplication methods.
- The application can handle large samples well above the recommended sequencing depth. Samples with more than 200M reads are down sampled to 200M reads prior to initiating the actual sample analysis. Down-sampling has successfully been tested with samples up to ~2x10GB zipped FASTQ input files.
- The application can take a dedicated type ("targeted gene panel") of in silico filters ("virtual panels") into account.

The application is compatible with existing general functionality such as:

- Downloadable files including VCF file, PDF report, etc.
- QC dashboard.

- RNA fusion viewer.
- Automated or on-demand transfer of all relevant analysis results to Alissa Interpret (including exon skipping results).
- Automated import of sequencing files from and export of results to Amazon Web Services (AWS).

SureSelect All Human Exon v7/v8 and Custom assays.

- The SSEL All Human Exon v7/v8 and SSEL custom algorithms support germline and somatic copy number variant (CNV), insertion and deletion (indel), single nucleotide variant (SNV) and ITD calling.

Implementation of Agilent's curated Alissa Annotation Server

- All annotation is now coming directly from Alissa Annotation Server and are no longer being fetched from Alissa Interpret.
- Annotations are improved, less error prone (and more in sync with Alissa Interpret). This includes, but is not limited to:
 - SNV/indel table variant information such as variant impact (e.g., "non-synonymous").
 - The SNV/indel "Annotation tab".
 - All gene/transcript annotation in variant tables (e.g., gene symbols in the CNV table).
 - All gene/transcripts information in visualizations (e.g., gene and transcript structures in the SNV/indel pileup).
 - Design annotation during import of (custom) designs. All regions and/or probes are now annotated on-the-fly against Annotation Server sources.
- All in silico filter content is based on the Annotation Server (custom) design annotation.
- There are no backwards compatibility issues. The current annotation that is fetched from Annotation Server is identical to the annotation that was historically retrieved through Alissa Interpret.
- Performance improvements have been observed by switching from Alissa Interpret to Annotation Server as an annotation source.
- All references to Alissa Interpret with respect to annotation (user interface, downloadable files, help text, ...) have been replaced by applicable references to Annotation Server (and specific used datasets).
- Switch to new variant storage architecture
- All variants (SNV/indel, CNV, translocations, ...) are now stored in the "Parquet" technology.
- The new variant storage solution is robust for increasing volumes, faster and more flexible.

Superior visualization tools to aid review of called variants in context

- SNV/indel/ITD and CNV variants are visualized in a sortable and filterable table (for example, on variant type (SNV/indel), CNV size, and quality).
- The CNV table contains an additional column with the stop position and displays a button to directly open the CNV viewer to assess the CNV calling data.
- The SNV/indel pileup and CNV viewer views can be synchronized within the same sample or across different samples.

Built-in QC dashboard to provide relevant metrics

- The relevant QC metrics are calculated and graphically represented per sample in a dedicated 'sample QC dashboard'.
- The sample QC dashboard and run QC dashboard offer trending information, allowing evaluation of the QC metrics over time.
- The QC metrics in the QC dashboards include preset minimum and recommended thresholds that generate errors and warnings, respectively, when a sample fails to meet a threshold.
- Alissa Reporter calculates and reports inaccessible regions
 - regions below a certain coverage threshold that are inaccessible for data analysis.
- Before this release, the QC metrics and plots related to the "Mapping", "Enrichment", and "Uniformity" for custom applications use the stripped BAM on the QC tiles and plots. Now, the full BAM is used on the QC tiles and plots. The stripped BAM values are shown as secondary values on the QC tiles.
- 200x, 300x, 400x, and 500x values are added as secondary values to the "Fraction of targeted bases at 100x coverage" QC tile for all somatic applications. Changes in underlying tools are needed to calculate these additional values. As a result, all reported values (e.g., 10x, 20x etc) are slightly higher (1-2%) than before.
- The SNV/indel results table is now sortable on the variant allele frequency (AF) column.
- The QC metric "fold 80 base penalty" is added to the uniformity category in the QC dashboard. This metric is defined as the fold change of non-zero read coverage needed to bring 80% of the targeted bases to the observed mean coverage.

Useful result output files

- Variant results can be exported into multiple human-readable format files, including VCF (v.4.3) files.
- The creation of multi-sample VCF files is supported by all applications.
- The QC metrics file contains both primary and secondary QC metrics values and reports the analysis options used in the footer.
- A new output file (gene/exon dropout file) gives an overview of the fraction of exons/genes that have zero, < 20X, or < 30X coverage.
- Other output files including the inaccessible regions, QC metrics, BAM and BAI files, and summary of the analysis results, can be batch exported from the software.

Integration to Data Interpretation tools

- Users with an active Alissa Interpret account have the option to link their Alissa Interpret instance (v.5.3 or higher) to their Alissa Reporter account for automatic transmission of analysis results.
- Availability of downloadable analysis results (VCF file, QC metrics files, inaccessible regions file) enables transfer to data interpretation tools of the user's choice.

Automated import/export via Amazon Web Services

- Users with an AWS account have the option to link their account to their Alissa Reporter account using an "AWS policy" (no password use required).
- After successful configuration of the AWS account, an automated process that uploads FASTQ files from AWS directly to the user's Alissa Reporter account takes place, it is possible to start analysis automatically after upload. A user is also able to export analysis results, such as VCF or PDF reports, to the Alissa Reporter user's AWS account after analysis has completed.
- AWS automatic upload allows an existing in silico filter to be specified in an attributed file (.yaml) prior to import, which will be applied for the analysis in Alissa Reporter. Different in silico filter can be specified for different sample groups and can be indicated in one single attribute file.
- Additional file format checks are introduced, to be in line with the file format checks used in the "attribute file upload" feature.
- The AWS automation now supports non-standard FASTQ file names, like the "attribute file upload" feature.
- The optional analysis settings 'interpret' (to send the results to your Alissa Interpret instance), and 'email' (to receive an email when analysis completed) can now be specified in the summary.yaml file.

Intuitive upload wizard

- Users can upload a sequencing run containing up to 768 zipped FASTQ files (< 100 GB per file) in a single action (from Illumina HiSeq 3000/4000, or Illumina NovaSeq 6000 (named before v1.3.0: Illumina NovaSeq) and NextSeq 1000/2000).
- Users can select an attribute file (YAML format) in the upload wizard (together with the FASTQ files). This attribute file should contain the run and sample information that is normally filled in manually during upload.
- The attribute file upload feature uses a YAML file format, like AWS automation file format.
- When clicking the "parse" button, the provided YAML file is parsed, and all the fields are populated in the upload wizard. A user can still change the field values afterwards.
- File format checks and consistency checks are in place, similarly to the checks that are performed when filling out the upload wizard fields manually.
- The attribute file upload feature is available both via the local upload or S3 upload in the upload wizard.
- The attribute file upload feature is not compatible with newly defined in silico filters and sample groups.
- The attribute file upload feature is compatible with non-standard FASTQ file names. A user can define (multiple) R1, R2, R3 files per sample that do not follow the standard Illumina naming format.
- The attribute file upload feature is compatible with the "resume upload" feature.
- Additional file format checks are introduced, to be in line with the file format checks used in the "attribute file upload" feature.
- The AWS automation now supports non-standard FASTQ file names, like the "attribute file upload" feature.
- The optional analysis settings 'interpret' (to send the results to your Alissa Interpret instance), and 'email' (to receive an email when analysis completed) can now be specified in the summary.yaml file.

Credit system functionality

- The first re-analysis of a successfully analyzed sample does no longer consume a credit. The second and subsequent analyses do consume credits.
- Custom design imports from SureDesign.
- When importing custom designs from SureDesign, partial BED files (*_partial.bed) are now supported. It is now possible to upload custom designs including a Covered.bed file and Regions_partial.bed file, as well as custom designs including a Covered_partial.bed file and Regions.

bed file. In other cases, custom designs including partial design files cannot be imported.

Textual

- “Gender” is replaced with “sex” throughout the application. The “Gender” entries in the AWS automation files are still functional for backward compatibility reasons. The words “Female” and “Male” are no longer used; XX and XY are now used for sex assignment.
- Small changes to the user interface, help text snippets, warning messages, and error messages to clarify existing functionality or expand for new functionality. A non-exhaustive selection of changes:
- “Sequencer” is replaced by “sequencing instrument” throughout the application.
- The PDF report limitations have been updated for all existing and new applications.
- Analysis option help texts have been updated to clarify whether they are applicable for germline and/or somatic and for SNV/indel, CNV or translocation calling.

Other

- In case an in silico filter is used, the gene list to search or filter on (e.g. SNV/indel variant overview or “find gene” in CNV viewer) is now limited to the genes defined in the in silico filter.
- Searching or filtering on a gene (e.g., SNV/indel variant overview or “find gene” in CNV viewer) is now case-insensitive.
- The analysis options are re-ordered and grouped per variant type on the sample view page, the PDF report, QC metrics file, and analysis parameter settings popups.
- Users can provide feedback via an online questionnaire that is available in the footer of the application.

Deployment availability

European deployment

[Alissa Reporter–Europe](#) (located in Ireland) is available for all commercial activities outside of the USA, Australia, and Asia Pacific.

USA deployment

[Alissa Reporter–US](#) (located in N. Virginia, USA) is available for all commercial activities within the USA.

APAC deployment

[Alissa Reporter–APAC](#) (located in Sydney, Australia) is available for all commercial activities within Australia and Asia Pacific.

Alissa Reporter new account set up is handled by Agilent support personal. Upon customer account creation, a user with elevated permissions (for example, 'admin' role) has additional functionality within a certain account, including but not limited to:

- Creating new logins
- Changing passwords of existing logins
- Managing roles of existing logins
- Creating and managing labels
- Creating and managing in silico filters

Software system requirements

Supported browsers

Agilent Alissa Reporter supports recent versions of the following web browsers:

- Google Chrome (v119 and higher)
- Safari (V17.1 and higher)
- Mozilla Firefox (v119 and higher)
- Microsoft Edge (v119 and higher)

Data analysis limitations

- Alignment of reads in pseudogene, homologous, or other difficult-to-map regions are not supported. Multi-mapping reads are removed during analysis and only primary reads are retained [AR-130] [AR-590].
- Custom designs imported prior to the release of version 1.2.0, are not compatible with the analysis of the mitochondrial chromosome (the analysis will fail). Re-importing the design allows analysis of the mitochondrial chromosome [AR-5021].
- In very rare cases, the variant annotation (e.g. exon position) shown in the SNV/indel table and what is observed in the SNV/indel pileup transcript track do not match (only observed for frameshift variants for PKD1 gene). This is related to inaccuracies in the annotation sources (Platform DataSet 37 in Alissa Interpret) used within Alissa Reporter. See this link for more details on Alissa Interpret [AR-947].

Performance limitations

- In rare cases, deleting a large batch of sequencing runs in a single operation can fail due to a timeout [AR-389].
- The Cancer CGP RNA application allows 2x100GB FASTQ files to be uploaded but has only been tested with files up to 2x7.5GB. This should cover all relevant use cases.
- In case a tumor and reference sample of both close to 100GB are analyzed together in tumor-normal mode, the analysis might fail [AR-2849].

Annotation limitations

- The Alissa Platform gene endpoints do not allow to retrieve gene information for those NCBI genes that lack any kind of nomenclature annotations (i.e., HGNC ID, approved gene symbol, previous symbols, ...). As such they are not displayed in Alissa Reporter.
- Some fields on the SNV/indel variant detailed view can be missing for old analyses due to the switch to the Annotation Server (HGVS protein, HGVS genomic, and Gene ID HGNC).

Other minor limitations

- When the list of enabled applications is modified or custom applications are activated/inactivated, the web application is not perfectly in sync for the user performing the action and for users that are concurrently using the web application (in the same account). The following limitations hold [AR-1774]:
- Users having the manage options open when a custom design is being inactivated (by another user) can still modify the options for the inactivated custom design during their current login session.
- The “Manage custom designs” button is out-of-sync for concurrent users until they close and reopen the “My account” view (e.g., a user is on the “My account” view when the “Custom DNA Germline” application is disabled).
- For an active user, the enabled assay dropdown lists are out-of-sync for opened views when a concurrent user inactivates a custom design (e.g., dropdown list when filtering in the sample overview).

- For an active user, the list of enabled applications (after they loaded it) is out-of-sync when a concurrent user enables/disables an application:
- Newly enabled applications will not show up for upload, give an error for analysis, and will not show up on the home page or in dropdown lists.
- Newly disabled applications will not be removed from the views and will give server errors when trying to apply during upload or analysis.
- The following concurrency limitations exist for the “resume upload” functionality [AR-1773]:
- Multiple concurrent resume uploads of the same run for which one of concurrent threads fails, might put the run as “failed” although one of the threads succeeded.
- When user A is resuming an upload of a run and user B is editing or removing existing (succeeded) samples from the same run at the same time, starting the analysis automatically after completing the resume run upload might fail or the analysis might start but result in a failed analysis.
- When an assay is disabled of a run that is being resumed and you want to start the analysis, the error mentions insufficient credits instead of the analysis being disabled.
- The scroll bar of the footer on the home page may disappear when changing the screen size from a full-width screen to a small width screen.

Please contact our support team for any questions at:

Informatics_Support@agilent.com

For Research Use Only. Not for use in diagnostic procedures. Alissa Interpret is a Class I Exempt Medical Device in the US, a CE-IVD in the EU and a Class I IVD in Canada and Australia. PR7001-2076

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