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Illumina Connected Run TruSight Oncology Comprehensive HRD (EU, DRAGEN) Analysis Module

Workflow Guide

ILLUMINA PROPRIETARY
Document # 200080937 v00
May 2026

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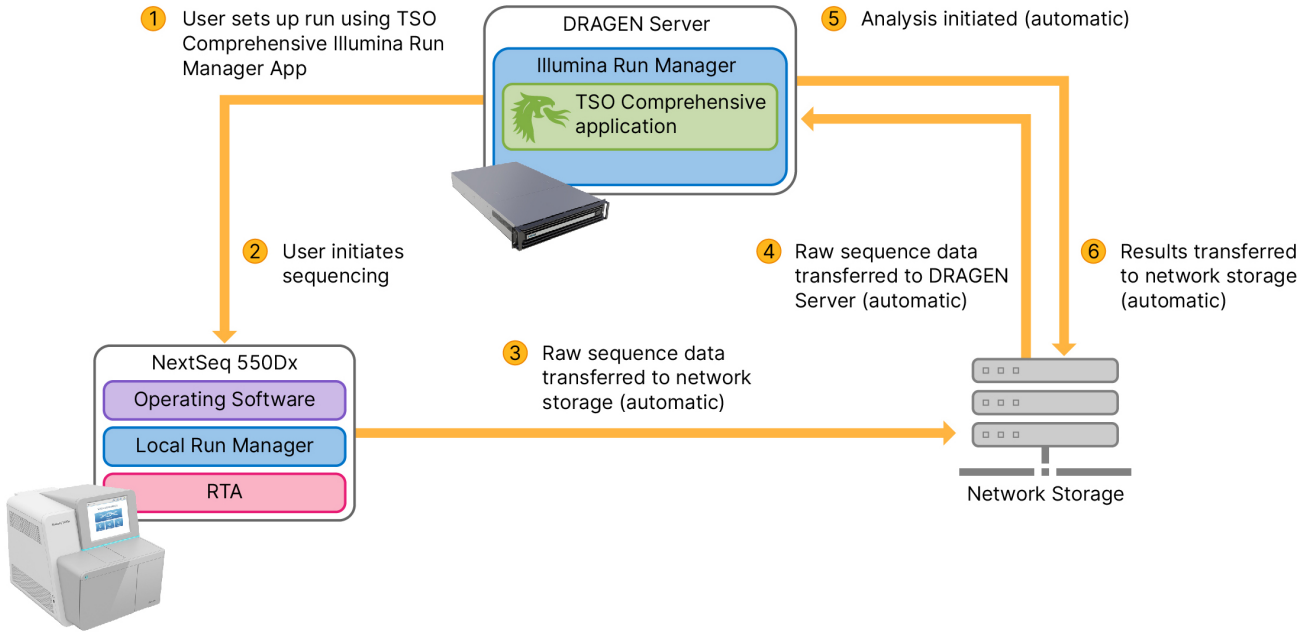
Overview

The Illumina® Connected Run TruSight™ Oncology Comprehensive (EU, DRAGEN) Analysis Module analyzes sequencing reads of DNA libraries prepared using TruSight Oncology Comprehensive HRD (DRAGEN) (TSO Comprehensive HRD (DRAGEN)).

- Use Illumina Run Manager to set up a run on the NextSeq 550Dx instrument, monitor status, set up sequencing data analysis, and raw data generation for the TSO Comprehensive HRD (DRAGEN) assay. For more information, refer to the *Illumina Run Manager for NextSeq 550Dx Software Guide (document # 200025239)*.
- Illumina Connected Run TSO Comprehensive (EU, DRAGEN) Analysis Module is a software application that runs on a DRAGEN Server for NextSeq 550Dx and supports analysis and reporting for prepared DNA libraries.

Figure 1 Overview of a TSO Comprehensive HRD (DRAGEN) Run Using Illumina Run Manager



For patient samples, the Illumina Connected Run TSO Comprehensive (EU, DRAGEN) Analysis Module generates the following reports:

- A TruSight Oncology Comprehensive (EU, DRAGEN) report for each patient sample. This report includes companion diagnostic and quality control results. This report is available in PDF and JSON formats.
- A Low Depth Report for each patient sample. This report includes a list of genomic positions (annotated with gene symbols) having insufficient sequencing depth to rule out the presence of a small variant in a DNA library.

- A Quality Control Metrics file (*.tsv). This report includes analysis status and quality control metrics for all patient samples in the sequencing run.

For control samples, the analysis module generates a control output report (*.tsv). This report includes quality control results for any control samples in the sequencing run.

About This Guide

This guide provides instructions for setting up run parameters for sequencing and analysis parameters for the TSO Comprehensive (EU, DRAGEN) Analysis Module. Use of the software requires knowledge of the Illumina Run Manager for NextSeq 550Dx. For information about TSO Comprehensive (EU, DRAGEN) Analysis Module and system settings, refer to the *Illumina DRAGEN Server for NextSeq 550Dx Site Prep and Installation Guide (document # 200025560)* and the *Illumina Run Manager for NextSeq 550Dx Software Guide (document # 200025239)*.

Enter Run Information

To set up a TSO Comprehensive HRD (DRAGEN) run, open a web browser on the networked DRAGEN Server to use the Illumina Connected Run TSO Comprehensive (EU, DRAGEN) Analysis Module software. For more information, refer to the *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

TSO Comprehensive (EU, DRAGEN) Analysis Module Application Configuration

The TSO Comprehensive (EU, DRAGEN) Analysis Module Application Configuration screen provides application, Knowledge Base (KB), and claims package information. Access this information as follows.

1. Open Illumina Run Manager and log in.
2. In the side navigation bar, select **Applications** to navigate to the Applications screen and view installed applications.
   If the application name is truncated, zoom out in the window.
3. From the Applications screen, select **TSO Comp (EU, DRAGEN)** to access the configuration for that application.

The configuration screen also displays information about installed resources for the application. For the TSO Comprehensive (EU, DRAGEN) Analysis Module, the required KB and claims package resources are configured in the Analysis section. Only one KB and one claims package can be installed at one time.

- **KB**—The TSO Comprehensive (EU, DRAGEN) Analysis Module requires an installed KB to perform analysis. The Analysis section of the Application Configuration screen displays the installed KB package.
- **Claims Package**—The TSO Comprehensive (EU, DRAGEN) Analysis Module requires an installed claims package to perform analysis. The claims package includes the companion diagnostic intended use claims that are available for analysis. The Analysis section of the Application Configuration screen displays the installed claims package.

Setting Up Runs

Use one of the following options to specify samples for the planned run:

- **[Set up run manually]** To set run parameters manually, refer to *Set Up Run Manually* on page 4.
- **[Import sample sheet]** To import an external file in a comma-separated values (CSV) format, refer to *Import Sample Sheet* on page 7.

NOTE For storage considerations, analysis output requires a minimum of 600 GB of free disk space in the selected output location. To set up the storage location in Illumina Run Manager, select External Storage for Analysis Results from the upper left drop-down list.

Set Up Run Manually

Select **Create Run** from the Planned tab of the Runs screen to set run parameters manually. The Create Run workflow allows for the selection of application and editing of run settings, sample data, and sample settings.

Select Application

- Select **TSO Comp (EU, DRAGEN)**, and then select **Next**.

If the name of the application is truncated in the Application screen, zoom out in the window.

Run Settings

Specify run parameters on the Run Settings screen as follows.

1. **Run Name**—Enter a run name that identifies the run from sequencing through analysis, with the following criteria:
   - 1–40 characters.
   - Only alphanumeric characters, spaces, underscores, or dashes.
   - Use an alphanumeric character immediately before and after spaces, underscores, and dashes.
   - Unique across all runs on the instrument.
2. **[Optional] Run Description**—Enter a run description to identify the run, with the following criteria.
   - 1–150 characters.
   - Only alphanumeric characters or spaces.
   - Use an alphanumeric character immediately before and after spaces.

The following additional fields are listed on the Run Settings screen. These fields are not editable.

- **Library Prep Kit**—The library prep kit is listed (TruSight Oncology Comprehensive (DRAGEN) Kit).
- **Index Adapter Kit**—The index adapter kit is listed (TruSight Oncology Comprehensive (DRAGEN) Kit).

- **Read and Index Lengths**—The read and index lengths (both 1 and 2) are listed with the following values:
  - Read 1 = 101
  - Index 1 = 8
  - Read 2 = 101
  - Index 2 = 8

Sample Data

The Sample Data screen is the first of two screens that captures sample information. Manually enter sample information or use a CSV template to upload sample information to the Sample Data and Sample Settings screens. Use the Download Template on the Sample Data screen to download a CSV template. The template contains the required column headings and format for import.

Refer to *Appendix D Sample Information Examples* on page 56 for Sample Data screen and Sample Settings screen examples.

1. Identify the number of samples to enter, including positive and negative controls for DNA.
   HRD enriched samples only require one row for DNA.
   A separate HRD line is not required. HRD must be assigned to DNA samples and DNA controls in the Sample Feature column on the Sample Settings screen.
2. **Sample ID**—Enter a unique sample ID with the following criteria.
   - 1–40 characters.
   - Only alphanumeric characters, underscores, or dashes.
   - Use an alphanumeric character immediately before and after underscores and dashes.
3. **Index Set ID**—Select an index set ID for the DNA library prepared from the sample.
   - For a DNA sample library and DNA controls, select a unique index ID (UPxx or CPxx indexes) from the DNA Index ID drop-down list.
   - If there are three libraries in total in the run, follow the index selection guidelines in the *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)*.

The following additional fields are listed on the Sample Data screen:

- **I7 Index**—This field is automatically populated based on the selection made in the Index Set ID drop-down list.
- **Index 1**—This field is automatically populated based on the selection made in the Index Set ID drop-down list.
- **I5 Index**—This field is automatically populated based on the selection made in the Index Set ID drop-down list.
- **Index 2**—This field is automatically populated based on the selection made in the Index Set ID drop-down list.

- [Optional] **Library Name**—An optional field that can be left blank. If left blank, the app automatically populates a value after submitting the run.

Sample Settings

The following columns appear on the Sample Settings screen:

1. **Sample ID**—The value in this field is carried over from the entries in the Sample ID column on the Sample Data screen.
2. [Optional] **Case ID**—Entering Case ID is not required, and will be automatically populated once proceeding to the next page.
3. **Sample Type**—DNA is automatically selected.
4. **Specimen Type**—Choose a specimen type or control type for the sample from a list of options determined by the installed claims package. The options can include the following:
   - Solid-FFPE
   - DNA Positive Control
   - DNA No-Template Control

   NOTE TSO Comprehensive HRD (DRAGEN) requires the use of DNA TruSight Oncology Control v2. DNA Positive Control and DNA No-Template Control must be included in each run. For more information on the DNA TruSight Oncology Control v2, refer to the *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)*.
5. **Cancer Type**—Use this field to assign a tumor type for each sample. Select the most specific tumor type available.
   - For Controls, the only option is N/A and is selected automatically.
   - Search the list of available Cancer Types. Select from the drop-down list, use a keyword search, or use the Search button. Refer to *Select a Cancer Type* on page 10.
6. **Sex**—Assign the Sex for each sample. The options are Male, Female, or Unknown.
   - For Controls, the only option is Unknown and is selected automatically.
7. **Sample Feature**—Select a Sample Feature value for each sample. The installed claims package determines the Sample Feature options.
   - Select **HRD** for samples to be processed with the TSO Comprehensive HRD (DRAGEN) assay.
   - If a Sample Feature is applied to a sample, it must also be applied to the associated controls.
8. [Optional] **Sample Description**—Enter a sample description with the following criteria:
   - 1–50 characters.
   - Only alphanumeric characters, spaces, underscores, or dashes.
   - Use an alphanumeric character immediately before and after spaces, underscores, and dashes.

Analysis Settings

The Analysis Settings screen displays information about the analysis settings, installed KB, and claims package.

The KB information includes the following:

- Name
- Version
- Description
- Published Date
- RefSeq Version

The claims package information includes the following:

- Name
- Version
- Description—A list of the biomarker, therapy, and cancer type for all CDx claims included in the Claims Package.

Run Review

The Run Review screen is the final step in Run Setup workflow. This section contains a summary of all previous screens:

- Application Details
- Run Settings
- Sample Data
- Sample Settings
- Analysis Settings

Select **Back** to edit the run, select **Exit** to exit the run setup workflow without saving the run, or select **Save** to save the run.

Import Sample Sheet

Sample sheets contain run setup information and provide a list of samples and their associated index sequences in the library prep. For information on index adapters, refer to the *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)*.

Create a run using a sample sheet as follows.

1. From the Planned tab of the Runs screen, select **Import Sample Sheet**.
2. Navigate to and select an external file in CSV format.
3. Choose from the following next steps:

- Exit the import process.
- Retry the import.
- Save the run.
- Select **Next** to continue through the Create Run screens. Review the run information and correct any errors found during sample sheet validation.

An error message displays when invalid input is entered. You can encounter multiple grouped error messages.

Configure a Sample Sheet

The following steps describe how to configure a v2 sample sheet to work with the TSO Comprehensive (EU, DRAGEN) Analysis Module.

CAUTION

Mismatches between the samples and index primers can cause incorrect result reporting due to loss of positive sample identification. Enter sample IDs and assign indexes in the sample sheet before beginning library preparation. Record sample IDs, indexes, and plate well orientation for reference during library preparation.

1. Open the sample sheet in a spreadsheet application.
2. In the **[appName_Data]** section, enter information for each sample. The following table describes the available sample parameters.

Data Column	Required	Description
Sample_ID	Y	ID given to the sample. This value is not case sensitive. The ID must meet the following criteria: • Unique for the run. • Between 1–40 characters in length. • Contain alphanumeric characters with underscores and dashes. Use an alphanumeric character immediately before and after underscores and dashes. No spaces allowed. • Match the Sample_ID information listed in the BCLConvert_Data section.
Index_ID	Y	Index adapter ID used for the sample. Must be unique per sample. Indexes UPxx and CPxx are valid for DNA samples.
Specimen_Type	Y	Valid values are as follows. • Solid-FFPE • DNAPositiveControl • NTCDNAControl

Data Column	Required	Description
Cancer_Type	Y	Valid and most specific cancer type code from the KB or claims package. **N/A** is the only acceptable value for control samples. Some cancer types are restricted to particular Specimen_Types. Refer to *Select a Cancer Type on page 10* for more information.
Sex	Y	Gender of sample. Valid values are as follows. • Female • Male • Unknown Value must be Unknown for positive control and NTC.
Sample_Type	Y	Valid value is as follows: • DNA
Case_ID	Y	Blank values are interpreted to be the same as Sample_ID.
Sample_ Description	N	Description for sample. Must meet the following criteria: • Between 1–50 characters in length. • Alphanumeric characters with underscores, dashes, and spaces. Use an alphanumeric character immediately before and after underscores, dashes, and spaces.
Sample_Feature	Y	Valid value is as follows: • HRD

3. In the **[BCLConvert_Data]** section, enter information for each sample. The following table describes the required fields.

Data Column	Required	Description
Sample_ID	Y	Must match the Sample_ID information listed in the [appName_Data] section.
Index1	Y	Index 1 sequence valid for Index_ID assigned to sample in [appName_Data] section. The index sequence can be found in *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)*.
Index2	Y	Index 2 sequence valid for Index_ID assigned to sample in [appName_Data] section. The index sequence can be found in *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)*.

4. Remove the **[BCLConvert_Settings]** section from the sample sheet. Including this section prevents the TSO Comprehensive (EU, DRAGEN) Analysis Module from successfully importing a sample sheet.

5. Make sure that the sample sheet file ends with the last row of CSV data and has no blank lines at the end of the file.

NOTE If the sample total for the run is three (excluding the NTC DNA Control sample), follow the index selection guidelines in the *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)*.

Select a Cancer Type

You must specify a cancer type for each sample. The available cancer types are derived from the installed KB.

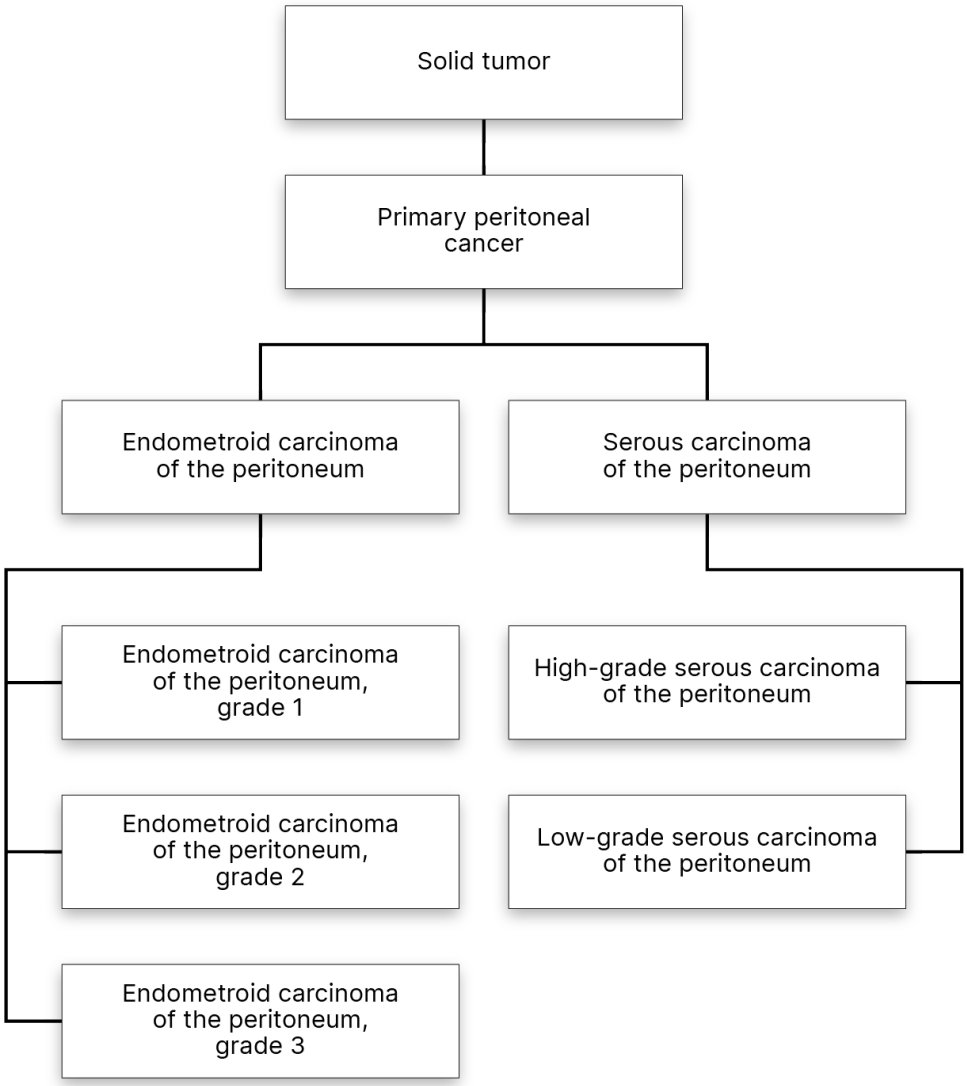
⚠ CAUTION

Incorrect selection of cancer type can cause incorrect results.

Companion diagnostics information is often cancer sub-type specific. Be as specific as possible when selecting the tumor type to retrieve the correct information for variant and biomarker calling and interpretation. The selected cancer type for a patient sample impacts which companion diagnostic intended uses are evaluated for the sample. Only patient samples with a cancer type that is an exact match or a descendent of the cancer type for a companion diagnostic intended use are evaluated for that claim.

For example, the term serous carcinoma of the peritoneum is a child of primary peritoneal cancer because serous carcinoma of the peritoneum is a type of primary peritoneal cancer. Figure 2 depicts a subset of an example disease ontology showing solid tumor as the root term, and the terms associated with primary peritoneal cancer (other tumor types are not shown). A term that is connected through parent-child relationships to lower-level terms is called an ancestor. The connected lower-level terms are descendants of the ancestor term. For example, primary peritoneal cancer is an ancestor of endometriod carcinoma of the peritoneum and low-grade serous carcinoma of the peritomeum. Serous carcinoma of the peritoneum is a descendant of both primary peritoneal cancer and solid tumor.

Figure 2 Example of a Disease Ontology Subset



Runs created with the **Import Sample Sheet** option must contain cancer types that match a tumor type code from the installed KB. The following table includes some examples of allowed cancer types for sample sheet import.

Cancer	Cancer_Type
Fallopian tube cancer	363444001_00340
Malignant epithelial ovarian cancer	254849005_00315
Primary peritoneal cancer	372016004_00341

Analysis Methods

After collecting the sequencing data, the TSO Comprehensive (EU, DRAGEN) Analysis Module processes it to:

- Perform quality control.
- Detect variants.
- Determine Genomic Instability Score (GIS) status.
- Determine companion diagnostic results.
- Report results.

The following sections describe the analysis methods.

Run Quality Control

Sequencing run quality metrics are evaluated to determine if they are within an acceptable range. The overall percentage of reads passing filter is compared to a minimum threshold. For Read 1 and Read 2, the average percentage of bases ≥ Q30 is compared to a minimum threshold. Q30 gives a prediction of probability of an incorrect base call (Q-score). If values for each of these three metrics meet the specifications, Run QC is reported as PASS and analysis continues. If a value for any one of the metrics fails to meet the specification, Run QC is reported as FAIL and analysis does not proceed. For more information, refer to *Run QC Metrics* on page 51.

FASTQ Generation

Sequencing data stored in BCL format is demultiplexed using index sequences unique to each sample added during the library preparation step to assign clusters to the library from which they originated. Each cluster contains two indexes (i5 and i7 sequences, one at each end of the library fragment). The combination of those index sequences is used to demultiplex the pooled libraries.

After demultiplexing, FASTQ files are generated. These files contain the sequencing reads for each individual sample library and the associated quality scores for each base call, excluding reads from any clusters that did not pass filter.

DNA Alignment and Error Correction

DNA alignment and error correction aligns sequencing reads derived from DNA sample libraries to a reference genome and corrects errors in the sequencing reads prior to variant calling.

DRAGEN unique molecular identifier (UMI) error correction consists of the following main steps:

1. DRAGEN UMI uses its hardware-accelerated mapper (based on a hash table implementation) to align DNA sequences in FASTQ files to the hg19 reference genome. These alignments are not written to a BAM file.
2. The raw alignments are processed to remove errors (including errors introduced during FFPE preservation, PCR amplification, and sequencing). Reads from the same original DNA molecule are tagged with the same UMI during library preparation. The UMI markers allow DRAGEN to compare related reads, remove outlier signals, and collapse these multiple reads into a single high-quality sequence.
3. DRAGEN performs a final alignment step on the UMI-collapsed reads. These final alignments are written to a BAM file and a corresponding BAM index file is created. DRAGEN uses these final alignments as input for small variant calling (SNVs, insertions, deletions, MNVs), large rearrangement calling, Genomic Instability Score (GIS) determination, and DNA library quality control.

Small Variant Calling

DRAGEN supports calling SNVs, insertions, deletions (including delins which are combinations of the two with a net change in nucleotides that is either an insertion or deletion), and MNVs in tumor-only samples by taking in mapped and aligned DNA reads from a tumor sample as input. Variants are detected via both column wise pileup analysis and local *de novo* assembly of haplotypes. The *de novo* haplotypes allow the detection of larger MNVs and delins than possible through column-wise pileup analysis only. The tumor-only pipeline produces a VCF file containing both germline and somatic variants that can be further analyzed to identify tumor mutations. The pipeline makes no ploidy assumptions, enabling detection of low-frequency alleles.

The main steps used in DRAGEN small variant calling are as follows.

1. Detection of callable regions (regions with sufficient read coverage).
2. Detection of active regions (regions where the reads deviate from the reference and there is a possibility of a germline or somatic call).
3. Haplotype assembly (*de novo* graph haplotypes are assembled from reads).
4. Events (possible somatic or germline calls) are extracted from column-wise pileup analysis.
5. Read base qualities are calibrated to account for FFPE noise.
6. Read likelihoods are computed for each read/haplotype pair.
7. Mutation calling is performed by summing the genotype probabilities across all reads/haplotype pairs.
8. Additional filtering is performed to improve variant calling accuracy, which includes using a systematic noise file. The systematic noise file indicates the statistical probability of noise at specific positions in the genome. This noise file is constructed using clean (normal) samples. Regions where noise is common (for example, difficult to map regions) have higher noise values at those locations. The small variant caller penalizes those regions, reducing the probability of making false positive calls.

Small Variant Annotation

Detected small variants are annotated using the Nirvana annotation engine with information from the RefSeq database and various population databases (COSMIC, ClinVar, dbSNP, 1000 Genomes, and gnomAD). Annotation of small variants is performed multiple times independently as described in the following section.

Static Annotation Databases for Companion Diagnostic Calling

Nirvana annotates filtered small variant calls with static (not updatable) annotation databases for use by downstream Companion Diagnostic calling (refer to *Companion Diagnostic Calling* on page 16). The VCF from the Small Variant Calling step is used as input (refer to *Small Variant Calling* on page 13).

Large Rearrangement Calling

The BRCA large rearrangement step generates segmentation of the BRCA1 and BRCA2 genes for exon level copy number variant (CNV) detection from the BAM file produced during DNA Alignment and Error Correction. The large rearrangement algorithm counts coverage of each target interval of the panel, performs normalization, and calculates the fold change values for each probe across the BRCA genes. Normalization includes GC bias correction, sequencing depth, and probe efficiency using a collection of normal FFPE genomic DNA samples. Initial segmentation is performed for each gene with circular binary segmentation. Amplitude, noise, and variance at adjacent segments using thresholds established with *in silico* data determine the merging of segments. A large rearrangement is called when merging of segments yields more than one distinct segment per gene. Start and end coordinates of the segment determined to be the rearrangement event, along with log2 mean fold change for the segment are included in the output for this step.

Large Rearrangement Annotation

The Nirvana annotation engine annotates detected large rearrangements for BRCA1 and BRCA2 with information from the RefSeq database, as well as various population databases (COSMIC, ClinVar, dbSNP, 1000 Genomes, and gnomAD). Annotation of large rearrangements is performed independently as described in *Large Rearrangement Calling* on page 14.

Static Annotation Databases for Companion Diagnostic Calling

Nirvana annotates large rearrangements with static (not updatable) annotation databases for use by downstream companion diagnostic calling (refer to *Companion Diagnostic Calling* on page 16). The VCF from Large Rearrangements Calling is used as input (refer to *Large Rearrangement Calling* on page 14).

Genomic Instability Score

Genomic Instability Score (GIS) is a whole genome signature for homologous recombination deficiency (HRD). GIS is composed of the sum of three components: loss of heterozygosity, telomeric allele imbalance, and large-scale state transition. These components are estimated using a GIS algorithm that uses an input of the b-allele frequency and coverage across a genome wide single nucleotide panel. A panel of normal samples is used for both bias reduction and normalization before GIS estimation.

Quality Control for DNA Sample Libraries

The contamination analysis step detects foreign human DNA contamination using the SNP error file and pileup file that are generated during the small variant calling, and the TMB trace file. The software determines whether a sample has foreign DNA using the contamination score. In contaminated samples, the VAFs in SNPs shift away from the expected values of 0%, 50%, or 100%. The algorithm collects all the positions that overlap with common SNPs that have VAFs of < 25% or > 75%. Then, the algorithm computes the likelihood that the positions are an error or a real mutation. The contamination score is the sum of all the log likelihood scores across the pre-defined SNP positions whose minor allele frequency is < 25% in the sample and not likely due to CNV events.

The larger the contamination score, the more likely there is foreign DNA contamination. If the contamination score is above predefined quality threshold, a sample is considered to be contaminated. If contamination is detected, DNA Library QC is reported as FAIL and no results are available for small variants, large rearrangements, or GIS. The contamination score was found also to be high in samples with highly rearranged genomes (or HRD samples), with 1% of HRD samples found to be above the threshold with no evidence for actual contamination.

QC metrics are used to assess the validity of small variant calling, large rearrangements, and GIS for DNA sample libraries that pass contamination quality control. If the sample library fails one or more quality metrics, the corresponding variant type or biomarker is not reported, and the associated QC category in the report header displays as FAIL.

DNA library QC results are available in the `MetricsOutput.tsv` file (refer to *Metrics Output* on page 40).

Low Depth Reporting for DNA Sample Libraries

A Low Depth Report is generated for each patient sample with a DNA library. The report includes a listing of genomic positions with a total sequencing depth < 100 and for which a passing small variant was not detected. These positions have insufficient sequencing depth to rule out the presence of a small variant. If there is sufficient sequencing depth of the variant allele, it is still possible to detect variants with a total sequencing depth < 100.

Contiguous positions of low depth overlapping the same genes are combined into genomic ranges in the Low Depth Report. Each genomic range in the report is annotated with one or more RefSeq gene symbols. The RefSeq annotation is based on the RefSeq database included as part of the KB.

Refer to *Low Depth Report* on page 43 for details on the content.

Control Reporting

A control output report is generated for each analysis and includes an assessment of each control sample included in the run. The TSO Comprehensive (EU, DRAGEN) Analysis Module automatically invalidates patient samples based on control sample results. Refer to *TSO Comprehensive (EU, DRAGEN) Report* on page 17 for more details.

The control output report is available in the `ControlOutput.tsv` file (refer to *Control Output Report* on page 36).

Companion Diagnostic Calling

For each installed companion diagnostic (CDx) intended use, the TSO Comprehensive (EU, DRAGEN) Analysis Module determines the applicability of the CDx intended use for each patient sample based on the tumor type of the patient sample. If the tumor type of the patient sample is an exact match or a descendent of the tumor type for a CDx intended use, it is considered applicable to that CDx intended use. Refer to *Select a Cancer Type* on page 10 for more information on the disease ontology. If the tumor type of the patient sample is not applicable to a CDx intended use, the CDx intended use is not evaluated for that sample.

If a required DNA sequencing library for a CDx intended use is not sequenced or fails DNA library QC contamination score, the patient sample is not evaluated for that CDx intended use. If a variant type (for example, small variants) or biomarker required for a CDx intended use fails QC, the patient sample is not evaluated for that variant type or biomarker. If at least one variant type or biomarker required for a CDx intended use passes QC and controls, the patient sample is evaluated for that CDx intended use. Refer to Table 5 for more information on what QC and control are required to pass for each variant type or biomarker for a CDx.

After determination that a CDx intended use is applicable for a patient sample, the required libraries are sequenced, and required QC measures pass, the companion diagnostic intended use is evaluated for the patient sample. Detected variants and/or biomarkers in the patient sample are evaluated to determine the result for the CDx intended use. An algorithm specific to the CDx intended use assesses the presence and/or absence of variants/biomarkers that match the CDx intended use.

CDx calling results are made available in the TSO Comprehensive (EU, DRAGEN) Analysis Module report. Positive CDx intended uses are reported in the *Companion Diagnostic Results* section of the TSO Comprehensive (EU, DRAGEN) Analysis Module report. Refer to Table 3 for more information.

Analysis Output

When the analysis is completed, TSO Comprehensive (EU, DRAGEN) Analysis Module generates an analysis folder with the following structure in the configured output folder:

- `IVD_Reports`
  - `ControlOutput.tsv`—Control Output report.
  - `MetricsOutput.tsv`—Metrics Output report.
  - `{Case ID}_TSOCompEU_KB{Knowledge Base Version}_Report.pdf`—TSO Comprehensive (EU, DRAGEN) report (PDF format) per patient sample.
  - `{Case ID}_TSOCompEU_KB{Knowledge Base Version}_Report.json`—TSO Comprehensive (EU, DRAGEN) report (JSON format) per patient sample.
  - `{Case ID}_LowDepthReport.tsv*`—Low depth report per patient sample.
- `Logs_Intermediates`—Logs and intermediate files generated during the analysis workflow. Intermediate files are intended to help with troubleshooting only. The information contained in the intermediate files is not intended to be used for clinical reporting or patient management. Performance of any variants identified in these files, other than validated variants, has not been demonstrated. Validated variants are variants with demonstrated performance characteristics. Each folder represents one step of the analysis workflow.

To view analysis output:

1. Navigate to the directory that contains the analysis folder.
2. Open the analysis folder to view output files.

Results Reports

TSO Comprehensive (EU, DRAGEN) Analysis Module reports in PDF and JSON formats are produced for each patient sample that completed analysis successfully. Refer to the analysis output folder for TSO Comprehensive (EU, DRAGEN) reports for all completed samples.

TSO Comprehensive (EU, DRAGEN) Report

The following tables describe the sections that make up the TSO Comprehensive (EU, DRAGEN) reports produced for each patient sample in PDF and JSON formats. The PDF report is human readable. The JSON report comprises data structures that are intended for machines to parse. Information found only in the JSON report and not reflected in the PDF report is marked as not applicable (N/A) for the PDF report. Variants not reported in Companion Diagnostic Results are not included in the reports.

Refer to the *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)* for interpretation of results.

- **Sample, Run, and Analysis Information**—Contains general information about the patient sample and the report.

Table 1 Sample, Run, and Analysis Information

PDF Report Field	JSON Report Field	Description
Report Date	reportDate	Date that the report was generated.
N/A	reportTime	Time that the report was generated.
Case ID	sampleInformation / sampleId	Sample Identifier. Patient demographics are not included.
Cancer Type	sampleInformation / cancerType	Cancer type associated with the patient sample.
N/A	sampleInformation / cancerTypeCode	Cancer type code associated with the patient sample.
N/A	sampleInformation / cancerTypePath	Cancer type path (with respect to the disease ontology) associated with the patient sample.
N/A	sampleInformation / cancerTypeCodePath	Cancer type code path (with respect to the disease ontology) associated with the patient sample.
Sample Type	sampleInformation / sampleType	Sample type of the patient sample.
Specimen Type	sampleInformation / specimenType	Specimen type of the patient sample.
Sex	sampleInformation / sex	Patient sex (Male, Female, or Unknown).
Sample Feature	sampleInformation / sampleFeature	Sample feature of the patient sample.
Analysis Date	sampleInformation / analysisDate	Date that the secondary analysis was completed.

PDF Report Field	JSON Report Field	Description
N/A	sampleInformation / analysisTime	Time that the secondary analysis was completed.
Run ID	sampleInformation / analysisRunId	Sequencing run ID.
N/A	sampleInformation / analysisRunName	Sequencing run name.

- **TSO Comprehensive (EU, DRAGEN) Analysis Module and Knowledge Base Configuration**—Contains information on the software and KB versions used when the report was generated.

Table 2 TSO Comprehensive (EU, DRAGEN) Analysis Module and Knowledge Base Configuration

PDF Report Field	JSON Report Field	Description
Knowledge Base Version	softwareConfiguration / knowledgeBaseVersion	Version of the Knowledge Base installed with the TSO Comprehensive (EU, DRAGEN) Analysis Module software.
Knowledge Base Published Date	softwareConfiguration / knowledgeBasePublished Date	Date associated with the Knowledge Base that was used to generate the report.
Module Version	softwareConfiguration / moduleSoftwareVersion	Version of the TSO Comprehensive (EU, DRAGEN) Analysis Module used to generate the report.
Claims Package Version	softwareConfiguration / claimsPackageVersion	Version of the Claims Package installed with the TSO Comprehensive (EU, DRAGEN) Analysis Module.

- **Companion Diagnostic Results**—Results for companion diagnostic (CDx) intended uses where an associated variant or biomarker was detected are listed in the PDF and JSON reports. Additional companion diagnostic intended uses where an associated variant or biomarker was not detected or not evaluated are listed in the JSON report only. Refer to Table 5.

Table 3 Companion Diagnostic Results

PDF Report Field	JSON Report Field	Description
[Message box]	reportFindings / companionDiagnostic Results / results / noEntryText	A message is optionally displayed in this section. The following message is possible: **No Companion Diagnostic biomarkers for the stated sample tumor type were detected.** This message is included when any of the following is true for all CDx intended uses: • The sample passes QC, but no associated variant or biomarker was detected or its cancer type is inapplicable for the CDx intended use. • The sample fails required Control QC. • The sample fails required QC metrics.
[Message box]	reportFindings / companionDiagnostic Results / results / message	A message is optionally displayed in this section. The following message is possible: **One or more Companion Diagnostic claims not evaluated. See Companion Diagnostics Intended Uses Evaluated.** This message is included when at least one CDx intended use could not be evaluated due to a control failure, QC failure, tumor type mismatch, or missing required DNA sequencing library. Any detected CDx biomarkers appear in a table below this message. Refer to Table 5 for reasons why a CDx intended use was not evaluated.
N/A	reportFindings / companionDiagnostic Results / results / genomicFindings / (array item for CDx intended use) / companionDiagnostic Name	Name of the companion diagnostic intended use. Includes biomarker description, therapy, and cancer type.

PDF Report Field	JSON Report Field	Description
Detected Variants/ Biomarkers	reportFindings / companionDiagnostic Results / results / genomicFindings / (array item for CDx intended use) / variants reportFindings / companionDiagnostic Results / results / genomicFindings / (array item for CDx intended use) / biomarkers	A list of detected variants or biomarkers associated with a detected CDx intended use for the sample. In the JSON report, this field is empty for CDx intended uses if the result is not equal to detected.
Therapy	reportFindings / companionDiagnostic Results / results / genomicFindings / (array item for CDx intended use) / therapy	The therapy associated with the CDx intended use.
Usage	reportFindings / companionDiagnostic Results / results / genomicFindings / (array item for CDx intended use) / usage	Usage of the CDx therapy (Indicated or See Note). In the JSON report, this field is present for CDx intended uses when the result is detected. • **Indicated**—The associated therapy is indicated for use. • **See Note**—A note describes usage of the therapy.

PDF Report Field	JSON Report Field	Description
Details	reportFindings / companionDiagnostic Results / results / genomicFindings / (array item for CDx intended use) / note reportFindings / companionDiagnostic Results / results / genomicFindings / (array item for CDx intended use) / variants / (array item for variant in genomic finding) reportFindings / companionDiagnostic Results / results / genomicFindings / (array item for CDx intended use) / biomarkers / (array item for variant in genomic finding)	Contains an optional note and a list of variant and/or biomarker details. In the PDF report, the order of variant/biomarker details corresponds to the order of variants listed for Detected Variants/Biomarkers field. Refer to Table 8,Table 9, and Table 10 for a list of variant detail fields. In the JSON report, these fields are empty for CDx intended uses if the result is not equal to detected.

PDF Report Field	JSON Report Field	Description
N/A	reportFindings / companionDiagnostic Results / results / genomicFindings / (array item for CDx intended use) / detailedResult / result	A coded value for the result of the CDx intended use. Possible values include the following: • **detected**—The CDx intended use is applicable to the cancer type of the sample, and one or more variants or biomarkers associated with the CDx intended use was detected in the sample. • **notDetected**—The CDx intended use is applicable to the sample's cancer type, but no variants or biomarkers associated with the CDx intended use were detected in the sample. • **tumorTypeNonMatch**—The CDx intended use is not applicable to the sample's cancer type. • **nucleicAcidNA**—The sample did not have a DNA library sequenced, which is required for the CDx intended use. • **qcFail**—The CDx intended use was not evaluated due to a QC failure. • **didNotCompleteAnalysis**—Analysis did not complete successfully for the sample. • **controlFail**—One or more control samples required for this CDx intended use failed. • **partialControlFail**—At least one control category related to this CDx intended use did not pass and no variant of biomarker associated with the CDx intended use was detected. Refer to Table 5 for the control categories relevant to this CDx intended use. • **partialQcFail**—At least one QC category related to this CDx intended use did not pass and no variant or biomarker associated with the CDx intended use was detected. Refer to Table 5 for the control categories relevant to this CDx intended use. • **negative**—Placeholder value for future use.

- **Companion Diagnostics QC**—This section lists genomic positions associated with a CDx intended use that have insufficient depth to make a confident reference call. Only CDx intended uses involving small variants that were evaluated for a sample are listed.

Table 4 Companion Diagnostics QC

PDF Report Field	JSON Report Field	Description
[Position list]	reportFindings / companionDiagnostic Results / qualityControl / insufficientQuality / entries / (array item for CDx intended use) / positions	A list of genomic positions for the associated CDx intended use having insufficient coverage.

- **Companion Diagnostics Intended Uses Evaluated**—This section lists all installed CDx intended uses, and includes a field that indicates whether the CDx intended use was evaluated for the sample. If a CDx intended use was not evaluated, a reason is provided.

Table 5 Companion Diagnostics Intended Uses Evaluated

PDF Report Field	JSON Report Field	Description
Cancer Type	reportFindings / companionDiagnostic Results / qualityControl / intendedUsesEvaluated / companionDiagnostic Table / entries / (array item for CDx intended use) / cancerType	According to the Intended Use statement.

PDF Report Field	JSON Report Field	Description
Biomarkers	reportFindings / companionDiagnostic Results / qualityControl / intendedUsesEvaluated / companionDiagnostic Table / entries / (array item for CDx intended use) / biomarkers	According to the Intended Use statement.
Therapy	reportFindings / companionDiagnostic Results / qualityControl / intendedUsesEvaluated / companionDiagnostic Table / entries / (array item for CDx intended use) / therapy	According to the Intended Use statement.
CDx Intended Use Evaluated	reportFindings / companionDiagnostic Results / qualityControl / intendedUsesEvaluated / companionDiagnostic Table / entries / (array item for CDx intended use) / intendedUseEvaluated	Indicates if the CDx intended use was evaluated for the sample (Evaluated/Not Evaluated). Evaluation of the CDx intended use requires passing the specific QC and control categories of the nucleic acid or variant/biomarker type associated with the CDx intended use. Additional gene and variant type specific evaluation statuses indicate if each biomarker applicable to the CDx was evaluated for the sample.

PDF Report Field	JSON Report Field	Description
CDx Intended Use Evaluated (continued)	reportFindings / companionDiagnostic Results / qualityControl / intendedUsesEvaluated / companionDiagnostic Table / entries / (array item for CDx intended use) / intendedUseEvaluated	CDx intended uses associated with the detection of an HRD biomarker in FFPE specimen types require DNA to be processed with the TSO Comprehensive HRD (DRAGEN) assay, Run QC to pass, DNA Library QC to pass, and at least one of the following: • DNA Small Variant &TMB QC, DNA No-Template Control, and DNA Positive Control—Small Variants all pass. For gene level control validation, at least one representative small variant for the biomarker must be detected in the DNA Positive Control. • DNA Copy Number Variant QC, DNA No-Template Control, and DNA Positive Control—Gene Amplification all pass. • DNA GIS QC, DNA GIS No-Template Control, and DNA GIS Positive Control all pass.
Comment	reportFindings / companionDiagnostic Results / qualityControl / intendedUsesEvaluated / companionDiagnostic Table / entries / (array item for CDx intended use) / comment	If CDx Intended Use Evaluated field is **Evaluated** and there are no additional comments needed, this field displays a dash. If CDx Intended Use Evaluated field is **Evaluated** and there are additional comments to list, a comment such as the following can be displayed. Example: • Some genomic positions associated with the CDx claim had insufficient coverage. Refer to Table 4 for details. If CDx Intended Use Evaluated field is **Not Evaluated**, a comment such as the following is displayed to indicate why the CDx Intended Use was not evaluated. Examples: • Tumor Type of sample does not match tumor type corresponding to the CDx Intended Use. • DNA data associated with a CDx biomarker is not available.

- **Variant Types Evaluated**—Contains information on which variant or biomarker types are eligible to be reported due to QC and control results.

Table 6 Variant Types Evaluated

PDF Report Field	JSON Report Field	Description
Variant Type	qualityControl / variantResults / variantResultsTable / entries / (array item for Variant or Biomarker type) / variantType	Variant or biomarker type. Possible values include the following: • Small Variants • Large Rearrangements • GIS
Control QC	qualityControl / variantResults / variantResultsTable / entries/ (array item for Variant or Biomarker type) / controlResult	Control QC (PASS, FAIL, or N/A) for the variant or biomarker type. • **PASS**—All required controls passed. • **FAIL**—One or more required controls failed. • **N/A**—Variant type is not available for the sample or Run QC has a value of FAIL. Small Variants require DNA No-Template Control and DNA Positive Control—Small Variants and TMB pass. Large Rearrangements require DNA No-Template Control and DNA Positive Control—Gene Amplifications to pass. GIS requires DNA GIS No-Template Control and DNA GIS Positive Control to pass. For more information on how control results are evaluated, refer to *Control Output Report* on page 36.

PDF Report Field	JSON Report Field	Description
Library QC	qualityControl / variantResults / variantResultsTable / entries/ (array item for Variant or Biomarker type) / qcResult	QC result (PASS, FAIL, or N/A) for the variant or biomarker type. • **PASS**—All required QC categories passed. • **FAIL**—One or more required QC categories failed. • **N/A**—Variant type is not available for the sample or Run QC has a value of FAIL. Small Variants require DNA Library QC and DNA Small Variant and TMB QC to pass. Large Rearrangements require DNA Library QC and DNA CNV QC to pass. GIS requires DNA Library QC and DNA GIS QC to pass. For more information on how quality control is evaluated, refer to *Appendix A QC Metrics Flowchart* on page 49.
Evaluated	qualityControl / variantResults / variantResultsTable / entries / (array item for Variant or Biomarker type) / reported	Indicates whether a variant type is reported (TRUE, FALSE, or N/A). • **TRUE**—Control Result and QC Result are both PASS. • **FALSE**—Either or both Control Result and QC Result are FAIL. • **N/A**—Variant type is not available for the sample or Run QC has a value of FAIL.
Comment	qualityControl / variantResults / variantResultsTable / entries / (array item for Variant or Biomarker type) / comment	If Reported field is TRUE, this field displays a dash. If Reported field is FALSE, a comment listing QC and control categories that failed is shown. Possible values include: • No DNA Library available. • Control Failure—List of all required controls that failed. • QC Failure—List of all required QC categories that failed.

- **About the Test**—Contains general information about the test.

Table 7 About the Test

PDF Report Field	JSON Report Field	Description
About the Test	about / description	Test description.

- **Small Variant Details in Report**—This section contains details about the detected small variants.

Table 8 Small Variant Details in Report

PDF Report Field	JSON Report Field	Description
Type	type / value	The detailed type of variant. Possible values for small variants include: • **SNV**—Single nucleotide variant. • **Insertion**—Addition of nucleotides of up to 25 bp. • **Deletion**—Removal of nucleotides of up to 25 bp. • **MNV**—Multi-nucleotide variant, being a substitution of two or three nucleotides with the same number of nucleotides. • **Indel**—One or more nucleotides replaced by one or more nucleotides and is not an SNV or MNV. This variant is commonly referred to as delins.
VAF	additionalInfo / (array item having label property = "VAF") / value	Variant allele frequency (as a percentage).
Consequence	additionalInfo / (array item having label property = "Consequence") / value	Variant consequence from the Sequence Ontology.
Nucleotide Change	additionalInfo / (array item having label property = "Nucleotide Change") / value	Change to the coding DNA reference sequence (the RefSeq transcript) in HGVS nomenclature. If the variant does not impact a transcript, the change to the genomic reference sequence in HGVS nomenclature is included.
Genomic Position	additionalInfo / (array item having label property = "Genomic Position") / value	Genomic position (hg19) in chromosome:position format. Refers to the position of the first base in the reference allele.
Reference Allele	additionalInfo / (array item having label property = "Reference Allele") / value	Reference allele.

PDF Report Field	JSON Report Field	Description
Alternate Allele	additionalInfo / (array item having label property = “Alternate Allele”) / value	Alternate allele.
Related Phased Variant	additionalInfo / (array item having label property = “Related Phased Variant”) / value	Gene symbol and protein change, transcript change, or genomic change in HGVS format for the related phased variant, as applicable.
N/A	cosmicIds	List of genomic mutation IDs associated with the variant from the Catalogue of Somatic Mutations In Cancer (COSMIC) database, as applicable. Not included for Companion Diagnostics Results.
N/A	detailedSmallVariant Data / vcfChromosome	Chromosome.
N/A	detailedSmallVariant Data / vcfPosition	Genomic position (hg19). Refers to the position of the first base in the reference allele (detailedSmallVariantData/referenceAllele field).
N/A	detailedSmallVariant Data / vcfRefAllele	The reference allele.
N/A	detailedSmallVariant Data / vcfVariantFrequency	Variant allele frequency.
N/A	detailedSmallVariant Data / annotation / transcripts	Detailed transcript-level annotations for a transcript (as applicable). Only a single preferred transcript is included.
N/A	detailedSmallVariant Data / annotation / transcripts / (first array item) / transcript	Transcript ID.

PDF Report Field	JSON Report Field	Description
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / source	Transcript source (RefSeq).
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / bioType	An Ensembl biotype classification for the transcript.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / aminoAcids	The change in amino acids, as applicable (for example, G/D).
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / cdnaPos	cDNA position.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / codons	Codon sequence change (for example, gGt/gAt), as applicable.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / cdsPos	Coding sequence position, as applicable.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / exons	The exon(s) affected by the variant, and total exon count, as applicable. For example, 4-6/7 indicates that exons 4, 5, and 6 were affected and that this transcript contains 7 exons in total.

PDF Report Field	JSON Report Field	Description
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / introns	The introns affected by the variant, as applicable.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / geneId	National Center for Biotechnology Information (NCBI) gene ID.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / hgnc	HUGO Gene Nomenclature Committee (HGNC) gene symbol.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / consequence	Array of variant consequences from the Sequence Ontology.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / hgvsc	Change to the coding DNA reference sequence (the RefSeq transcript) in HGVS nomenclature, as applicable.
N/A	detailedSmallVariantData / annotation / transcripts / (first array item) / hgvsp	Change to the protein sequence in HGVS nomenclature, as applicable.

PDF Report Field	JSON Report Field	Description
N/A	detailedSmallVariant Data / annotation / transcripts / (first array item) / isCanonical	Displays true if this transcript is considered the canonical transcript of the gene, otherwise false. A canonical transcript for a gene is determined as follows: Only NM & NR transcripts are included. Transcripts for a gene are sorted in the following order: • Locus Reference Genomic (LRG) entries come before non-LRG entries. • Descending CDS length. • Descending transcript length. • Accession number. With this sorting, the first transcript is considered canonical.
N/A	detailedSmallVariant Data / annotation / transcripts / (first array item) / proteinId	Protein ID.
N/A	detailedSmallVariant Data / annotation / transcripts / (first array item) / proteinPos	Protein position.

- **Large Rearrangement Details in Report for Companion Diagnostics Results**—This section contains details about the detected large rearrangements (exon-level CNVs).

Table 9 Large Rearrangement Details in Report

PDF Report Field	JSON Report Field	Description
Type	type / value	The detailed type of variant. Possible values for large rearrangements include: • **Large Rearrangement**.
Affected Exon(s)	additionalInfo / (array item having label property = “Affected Exon(s)”)	The exon(s) affected by the large rearrangement. For example, 4–6 indicates that exons 4, 5, and 6 were affected.
Transcript	additionalInfo / (array item having label property = “Transcript")	Transcript ID (RefSeq).
Genomic Position	additionalInfo / (array item having label property = “Genomic Position”)	Genomic position of the large rearrangement represented as a range. Chromsome:position - position format (hg19).
N/A	exonLevelCnvVariant Details / gene	Gene symbol.
N/A	exonLevelCnvVariant Details / chromosome	Chromosome of large rearrangement.
N/A	exonLevelCnvVariant Details / begin	Start position (hg19) of large rearrangement.
N/A	exonLevelCnvVariant Details / end	End position (hg19) of large rearrangement.
N/A	exonLevelCnvVariant Details / cnvType	Value is LOSS.

PDF Report Field	JSON Report Field	Description
N/A	exonLevelCnvVariant Details / annotation / source	Transcript source (RefSeq).
N/A	exonLevelCnvVariant Details / annotation / affectedExons	The exon(s) affected by the large rearrangement. For example, 4–6 indicates that exons 4, 5, and 6 were affected.
N/A	exonLevelCnvVariant Details / annotation / transcript	Transcript ID.

- **Genomic Instability Score Details in Report for Companion Diagnostics Results**—This section contains details about the Genomic Instability Score.

Table 10 Genomic Instability Score Details in Report

PDF Report Field	JSON Report Field	Description
Type	type / value	Description of the biomarker type. Possible values for Genomic Instability Score include: • **Biomarker**.
N/A	genomicInstability / additionalInfo / (array item having label property = “GIS Score”) / value	The Genomic Instability Score value.

Control Output Report

File name: `ControlOutput.tsv`

The control output report is a tab-delimited file that provides quality control information for any control samples that were included in the run. The TSO Comprehensive (EU, DRAGEN) Analysis Module automatically invalidates patient sample results based on control sample results.

Refer to the *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)* for guidance on run validity and patient sample validity based on results for controls.

The control output report contains the following sections and associated fields (run ID is included before the first section):

- **Analysis Details**—Contains information on the analysis.

Field	Description
Report Date	The date the control report was generated.
Report Time	The time the control report was generated.
Module Version	The version of the TSO Comprehensive (EU, DRAGEN) Analysis Module.
Pipeline Version	The version of the analysis pipeline/workflow.
Claims Package Version	The version of the claims package.

- **Sequencing Run Details**—Contains information on the sequencing run.

Field	Description
Run Name	The name of the sequencing run.
Run Date	The date of the sequencing run.
Instrument ID	The unique ID associated with the sequencing instrument.
Instrument Control Software Version	NextSeq Operating Software (NOS) version in use for the run.
Instrument Type	The sequencing instrument type.
RTA Version	Real-Time Analysis (RTA) software version in use for the sequencing run.
Reagent Cartridge Lot Number	The lot number of the reagent cartridge used for the run.

- **Analysis Status**—Contains information on whether analysis completed for each control sample, and whether any samples failed due to a software error.

Field	Description
Sample_ID	Sample ID of the control sample. Value is (Not Run) for control type(s) not included in the run.
COMPLETED_ALL_STEPS	Indicates whether the control sample completed all the analysis steps. Possible values include TRUE, FALSE, and N/A. If the value is FALSE, contact Illumina Technical Support for more information.
FAILED_STEPS	A list of failed analysis steps due to a software error. Contact Illumina Technical Support for more information if there are any failed analysis steps.
STEPS_NOT_EXECUTED	A list of analysis steps that were not executed due to a software error. Contact Illumina Technical Support for more information if there any steps that were not executed.

- **DNA NTC Library QC Metrics**—Contains information on the quality control metric that was evaluated for the DNA No-Template Control. The status of PASS indicates that the value for the metric is within the lower specification limit (LSL) and upper specification limit (USL) ranges. The status of FAIL indicates that value for the metric is outside of LSL or USL range. N/A values are listed if the DNA No-Template Control was not included in the sequencing run.

Metric	Description	Units	Quality Threshold
MEDIAN_EXON_COVERAGE	Median exon fragment coverage across all exon bases.	Count	≤ 8

- **DNA—GIS NTC Library QC Metrics**—Contains information about whether the GIS control passed in the DNA No-Template Control. The status of PASS indicates that the value for the metric is within the lower specification limit (LSL) and upper specification limit (USL) ranges. The status of FAIL indicates that value for the metric is outside of LSL or USL range. N/A values are listed if the DNA—GIS No-Template Control was not included in the sequencing run. These control results are only applicable to GIS results for patient samples.

Metric	Description	Units	Quality Threshold
MEDIAN_TARGET_COVERAGE	Median target coverage across all exon bases.	Count	≤ 2

- **RNA NTC Library QC Metrics**—Contains information on the quality control metric that was evaluated for the RNA No-Template Control. This does not apply for TSO Comprehensive HRD (DRAGEN). N/A values are listed.
- **No-Template Control Types**—Contains information about the no-template control sample included in the run.

Field	Description
Control Type	The control type of the control sample. Possible values include: • DNA No-Template Control • DNA-GIS No-Template Control
Sample_ID	Sample ID of the control sample. Value is (Not Run) if this control type was not included in the run.
AnalysisComplete	Indication of whether analysis completed for this control sample. Possible values include TRUE, FALSE, and N/A.
Overall Result	The QC result for the control sample based on the associated NTC Library QC metrics. Possible values include PASS, FAIL, and N/A.

- **Positive Control Types—[Specimen Type]**—Contains information about the positive control samples included in the run, which are applicable to patient samples with a specimen type matching the section header (for example, Solid-FFPE). This section is repeated for each specimen type included in the sequencing run.

Field	Description
Control Type	The control type of the control sample. Possible values include: • DNA Positive Control—Small Variants and TMB • DNA Positive Control—Gene Amplifications • DNA-GIS Positive Control
Sample_ID	Sample ID of the control sample. Value is (Not Run) if this control type was not included in the run.
AnalysisComplete	Indication of whether analysis completed for this control sample. Possible values include TRUE, FALSE, and N/A.
Overall Result	The QC result for the control sample. Possible values include PASS, FAIL, and N/A.
Sensitivity Value	The calculated sensitivity value for the control sample. Represents the ratio of detected control variants to the total number of expected control variants in the control sample. Only applicable for DNA Positive Control.
Sensitivity Threshold	The minimum sensitivity value required for the control sample to have a QC result of PASS. Only applicable for DNA Positive Control.

- **DNA-GIS Positive Control Results—[Specimen Type]**—Contains information about whether the GIS control passed in the DNA Positive Control. The status of PASS indicates that the value for the metric is within the lower specification limit (LSL) and upper specification limit (USL) ranges. The status of FAIL indicates that value for the metric is outside of LSL or USL range. N/A values are listed if the DNA—GIS Positive Control was not included in the sequencing run.

Metric	Description	Units	Quality Threshold
PCT_TARGET_HRD_50X	Percent HRD probes with 50X fragment coverage.	%	≥ 50

- **Small Variants Truth Table Results—[Specimen Type]**—Contains information on which control DNA small variants applicable to the specimen type were detected or not detected (one row per control variant) in the DNA Positive Control. N/A values are listed if the DNA Positive Control was not included in the sequencing run. This section is repeated for each specimen type included in the sequencing run.

Field	Description
Detected	Indicates whether the control DNA small variant was detected in the control sample. Possible values include TRUE, FALSE, and N/A.
HGNC Gene Name	HUGO Gene Nomenclature Committee (HGNC) gene symbol associated with the control DNA small variant.
Chromosome	Chromosome of the control DNA small variant.
Position	Position (hg19) of the control DNA small variant.
Reference Allele	Reference allele of the control DNA small variant.
Alternative Allele	Alternate/alternative allele of the control DNA small variant.

- **Gene Amplification Truth Table Results—[Specimen Type]**—Contains information on which control DNA gene amplifications in the DNA Positive Control were detected or not detected (one row per control variant). N/A values are listed if the DNA Positive Control was not included samples in the sequencing run.

Field	Description
Detected	Indicates whether the control DNA gene amplification was detected in the control. Possible values include TRUE, FALSE, and N/A.
Type	Type of the control DNA gene amplification.
HGNC Gene Name	HUGO Gene Nomenclature Committee (HGNC) gene symbol associated with the control DNA gene amplification.

- **MSI Truth Table Results**—Contains information on MS status of the DNA Positive Control was detected or not detected. This does not apply for TSO Comprehensive HRD (DRAGEN). N/A values are listed.
- **Splice Variants Truth Table Results**—Contains information on which control RNA splice variants applicable to the specimen type were detected or not detected (one row per control variant) in the RNA Positive Control. This does not apply for TSO Comprehensive HRD (DRAGEN). N/A values are listed.
- **Fusions Truth Table Results**—Contains information on which control RNA fusion variants in the RNA External Control (positive RNA control) were detected or not detected (one row per control variant). This does not apply for TSO Comprehensive HRD (DRAGEN). N/A values are listed.

Metrics Output

File name: `MetricsOutput.tsv`

The metrics output is a tab-delimited file that provides quality control information for patient samples included in the run.

The metrics output file contains the following sections and associated fields:

- **Header**—Contains general information about the file and the run.

Field	Description
Output Date	Date this file was created.
Output Time	Time this file was created.
Workflow Version	The version of the analysis pipeline/workflow.
Module Version	The version of the TSO Comprehensive (EU, DRAGEN) Analysis Module.
Run ID	The ID of the sequencing run.
Run Name	The name of the sequencing run.

- **Run QC Metrics**—Contains quality control information for the sequencing run. This section corresponds to the Run QC status in the TSO Comprehensive (EU, DRAGEN) report and contains one row per QC metric that contributes to Run QC status. All QC metrics in this section must pass for Run QC to pass. Refer to *Run Quality Control* on page 12 for analysis details.

Column	Description
Metric (UOM)	QC metric name and unit of measurement.
LSL	Lower specification limit (inclusive).
USL	Upper specification limit (inclusive).

Column	Description
Value	QC metric value.
PASS/FAIL	Indicates whether the sample passed or failed the quality control metric. Possible values include PASS, FAIL, and N/A.

- **Analysis Status—[Specimen Type]**—Contains information on whether analysis completed for each patient sample matching the specimen type in the section header, and whether any of those samples failed due to a software error. Each column in this section corresponds to a patient sample (Case ID is used for the column name). This section is repeated for each specimen type included in the sequencing run.

Field	Description
COMPLETED_ALL_STEPS	Indicates whether the sample completed all steps of the analysis. Possible values include TRUE and FALSE. If the value is FALSE, refer to *Troubleshooting* on page 48.
FAILED_STEPS	A list of any failed analysis steps due to a software error. Refer to *Troubleshooting* on page 48 if any step is listed here.
STEPS_NOT_EXECUTED	A list of any analysis steps not executed due to a software error. Refer to *Troubleshooting* on page 48 if any step is listed here.

- **QC Metrics Sections for Patient Samples**—The following table notes where a quality control status in the TSO Comprehensive (EU, DRAGEN) report corresponds to a section. A section is included for each type of quality control used for patient samples.

Section	Description	Report QC Category
DNA Library QC Metrics—[Specimen Type]	This section is repeated for each specimen type included in the sequencing run. Includes QC metrics used as validity criteria for DNA sample libraries processed with the specimen type specified in the section header. Refer to *Quality Control for DNA Sample Libraries* on page 15 for analysis details.	DNA Library QC

Section	Description	Report QC Category
DNA Library QC Metrics for Small Variant Calling and TMB—Solid-FFPE	QC metrics used as validity criteria for small variants and TMB in a DNA sample library with a specimen type of Solid-FFPE. Refer to *Quality Control for DNA Sample Libraries* on page 15 for analysis details. Refer to *DNA Library QC for Small Variant Calling and TMB Metrics and Boundaries* on page 51 for metric descriptions and thresholds.	DNA Small Variant & TMB QC
DNA Library QC Metrics for MSI—Solid-FFPE	Does not apply for TSO Comprehensive HRD (DRAGEN). N/A values are listed.	DNA MSI QC
DNA Library QC Metrics for CNV—Solid-FFPE	QC metrics used as validity criteria for large rearrangements in a DNA sample library with a specimen type of Solid-FFPE. Refer to *Quality Control for DNA Sample Libraries* on page 15 for analysis details. Refer to *DNA Library QC for CNV Metrics and Boundaries* on page 52 for metric descriptions and thresholds.	DNA Copy Number Variant QC
DNA Library QC Metrics for GIS—Solid-FFPE	QC metrics used as validity criteria for GIS in a DNA sample library with a specimen type of Solid-FFPE. Refer to *Quality Control for DNA Sample Libraries* on page 15 for analysis details.	DNA GIS QC
DNA Expanded Metrics	DNA Expanded Metrics are for information only and do not directly indicate the quality of DNA libraries. Refer to *Quality Control for DNA Sample Libraries* on page 15 for analysis details. Refer to *DNA Expanded Metrics* on page 52 for metric descriptions.	N/A
RNA Library QC Metrics—Solid-FFPE	QC metrics used as validity criteria for RNA sample libraries with a specimen type of Solid-FFPE. This does not apply for TSO Comprehensive HRD (DRAGEN). N/A values are listed.	RNA Library QC

Section	Description	Report QC Category
RNA Expanded Metrics	RNA Expanded Metrics are for information only and do not directly indicate the quality of RNA libraries. This does not apply for TSO Comprehensive HRD (DRAGEN). N/A values are listed.	N/A

Each section contains the following columns:

- One column per sample (named with Sample ID).
- **Metric (UOM)**—The QC metric name and unit of measurement.
- **LSL**—Lower specification limit (inclusive).
- **USL**—Upper specification limit (inclusive).

Each section contains the following rows:

- One row per QC metric.
- **PASS/FAIL**—Indicates whether the sample passed or failed for the type of quality control. A status of PASS indicates that the sample values for the metrics are within LSL and USL range. A status of FAIL indicates that sample values for one or more of the metrics are outside of the LSL or USL range. This row is not included for DNA Expanded Metrics or RNA Expanded Metrics.
- **Notes**—Contains a list of notes describing the content of the file.

Low Depth Report

File name: `{SAMPLE_ID}_LowDepthReport.tsv`

The low depth report is a tab-delimited file created for each patient sample. The report includes a listing of genomic position ranges with a total sequencing depth < 100 and for which a passing variant was not detected. These positions have insufficient sequencing depth to rule out the presence of a small variant. Positions on the block list are excluded from the report.

The low depth report is not regenerated during Report Regeneration.

The low depth report contains the following sections and associated fields:

- **Header**—Contains general information about the file and the run.

Field	Description
Report Title	The name of the report.
Product Title	The name of the product.
Sample ID	Sample ID of the patient sample.
Report Date	The date the low depth report was generated.

Field	Description
Run ID	The ID of the sequencing run.
Run Date	The date of the sequencing run.
Knowledge Base version	The version of the KB that was installed when the low depth report was generated.
Knowledge Base published date	The date associated with KB that was installed when the low depth report was generated.
Module Version	The version of the Illumina Run Manager used in the analysis.
Regulatory statement	The regulatory statement defining the proper use of the report.

- **Genomic Range List**—Contains a list of genomic position ranges with low depth. Contiguous genomic positions with low depth overlapping the same gene(s) are combined into a single row.

Column	Description
Chrom	Chromosome.
Start	Start position (hg19).
End	End position (hg19).
Gene	Gene symbol(s) overlapping the genomic range based on the RefSeq database included in the KB.

View Analysis Results

From the Completed tab of the Runs screen, select the run name to view analysis results.

Run Details Screen

The Run Details screen displays the analysis results associated with the selected run and provides the option to reanalyze the run with different parameters. The top of the screen includes the run details, such as the run name, run description, and application details. The Status section updates with the start and finish times of steps in the run.

NOTE Analysis Complete status indicates that the analysis completed but does not indicate that the analysis was successful. The Analysis Status field in the Sample Level Metrics table indicates whether all steps in the pipeline complete successfully. Review the *Metrics Output* on page 40 for details on any failed steps.

Sequencing information is displayed, including the instrument name, library prep kit, index adapter kit, read type, and read lengths. Users can also choose to requeue their analysis in this section (refer to *Requeue Analysis* on page 47).

Results Section

The Results section includes links to the TSO Comprehensive (EU, DRAGEN) report for each patient sample in the run. This section also includes the Output Folder path for the analysis. Navigate to this location to access results and intermediate files. QC and control statuses in the Results section are sourced from the *Metrics Output* on page 40 and *Control Output Report* on page 36.

Run Level Metrics

The Run Level Metrics section of the Samples & Results screen displays a status of PASS or FAIL for each run level metric including control sample status. Refer to the following table for outcome value interpretation.

Metric	Outcome	Interpretation
RunQc	PASS	Sequencing run is valid.
	FAIL	Quality metrics for the sequencing run have not been met. Samples in the sequencing run are invalid.

Metric	Outcome	Interpretation
DNA Positive Control—Small Variants and TMB	PASS	Expected variants have been detected.
	FAIL	Variant calling specifications have not been met and small variants in the sequencing run are invalid.
DNA Positive Control—Gene Amplifications and Large Rearrangements	PASS	Expected variants have been detected.
	FAIL	Variant calling specifications have not been met and large rearrangements in the sequencing run are invalid.
DNA Positive Control—MSI This does not apply for TSO Comprehensive HRD (DRAGEN)	PASS	Expected variants have been detected.
	FAIL	Variant calling specifications have not been met and MSI scores in the sequencing run are invalid.
DNA No-Template Control	PASS	Cross-contamination between libraries is not indicated.
	FAIL	Cross-contamination between libraries is indicated. DNA samples in the library preparation event and all associated sequencing runs are invalid.
DNA GIS Positive Control	PASS	Sufficient sequencing coverage and uniformity to calculate GIS.
	FAIL	Insufficient sequencing coverage and uniformity to calculate GIS in the sequencing run.
DNA GIS No-Template Control	PASS	Cross-contamination between libraries is not indicated.
	FAIL	Cross-contamination between libraries is indicated. GIS in the sequencing run are invalid.

Sample Level Metrics

The Sample Level Metrics section of the Run Details screen displays quality control information for patient samples included in the run. Sample level metrics are evaluated independently of run level metrics.

- **Sample**—The sample ID.
- **Analysis Status**—Possible values are PASS and FAIL. Indicates whether all steps have completed successfully for the sample. Review *Metrics Output* on page 40 for details on any failed steps.

- **DNA Library QC Metrics**—Possible values are PASS and FAIL. Indicates whether the sample passed or failed DNA library QC, which applies to the DNA library that was sequenced. Corresponds to DNA Library QC in the TSO Comprehensive (EU, DRAGEN) report. An N/A is shown if a DNA library was not sequenced, or Run QC has a value of FAIL.
- **RNA Library QC Metrics**—Indicates whether the sample passed or failed RNA library QC. This does not apply for TSO Comprehensive HRD (DRAGEN). N/A values are listed.
- **DNA Library QC Metrics for GIS**—Possible values are PASS and FAIL. Indicates whether the sample passed or failed QC for GIS in the DNA library. Corresponds to DNA GIS QC in the TSO Comprehensive (EU, DRAGEN) report. An N/A is shown if a DNA library was not sequenced, Run QC has a value of FAIL, or DNA Library QC has a value of FAIL.
- **DNA Library QC Metrics for MSI**—Indicates whether sample passed or failed QC for MSI in the DNA library. This does not apply for TSO Comprehensive HRD (DRAGEN). N/A values are listed.
- **DNA Library QC Metrics for CNV**—Possible values are PASS and FAIL. Indicates whether the sample passed or failed QC for large rearrangements in the DNA library. Corresponds to DNA Copy Number Variant QC in the TSO Comprehensive (EU, DRAGEN) report. An N/A is shown if a DNA library was not sequenced, Run QC has a value of FAIL, or DNA Library QC has a value of FAIL.
- **DNA Library QC Metrics for Small Variants and TMB**—Possible values are PASS and FAIL. Indicates whether the sample passed or failed QC for small variants and TMB in the DNA library. Corresponds to DNA Small Variant and TMB QC in the TSO Comprehensive (EU, DRAGEN) report. An N/A is shown if a DNA library was not sequenced, Run QC has a value of FAIL, or DNA Library QC has a value of FAIL.

Requeue Analysis

You can requeue a completed analysis run on TSO Comprehensive (EU, DRAGEN) Analysis Module.

1. From the Completed tab of the Runs screen, select the run name to view analysis results.
2. At the bottom of the Run Details page, select **Requeue Analysis**.
3. Select one of the following requeue options:
   - Requeue analysis with no changes.
   - Edit run settings and requeue analysis. The Run Name cannot be edited.
   - Requeue analysis with a different application.
     For detailed instructions, refer to the application workflow guide for your analysis application.
4. Select the external storage configuration where current sequencing data is located (automatically populated, but editable).
5. Enter a reason for the reanalysis.
6. Select **Requeue Analysis**.

Troubleshooting

The following table lists software issues that you might encounter when using TSO Comprehensive (EU, DRAGEN) assay software.

If the sample report indicates that the analysis failed due to a software error, use the following files to troubleshoot the issue based on the specific failed step. Both files are located in the `IVD_Reports` folder.

- `MetricsOutput.tsv`—Indicates the analysis step that did not complete for patient samples under FAILED_STEPS.
- `ControlOutput.tsv`—Indicates the analysis step that did not complete for control samples under FAILED_STEPS.

Use the following table to troubleshoot issues in the workflow:

Observation	Recommended Action
Analysis remains in the ***Analysis in Progress*** state for longer than 3 hours.	From the Active Runs page, select **Cancel Analysis** under the Actions column. After canceling the run, navigate to the Completed Runs page and requeue the analysis for the canceled run. Refer to *Requeue Analysis* on page 47 for details on the requeue process.
Error with FastqValidation step.	Possible causes are as follows: • Incorrect or nonexistent index resulting in no reads for the sample. If an incorrect index is suspected, repeat the analysis with the correct index identifier selected. Otherwise, repeat the TruSight Oncology Comprehensive HRD (DRAGEN) workflow with a new extraction of nucleic acid in accordance with the *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)*. • Run performance. Assess the run performance using Sequencing Analysis Viewer (SAV) and contact Illumina Technical Support for assistance.

For any other failed steps, review `LogsIntermediates/run.log` and the logs and intermediate files under the relevant step-specific subfolder in the `Logs_Intermediates` folder. Contact Illumina Technical Support for assistance.

Appendix A QC Metrics Flowchart

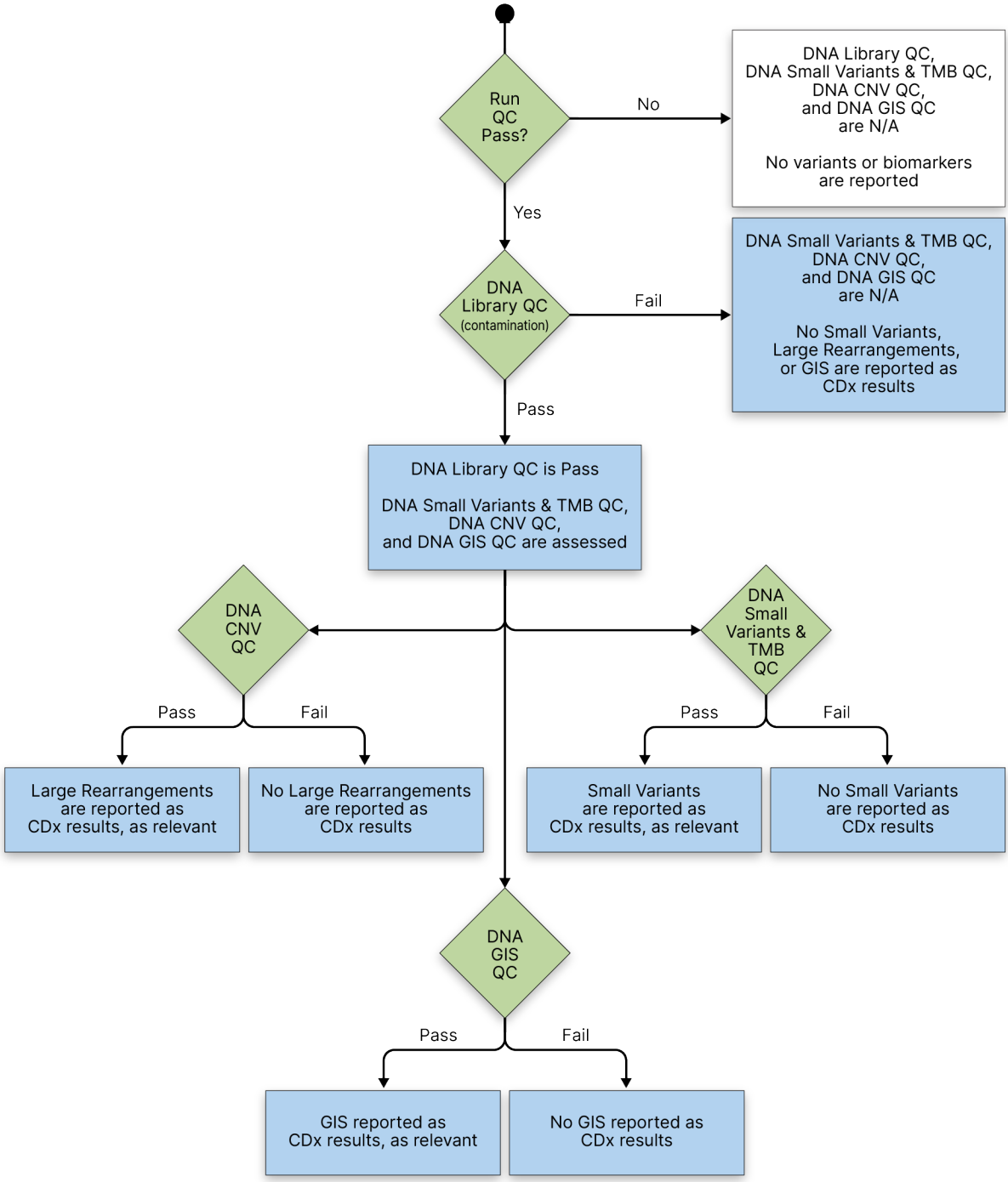
The following flowchart describes the QC metrics that are listed on the TSO Comprehensive (EU, DRAGEN) report. If Run QC fails, then no other QC steps are assessed, and all are marked as N/A. If DNA is not sequenced or fails Library QC, then any corresponding variant types are not included in Companion Diagnostic results. DNA Library QC is a measure of contamination. If DNA Library QC fails, the downstream DNA QC Metrics (DNA small variants and TMB QC, DNA CNV QC, and DNA GIS QC) are marked as N/A. For more information, refer to the following sections and tables:

- *Analysis Methods* on page 12
- *TSO Comprehensive (EU, DRAGEN) Report* on page 17
- *Quality Control for DNA Sample Libraries* on page 15
- *Metrics Output* on page 40
- *Appendix B QC Metrics* on page 51

The flowchart does not map the control samples. The results from the control samples do not impact the QC metrics on the TSO Comprehensive (EU, DRAGEN) PDF or JSON report. Failure of control samples invalidates sample results irrespective of QC results, as described in *Control Output Report* on page 36. For additional control sample information, refer to the *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)*.

The flowchart does not map the position-level QC results. These results are part of the Companion Diagnostic QC results, which are described in *Companion Diagnostics QC* on page 24.

Figure 3 QC Metrics Flowchart



Appendix B QC Metrics

Run QC Metrics

Metric	Description	Units	Quality Threshold
PCT_PF_READS	Percent of reads passing filter.	%	≥ 80%
PCT_Q30_R1	Average percent of base calls with quality score of Q30 or higher for Read 1.	%	≥ 80%
PCT_Q30_R2	Average percent of base calls with quality score of Q30 or higher for Read 2.	%	≥ 80%

DNA Library QC Metrics and Boundaries

Metric	Description	Units	Quality Threshold (FFPE)
CONTAMINATION_SCORE	The contamination score based on variant allele frequency (VAF) distribution of SNPs.	N/A	≤ 1457

DNA Library QC for Small Variant Calling and TMB Metrics and Boundaries

Metric	Description	Units	Quality Threshold (FFPE)
MEDIAN_INSERT_SIZE	The median fragment length in the sample.	Base pairs	≥ 70.0
MEDIAN_EXON_COVERAGE	Median exon fragment coverage across all exon bases.	Count	≥ 150.0
PCT_EXON_50X	Percent exon bases with 50x fragment coverage.	%	≥ 90.0
PCT_CHIMERIC_READS	Percent of chimeric reads.	%	≤ 8.0

DNA Library QC for GIS Metrics and Boundaries

Metric	Description	Units	Quality Threshold (FFPE)
PCT_TARGET_HRD_50X	Percent HRD probes with 50X fragment coverage.	%	≥ 50.0
EXCESSIVE_TF	Error mode metric that indicates whether tumor fraction is excessive and tumor and normal signals can no longer be differentiated for calculating GIS.	N/A	≥ 0

DNA Library QC for CNV Metrics and Boundaries

Metric	Description	Units	Quality Threshold (FFPE)
GENE_SCALED_MAD	The median of absolute deviations from the median of the normalized count of each CNV target region.	Count	0 ≤ x ≤ 0.134
MEDIAN_BIN_COUNT_CNV_TARGET	The median raw bin count per CNV target.	Count	≥ 1.0

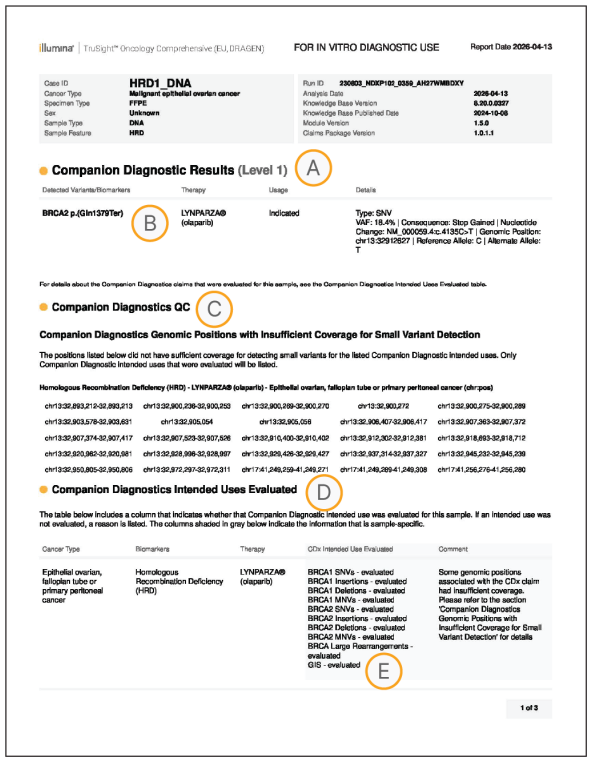
DNA Expanded Metrics

DNA expanded metrics are provided for information only. These metrics can be informative for troubleshooting but are provided without explicit specification limits and are not directly used for sample quality control. For additional guidance, contact Illumina Technical Support.

Metric	Description	Units
TOTAL_PF_READS	Total reads passing filter.	Count
MEAN_FAMILY_SIZE	The sum of the reads in each family divided by the number of families after correction, collapsing, and filtering on supporting reads.	Count
MEDIAN_TARGET_COVERAGE	The median coverage of bases.	Count

Metric	Description	Units
PCT_EXON_100X	Percent of exon bases with greater than 100X coverage.	%
PCT_READ_ ENRICHMENT	Percentage of reads that cross any part of the target region vs. total reads. The calculation of this metric considers only non-HRD probes. Therefore, if HRD probes are applied for the sample, lower values can be observed.	%
PCT_USABLE_UMI_ READS	The percentage of reads with usable UMIs.	%
MEAN_TARGET_ COVERAGE	The mean coverage of bases.	Count
PCT_ALIGNED_ READS	Percent of reads that aligned to the reference genome.	%
PCT_ CONTAMINATION_ EST	Percent of contamination of the sample.	%
PCT_PF_UQ_READS	Percent unique reads passing filter.	%
PCT_TARGET_0.4X_ MEAN	Percent target bases with target coverage greater than 0.4 times the mean.	%
PCT_TARGET_100X	Percent target bases with greater than 100X coverage.	%
PCT_TARGET_250X	Percent target bases with greater than 250X coverage.	%
MEDIAN_TARGET_ HRD_COVERAGE	Median target fragment coverage across all target positions in the genome. Coverage is the total number of non-duplicate pair alignments that overlap.	Count

Appendix C TSO Comprehensive HRD (DRAGEN) Report Reference



illumina | TruSight™ Oncology Comprehensive (EU, DRAGEN) FOR IN VITRO DIAGNOSTIC USE Report Date 2026-04-13

Case ID	HRD1_DNA	Run ID	230803_NDXP102_0350_AH37WMBDXY
Cancer Type	Malignant epithelial ovarian cancer	Analysis Date	2026-04-13
Specimen Type	FFPE	Knowledge Base Version	6.30.0.0327
Sex	Unknown	Knowledge Base Published Date	2024-10-08
Sample Type	DNA	Module Version	1.5.0
Sample Feature	HRD	Clarity Package Version	1.0.1.1

Companion Diagnostic Results (Level 1) A

Detected Variants/Biomarkers	Therapy	Usage	Details
BRCA2 p.(Gln1379Ter) B	LYNPARZA® (olaparib)	Indicated	Type: SNV VAF: 18.4% | Consequence: Stop Gained | Nucleotide Change: NM_000059.4:c.4135C>T | Genomic Position: chr13:32915827 | Reference Allele: C | Alternate Allele: T

For details about the Companion Diagnostics claims that were evaluated for this sample, see the Companion Diagnostics Intended Uses Evaluated table.

Companion Diagnostics QC C

Companion Diagnostics Genomic Positions with Insufficient Coverage for Small Variant Detection

The positions listed below did not have sufficient coverage for detecting small variants for the listed Companion Diagnostic Intended uses. Only Companion Diagnostic Intended uses that were evaluated will be listed.

Homologous Recombination Deficiency (HRD) - LYNPARZA® (olaparib) - Epithelial ovarian, fallopian tube or primary peritoneal cancer (chr:pos)

chr13:32,893,212-32,893,213	chr13:32,900,236-32,900,253	chr13:32,900,269-32,900,270	chr13:32,900,272	chr13:32,900,275-32,900,289	
chr13:32,903,578-32,903,631	chr13:32,905,054	chr13:32,905,058	chr13:32,906,407-32,906,417	chr13:32,907,363-32,907,372	
chr13:32,907,374-32,907,417	chr13:32,907,523-32,907,526	chr13:32,910,400-32,910,402	chr13:32,912,302-32,912,381	chr13:32,918,693-32,918,712	
chr13:32,920,962-32,920,981	chr13:32,928,996-32,928,997	chr13:32,926,426-32,926,427	chr13:32,937,314-32,937,327	chr13:32,945,232-32,945,239	
chr13:32,950,805-32,950,806	chr13:32,972,297-32,972,311	chr17:41,249,259-41,249,271	chr17:41,249,289-41,249,308	chr17:41,256,276-41,256,280	

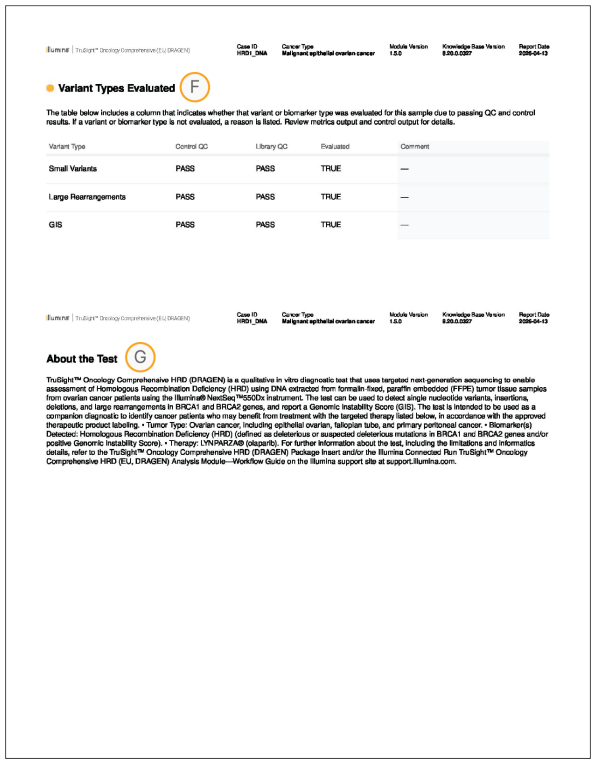
Companion Diagnostics Intended Uses Evaluated D

The table below includes a column that indicates whether that Companion Diagnostic intended use was evaluated for this sample. If an intended use was not evaluated, a reason is listed. The columns shaded in gray below indicate the information that is sample-specific.

Cancer Type	Biomarkers	Therapy	CDx Intended Use Evaluated	Comment
Epithelial ovarian, fallopian tube or primary peritoneal cancer	Homologous Recombination Deficiency (HRD)	LYNPARZA® (olaparib)	BRCA1 SNVs - evaluated BRCA1 Insertions - evaluated BRCA1 Deletions - evaluated BRCA1 MNVs - evaluated BRCA2 SNVs - evaluated BRCA2 Insertions - evaluated BRCA2 Deletions - evaluated BRCA2 MNVs - evaluated BRCA Large Rearrangements - evaluated GIS - evaluated E	Some genomic positions associated with the CDx claim had insufficient coverage. Please refer to the section 'Companion Diagnostics Genomic Positions with Insufficient Coverage for Small Variant Detection' for details

1 of 3

A. A CDx result indicates that the patient sample has a tumor type and biomarker that is targeted by the indicated therapy. For details, refer to *Companion Diagnostic Calling* on page 16. If there are no CDx results, the report states that no Companion Diagnostic biomarkers for the stated sample tumor type were detected.
B. The CDx biomarker observed in patient sample. Usage can be **Indicated** or **See Note**. If applicable, a note in the Details column provides additional information about the variant, such as information about possible drug resistance.
C. The Companion Diagnostics QC section provides position-level QC information about CDx biomarkers. If no positions are listed, there is sufficient coverage throughout the targeted variants and regions. For more information, refer to *Companion Diagnostics QC* on page 24.
D. The Companion Diagnostics Intended Uses Evaluated section lists all CDx Intended Uses and indicates whether they were evaluated in this sample. Refer to *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)* for more information about the TSO Comprehensive HRD (DRAGEN) intended use. Cancer type, Biomarkers, and Therapy are from the Intended Use statement.
E. Evaluation occurs if the tumor type is appropriate for a CDx and the sample passed required QC categories. For more information on CDx criteria required for samples to be evaluated, refer to *Companion Diagnostic Calling* on page 16.
    - ***Evaluated***—The sample was evaluated for this intended use biomarker. Specific results are identified in the Companion Diagnostics Results section of the report.
    - ***Not Evaluated***—The sample was not evaluated for this intended use biomarker and a comment explains why.



illumina | TruSight™ Oncology Comprehensive (EU, DRAGEN)

Case ID	Cancer Type	Module Version	Knowledge Base Version	Report Date
HRD1_DNA	Malignant epithelial ovarian cancer	1.0.0	0.20.0.0207	2026-04-13

Variant Types Evaluated F

The table below includes a column that indicates whether that variant or biomarker type was evaluated for this sample due to passing QC and control results. If a variant or biomarker type is not evaluated, a reason is listed. Review metrics output and control output for details.

Variant Type	Control QC	Library QC	Evaluated	Comment
Small Variants	PASS	PASS	TRUE	—
Large Rearrangements	PASS	PASS	TRUE	—
GIS	PASS	PASS	TRUE	—

illumina | TruSight™ Oncology Comprehensive (EU, DRAGEN)

Case ID	Cancer Type	Module Version	Knowledge Base Version	Report Date
HRD1_DNA	Malignant epithelial ovarian cancer	1.0.0	0.20.0.0207	2026-04-13

About the Test G

TruSight™ Oncology Comprehensive HRD (DRAGEN) is a qualitative in vitro diagnostic test that uses targeted next-generation sequencing to enable assessment of Homologous Recombination Deficiency (HRD) using DNA extracted from formalin-fixed, paraffin embedded (FFPE) tumor tissue samples from ovarian cancer patients using the Illumina® NextSeq™ 550Dx Instrument. The test can be used to detect single nucleotide variants, insertions, deletions, and large rearrangements in BRCA1 and BRCA2 genes, and report a Genomic Instability Score (GIS). The test is intended to be used as a companion diagnostic to identify cancer patients who may benefit from treatment with the targeted therapy listed below, in accordance with the approved therapeutic product labelling. • Tumor Type: Ovarian cancer, including epithelial ovarian, fallopian tube, and primary peritoneal cancer. • Biomarker(s) Detected: Homologous Recombination Deficiency (HRD) (defined as deleterious or suspected deleterious mutations in BRCA1 and BRCA2 genes and/or positive Genomic Instability Score). • Therapy: LYNPARZA® (olaparib). For further information about the test, including the limitations and informatics details, refer to the TruSight™ Oncology Comprehensive HRD (DRAGEN) Package Insert and/or the Illumina Connected Run TruSight™ Oncology Comprehensive HRD (EU, DRAGEN) Analysis Module—Workflow Guide on the Illumina support site at support.illumina.com.

F. Variant Types Evaluated indicates the variant or biomarker types that are evaluated for the patient samples due to passing QC and control results. Refer to *Variant Types Evaluated* on page 27 for more information.

G. For specific information regarding the TruSight Oncology Comprehensive HRD (DRAGEN) assay, including limitations and informatics details, refer to the *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)*.

Appendix D Sample Information Examples

Sample Data

A Add 1 Rows +

	Sample ID* C	Index Set ID*	I7 Index	Index 1	I5 Index	Index 2	Library Name
1	SampleA-DNA	CP01	D721	CATCGAGG	D507	ACGTCCTG	
2	SampleB-DNA	CP02 D	D723	CTCGACTG	D508	GTCAGTAC	
3	SampleC-DNA	UP01	D702	TCCGGAGA	D503	AGGATAGG	
4	SampleD-DNA	UP02	D707	CTGAAGCT	D504	TCAGAGCC	
5	SampleE-DNA	CP03	D709	CGGCTATG	D519	CCGTCGCC	
6	DNAPosControl B	UP03	D717	CGTAGCTC	D509	CATCCGAA	
7	DNA-NTControl	UP04	D706	GAATTCGT	D510	TTATGAGT	

Sample Settings

	Sample ID*	Case ID	Sample Type*	Specimen Type*	Cancer Type*	Sex*	Sample Feature E	Sample Description
1	SampleA-DNA		DNA	Solid-FFPE	Ovarian cancer	Female	HRD	
2	SampleB-DNA		DNA	Solid-FFPE	Ovarian cancer	Female	HRD	
3	SampleC-DNA		DNA	Solid-FFPE	Ovarian cancer	Female	HRD	
4	SampleD-DNA		DNA	Solid-FFPE	Ovarian cancer	Female	HRD	
5	SampleE-DNA		DNA	Solid-FFPE	Ovarian cancer	Female	HRD	
6	DNAPosControl		DNA	DNA Positive Control	NA	Unknown	HRD F	
7	DNA-NTControl		DNA	DNA No-Template Control	NA	Unknown	HRD	

* Required Fields

A. Make sure that the correct number of rows are added.

B. Include positive and negative controls for DNA when adding rows.

NOTE A sample processed with TSO Comprehensive HRD (DRAGEN) only requires one DNA line. A separate HRD line is not required. HRD must be assigned to DNA samples and DNA controls in the Sample Feature column on the Sample Settings screen.

C. Enter a unique sample ID.

D. Select a unique index set ID.
   - Index set IDs must be unique for all sample libraries and controls.
   - For a DNA sample library and DNA controls, select a unique index ID (UPxx or CPxx indexes) from the DNA Index ID drop-down list.
   - If there are three libraries in total in the run, follow the index selection guidelines in the *TruSight Oncology Comprehensive HRD (DRAGEN) Package Insert (document # 200080934)*.

E. Select **HRD** for samples processed with the TSO Comprehensive HRD (DRAGEN) assay.

F. If a Sample Feature is applied to a sample, it must also be applied to the associated controls.

Appendix E Cybersecurity

Overview

Illumina recommends customers apply the following to Illumina products.

Recommended Security Requirements

To continuously maintain a protected security posture for Illumina products deployed at the customer site, Illumina recommends following the security requirements listed below to avoid elevation of cybersecurity risks.

1. Make sure the Illumina instrument is positioned into a protected, well segmented network, internal to the customer organization, with access restricted to authorized personnel only.
2. Make sure the Illumina instrument is physically located within restricted environments within customer facilities, with access that is restricted to authorized personnel only.
3. Make sure communication with customer-provided network storage / server / PC is configured to use one of the latest secure protocols provided on the Illumina instrument. Secure protocols will ensure that there is authentication of the connection and will provide protection of the integrity and confidentiality of the transferred data. An example of a robust security protocol would be SMB v3.1.1 or newer with encryption and signing enabled.
4. Make sure no additional connections are configured on the Illumina instrument except for what is described in the instrument-specific documentation and configured by default as described below:
    a. For those instruments running Illumina Run Manager, make sure the inbound connection to the Illumina Run Manager module's Local Console on the instrument's operating software, which is used to pre-configure the sequencing run, is allowed only over TLS 1.2 or greater.
    b. Outbound connection to the customer-provided network storage is allowed only over secure protocols. The connection is used to make results and analyses produced by the Illumina instrument available to the customer.
    c. Optional one-way, outbound connection to Illumina cloud server, used to send telemetry on the Illumina instrument is allowed only over TLS 1.2 or greater.
5. Make sure the Illumina instrument is not used as a general use PC, where operating system level access is misused to perform actions, such as browsing the internet, downloading and installing additional non-Illumina software, etc.
6. Illumina suggests exercising caution when joining the product to Active Directory. This can overwrite Illumina's product security controls with policies from the Active Directory. If the decision is made to join Illumina's product to Active Directory, please ensure that security configuration pushed through Group Policy Objects (GPOs) does not overwrite instrument's security settings.

7. If you believe that your instrument has been impacted by a potential cybersecurity event, please contact Illumina at techsupport@illumina.com and report it as soon as possible.

Secure Device Decommissioning / Resale Protocol

Illumina provides the following guidance for securely decommissioning devices by sanitizing the Illumina instrument of sensitive, confidential, and proprietary data and software.

- In the case of a customer desiring to decommission an Illumina instrument, the hard drive must be extracted and either retained by the customer for data archiving as per customer policies or should be physically destroyed. For assistance, please contact Illumina Technical Support at techsupport@illumina.com to request a billable site visit.
- If the Illumina instrument will be resold, customers should contact Illumina Technical Support at techsupport@illumina.com as sensitive, confidential, and proprietary data, and software sanitization must be performed.

Patching

Illumina is committed to providing continuous cybersecurity support for its products, including timely communications regarding maintenance and updates of supported software and cybersecurity-specific Illumina application patching. For IVD instruments, software updates and cybersecurity patches for the Illumina instrument software will be delivered and installed by Illumina's Field Service Team. For operating system patches (for example Microsoft, Oracle) that have been tested by Illumina and made available via Illumina's Operating System Security Updates page, please work with your IT Department to apply patches to Illumina's products. Illumina is committed to providing this support on the most recent version of instrument software until the product is at its End of Service Life (EoSL) date.

Applying critical security updates on a regular schedule helps to protect Illumina instruments from the latest security threats and vulnerabilities.

Security Posture

Illumina instruments are shipped with set of software, features and configurations, enabling strong security posture at the operating system level, such as:

- Firewall configured to only allow necessary connections.
- Security audit logging is enabled with logs stored in a protected manner for Dx instruments.

Illumina recommends customers:

- Follow the instructions provided on the Antivirus Software section of the *Illumina Instrument Control Computer Security and Networking (document # 1000000085920)* and enable anti-virus tool in manual scan mode.

- Do not modify any security configurations once the anti-virus tool is enabled and configured or, if necessary, contact Illumina Technical Support for consultation before doing so to avoid introduction of additional cybersecurity risks on the product.

The Illumina instrument is shipped with user management functionality that follows best security practices and includes:

- Strong utilization of role-based access control and separation of User and Administrator roles (refer to Illumina Run Manager User Management).
- Introduction and enforcement of password complexity rules.
- Introduction and enforcement of brute force attack protection.
- Ability to maintain identity-based accounts.
- Storage of passwords.
- Identity based security audit logging for Illumina Run Manager enabled instruments.

Illumina recommends using the provided system user management functions to their full extent and only using non-shared accounts, with unique login accounts and passwords associated with each user. Illumina also recommends utilizing security best practices, including password conventions recommended by the National Institute of Standards and Technology (NIST). Those practices include not re-using passwords from another system, not using dictionary words as part of the password, and using passwords of sufficient length and complexity (at least 10 characters, with one upper case, one lower case, one digit, and one special character).

Illumina Run Manager Certificate Set Up

To ensure secure communication when accessing Illumina Run Manager, follow the steps below for managing certification.

Upon installation, an Illumina Field Service Engineer (FSE) will set up the certificate for the DRAGEN Server. If your own certificate is used, this set up will be done by an FSE during installation. To set up a certificate after installation, contact Illumina Technical Support for assistance. If one is not provided, a self-sign certificate will be generated during installation.

When connecting to the Illumina Run Manager from another computer that is not the instrument and a self-signed certificate is used, you must add the self-signed certificate to the computer's trusted certificate store. For additional support installing certificate, please contact your IT Department.

Revision History

Document	Date	Description of Change
Document # 200080937 v00	May 2026	Initial release.

Technical Assistance

For technical assistance, contact Illumina Technical Support.

Website:	www.illumina.com
Email:	techsupport@illumina.com

Safety data sheets (SDSs)—Available on the Illumina website at support.illumina.com/sds.html.

Product documentation—Available for download from support.illumina.com.

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