Release notes

Agilent Alissa Reporter v1.2.0

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, a built-in quality control (QC) dashboard, and seamless connectivity to the Agilent Alissa Interpret software. 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

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).
- The upload wizard supports automated lane merging of samples split across different lanes on applicable sequencing instruments (for example, Illumina NextSeq).
- The upload wizard enables 'resume upload' in case a sequencing run fails to upload.

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 Exon-level CNV calling	G5395AA
	Human All Exon V8		G5396AA
	Custom panels		G5398AA
Somatic	Human All Exon V7	SNV/indel Gene-level CNV calling	G9591AA
	Human All Exon V8		G9592AA
	Custom panels		G9593AA

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Trusted Answers

Optimized algorithms for variant analysis

- The algorithms support germline and, as the newest feature, somatic copy number variant (CNV), insertion and deletion (indel), and single nucleotide variant (SNV) calling.
- Samples can be analyzed with configurable and preconfigured alignment and calling parameters.
- Analysis options provide multiple duplication methods including positional, single molecular barcode (MBC) consensus, or duplex MBC consensus.
- SureSelect QXT, XT/LI, XTHS, XTHSv2, and newest SureSelect XTHSv2 without MBC, chemistries are supported in full.
- Users can apply a virtual in silico filter custom panel that limits the results shown to a subset of genes/regions/variants that are part of the original assay kit.
- Results include annotation of variants using Alissa Annotation Server annotation-rich sources.
- Somatic SNV/indels can be called down to a variant allele frequency of 0.1% and detect somatic CNVs down to gene level.
- Somatic applications: in case of the presence of a matched or unmatched reference sample, a tumor-normal mode can be used for analysis which will remove germline variants (SNV/indel) identified in the reference sample.
- Support of SNV/indel calling on the mitochondrial genome for all applications and, when chosen, will run a dedicated mitochondrial analysis pipeline. The full mitochondrial spike-in panels to be analyzed.

Superior visualization tools to aid review of called variants in context

- SNV/indel 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.
- A detailed variant view (including additional information per variant) is available for SNV/indel and CNV.
  A link to the detailed view is available from within the overview table.
- The SNV/indel pileup and CNV viewer have a cytoband track that displays cytoband information.
- SNV/indel variants have a detailed annotations tab available, populated from Alissa Interpret.
- SNV/indel variants can be visually inspected using an integrated genome browser (SNV/indel pileup), including read pileup. A link to the SNV/indel pileup is available from within the SNV/indel table.
- The SNV/indel pileup allows visualization of secondary alignments and the mapping quality (MapQ).
- CNV variants can visually be inspected using an integrated genome browser (CNV viewer). A link to the CNV viewer is available from within the CNV table.
- The SNV/indel pileup and CNV viewer views can be synchronized within the same sample or across different samples.
- Retention time for visualization (for example, SNV/indel pileup, CNV viewer) has been increased to six months.

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 relevant QC metrics are graphically represented per sequencer run in a dedicated 'run QC dashboard'. The run QC dashboard visualizes summary statistics (minimum, mean, maximum) of the QC metrics for the samples included in the run.
- 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.
- Two additional deduplication QC metrics are calculated for all applications (for example, fraction of PCR-duplicated reads and fraction of optical-duplicated reads).
- The fraction of bases at 20X coverage (germline) and fraction of bases at 100X coverage (somatic)QC metrics are enabled to also sow secondary values (for example, 10X, 30X, 40X, 50X, etc).
- The QC metrics calculate QC dropout and AT dropout values for all applications.
- For custom applications, QC metrics are calculated on both the original/raw BAM file and the BAM file generated after removal of off-target reads.

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.

Seamless integration with Alissa Interpret

- 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.
- After successful configuration of the Alissa Interpret link. An automatic transmission of analysis results (VCF file, QC metrics files, inaccessible regions file) to Alissa Interpret can take place at the end of sample analysis in Alissa Reporter.
  An on-demand transmission of analysis results (VCF file, QC metrics file, inaccessible regions file) to Alissa Interpret can take place for successfully analyzed samples.

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.

Custom design import from SureDesign

- Alissa Reporter enables the import of SureSelect custom designs, including published community panels and legacy catalog panels, from SureDesign into any account using existing SureDesign credentials.
- After a SureSelect DNA custom design is imported, a distinct analysis application is created allowing users to adjust certain analysis parameters.
- A tier level is calculated for each SureSelect DNA custom design upon import and is visualized on the detailed custom design view.

Convenient credit system functionality

- A credit system maintains the credit balance (for example, purchased analysis versus used analysis) per catalog application or per tier, in the case of custom applications.
- The credit/analysis balance is consultable for each user and allows at-a-glance run stats. The system notifies users in the Alissa Reporter interface and by email when the credit balance of certain applications or tiers falls below a warning threshold.

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 a 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 (v108 and higher)
- Safari (V15 and higher)
- Mozilla Firefox (v107 and higher)
- Microsoft Edge (v107 and higher)

Known limitations

- Alignment of reads in pseudogene, homologous, or other difficult-to-map regions is not supported. Multi-mapping reads are removed during analysis and only primary reads are retained.
- In very rare cases, the variant annotation, for example, 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. Click the following link for more details on Alissa Interpret.
- The Human All Exon V7/V8 germline and custom DNA germline application analysis can fail if CNV analysis is set to "Yes" and one of the samples included in the run has a very poor correlation (< 15%) with the other samples. A re-upload and re-analysis excluding the poorly correlating sample resolves the problem.
- Existing custom designs imported prior to the release of version 1.2.0 are not compatible with the analysis of the mitochondrial chromosome, and analysis will fail. To allow analysis of the mitochondrial chromosome of such designs, the design must be re-imported.
- In rare cases, deleting a large batch of sequencing runs in a single operation can fail due to a timeout.
- In case both the target and reference samples are close to 100 GB, and both are used for the analysis, the analysis might fail due to resource constraints.

Please contact our support team for any questions at:
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